UNIVERSIDAD AUTÓNOMA METROPOLITANA

DOCTORADO EN CIENCIAS BIOLÓGICAS Y DE LA SALUD

Monitoreo electrónico uterino y fetal durante el parto en perras con diferente experiencia materna y talla: respuestas fisiológicas, neurológicas y termográficas del neonato canino como modelo altricial

T E S I S

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P R E S E N T A

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Karina del Rocío Lezama García

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ÍNDICE GENERAL

INDICE DE CUADROS Y FIGURAS

ABREVIATURAS

A: Abdominal.

AB: After birth.

BAT: Brown adipose tissue.

Ca^{++}: Calcio.

FCF: Frecuencia cardiaca fetal.

HCO^{3-}: Bicarbonato de sodio.

HPA: Hypothalamic Pituitary Axis.

IRT: Infrared thermography.

MEF: Monitoreo electrónico fetal.

MTBB: Miembro torácico bíceps braquial.

MTC: Miembro torácico codo.

MTM: Miembro torácico metacarpos.

MPF: Miembro pélvico femoral.

N: Nasal.

T: Torácica.

POA: Preoptic area from hypothalamus.

pO_2: Presión parcial de oxígeno.

pCO_2: Presión parcial de oxígeno.

PSI: Párpado superior izquierdo.

SB: Stillbirth

SNA: Sistema Nervioso Autónomo.

SNC: Sistema Nervioso Central.

SNS: Sistema Nervioso Simpático.

SNP: Sistema Nervioso Parasimpático.

TIR: Termografía infrarroja.

TRP: Transient receptor potential.

RESUMEN

El objetivo general del presente estudio fue determinar cuál es el efecto de la experiencia materna y peso (tanto de la madre como del recién nacido) en las respuestas relacionadas con el monitoreo electrónico fetal y uterino, perfil fisio-metabólico sanguíneo, cambios en la temperatura corporal del neonato hipotérmico, así como la vitalidad y grado de tinción de meconio en la piel del recién nacido. Así mismo, se buscó determinar cuáles son las modificaciones en la dinámica uterina (intensidad, duración y número de contracciones) y los episodios de bradicardia fetal por efecto del peso y experiencia materna durante el monitoreo electrónico del parto en la perra; esclarecer cuáles son las alteraciones neurológicas y calificación de vitalidad del canídeo recién nacido por efecto del peso y experiencia materna, durante el monitoreo electrónico del parto en la perra; y finalmente, evaluar cuáles son las alteraciones en el grado de tinción de meconio en la piel, las respuestas termográficas y los desajustes fisiológicos sanguíneos del neonato, por efecto de la duración del parto y tamaño de camada en perras con diferente peso y experiencia materna. Este estudio se dividió para su elaboración en cuatro fases: 1) Dinámica uterina, perfil sanguíneo y monitoreo electrónico fetal en perras primíparas y multíparas clasificadas según su peso; 2) Evaluación de la vitalidad, perfil sanguíneo y grado de tinción de meconio en la piel en perros recién nacidos clasificados según su peso al nacer; 3) Relación entre el peso de la madre y la temperatura superficial de sus cachorros en diferentes etapas del postparto; 4) Relación entre el peso del cachorro recién nacido y su equilibrio térmico. La mortalidad perinatal es un aspecto que aqueja a todas las especies. En perros se han reportado cifras que oscilan entre el 5 y el 40%. Recientemente se ha utilizado el monitoreo electrónico fetal y uterino en animales domésticos para evaluar a la madre y al recién nacido antes y durante el parto. De esta forma se puede registrar la frecuencia cardíaca fetal y la dinámica uterina. Debido a las altas tasas de mortalidad en cachorros, detectar rápidamente las causas y evitar que los recién nacidos mueran es sumamente importante. Por ello, la evaluación de vitalidad, los parámetros sanguíneos y el grado de tinción de meconio en la piel son recursos valiosos en perinatología canina. La estabilidad térmica de los recién nacidos es un parámetro esencial que se puede registrar para llevar a cabo la evaluación neonatal. Conocer las ventanas térmicas para evaluar y mantener una temperatura constante ayuda a reducir significativamente la mortalidad neonatal. La hipotermia es un factor asociado con la mortalidad neonatal y puede ocurrir inmediatamente después del nacimiento como mecanismo protector para prevenir el daño hipóxico en los recién nacidos, o para reducir la tasa metabólica y con ello mejorar las posibilidades de supervivencia en las primeras horas de vida. El intercambio de calor y la temperatura superficial de los animales se pueden evaluar por medio de la termografía infrarroja (TIR). Para la elaboración de la primera fase del estudio se agruparon 96 perras y sus 476 cachorros en cuatro grupos experimentales de 24 individuos cada uno (12 primíparas y 12 multíparas), según su peso corporal: G_1 (4-8 kg), G_2 (8.1-16 kg), G_3 (16.1 a 32 kg) y G_4 (32.1 a 39.6 kg). Se evaluaron las desaceleraciones de la frecuencia cardíaca fetal (DIP 2), la dinámica uterina y el perfil sanguíneo, incluidos la glucosa, el lactato, la pCO_2, la pO_2, el pH, el HCO_3^- y el Ca^{++}. Esto con el objetivo de evaluar la dinámica uterina en perras con diferentes pesos y número de paridad. Se observó que el peso es un valor que puede afectar la vitalidad de los recién nacidos y la dinámica uterina de las perras, mostrando diferencias en la frecuencia, intensidad y duración de las contracciones miometriales. El intervalo de expulsión entre cachorros aumentó en las hembras primíparas más livianas y disminuyó en las multíparas más pesadas. La duración de la fase de expulsión, así como el número de nacidos muertos fue mayor en las hembras primíparas más pesadas. Los cachorros machos recién nacidos registraron pesos significativamente más altos que las hembras recién nacidas. Para la elaboración de la segunda fase de este estudio se reclutaron 435 cachorros de 85 perras cercanas al parto y se dividieron en cuatro cuartiles según el peso al nacer del cachorro: Q_1 (127–200 g) *n*=110 cachorros, Q_2 (201–269 g) *n*=108 cachorros, Q_3 (270–388 g) *n*=108 cachorros y Q_4 (389–464 g) *n*=109 cachorros. Esto con el fin de evaluar de manera integral el efecto del peso al nacer sobre las variables del perfil sanguíneo, la vitalidad de los cachorros recién nacidos y el grado de tinción de meconio. Se concluyó que el peso de los recién nacidos se correlacionaba con el grado de tinción de meconio, presentándose más casos de tinción de meconio severa en los cachorros del grupo de mayor peso al nacer. El peso de los recién nacidos se correlacionó con un mayor número de mortinatos y alteraciones en las variables sanguíneas, encontrándose que los casos más graves de acidosis metabólica, hipoxia e hipoglucemia en los cachorros fueron los agrupados

en el cuartil Q_4. Por el contrario, no se encontraron correlaciones estadísticamente significativas entre el peso de los recién nacidos y la vitalidad. El análisis de los resultados mostró que los cachorros más vigorosos se encontraron en el Q_1. Sin embargo, al minuto 60 después del nacimiento, todos los cachorros en los cuatro cuartiles estandarizaron sus puntuaciones de vitalidad. En la tercera fase de este estudio se evaluó la temperatura superficial mediante TIR en ocho ventanas térmicas y siete momentos diferentes: cuando el cachorro está húmedo debido al líquido amniótico inmediatamente después del nacimiento hasta las 24 h de vida en cachorros recién nacidos de hembras divididos en cuatro grupos de acuerdo con su peso corporal. Lo anterior con el objetivo de evaluar la temperatura superficial y las alteraciones en las distintas ventanas térmicas de cachorros con madres de diferentes pesos y sus repercusiones en dicha temperatura. Los resultados revelaron una correlación positiva entre el peso de las perras y la capacidad para alcanzar termoestabilidad en los cachorros recién nacidos en todas las ventanas térmicas evaluadas. En cuanto al efecto del tiempo en el que se evaluó a los cachorros, se encontró que las temperaturas más bajas se registraron cuando los cachorros todavía estaban húmedos y las temperaturas más altas se registraron a las 24 h después del nacimiento. Las ventanas térmicas con mayor temperatura fueron abdominal, torácica, nasal y palpebral superior izquierda y las de menor temperatura fueron la ventana térmica bíceps braquial del miembro torácico, torácico codo, torácico metacarpo y pélvico femoral. Del mismo modo, se observó un aumento significativo de las temperaturas en las ventanas térmicas abdominal, torácica y palpebral superior izquierdo inmediatamente después de la ingestión de calostro. Se puede concluir que el peso de las perras es un factor importante que interviene positivamente en la capacidad termorreguladora de los cachorros, especialmente cuando los recién nacidos están secos y han pasado 24 h después del nacimiento. Para la elaboración de la cuarta fase de este estudio se evaluaron las alteraciones microcirculatorias en 8 diferentes ventanas térmicas monitoreadas por medio de TIR, durante 7 etapas diferentes en 289 cachorros recién nacidos asignados en 4 grupos. Se tomaron tres termogramas de cuatro zonas de cada cachorro: la región facial, frontal, lateral derecha y lateral izquierda. Los cachorros recién nacidos se agruparon en 4 cuartiles según su peso: Q_1 (126–226 g) n=73, Q_2 (227–330 g) n=72, Q_3 (331–387 g) n=74 y Q_4 (388–452 g) n=70. Se consideraron un total de 8 ventanas térmicas y 7 tiempos de evaluación desde húmedo al nacer hasta 24 h después del nacimiento. Los resultados revelaron una correlación positiva entre el peso del cachorro y su capacidad para lograr termoestabilidad en todas las ventanas térmicas evaluadas. Diferencias estadísticamente significativas (p< 0,0001) en las temperaturas entre los 4 cuartiles (Q_1, Q_2, Q_3 y Q_4) fueron encontradas. Las temperaturas más bajas se registraron cuando los cachorros aún estaban húmedos y las más altas a las 24 h después del nacimiento. Las ventanas térmicas con temperaturas más altas fueron la abdominal (34.234 ± 0.056°C), torácica (33.705 ± 0. 049°C), nasal (30.671 ± 0,110°C) y palpebral superior izquierda (34.066 ± 0.052°C), mientras que las más bajas fueron bíceps braquial del miembro torácico (27.534 ± 0.051°C), miembro torácico codo (27.141 ± 0.049°C), miembro torácico metacarpo (27.024 ± 0.062°C) y miembro pélvico femoral (27.654 ± 0.055°C). Se concluye que evaluar la respuesta térmica en cachorros recién nacidos puede ayudar a identificar cambios drásticos de temperatura o una compensación termorreguladora deficiente durante las primeras horas de vida y, de esta manera, ayudar a prevenir las consecuencias de la hipotermia.

Palabras clave: monitoreo fetal, monitoreo uterino, tinción de meconio, TIR, gasometría, asfixia, peso al nacimiento, cachorros, perras, mortalidad intraparto, escala de vitalidad.

ABSTRACT

The general objective of the present study was to determine the effect of maternal experience and weight on the responses related to fetal and uterine electronic monitoring, blood physiometabolic profile, changes in body temperature of the hypothermic neonate, and vitality and degree of meconium staining on the skin of the newborn. Likewise, we sought to determine what are the changes in uterine dynamics (intensity, duration and the number of contractions) and episodes of fetal bradycardia due to the effect of weight and maternal experience during electronic monitoring of labor in the bitch; to clarify what are the neurological alterations and vitality score of the newborn canine due to the effect of height and maternal experience, during the electronic monitoring of parturition in the bitch; and finally, to evaluate which are the alterations in the degree of meconium staining in the skin, the thermographic responses and the physiological blood imbalances of the neonate, due to the effect of the duration of whelping and litter size in bitches with different sizes and maternal experience. This study was divided into four phases: 1) Uterine dynamics, blood profile and electronic fetal monitoring in primiparous and multiparous bitches classified according to their weight; 2) Assessment of vitality, blood profile and degree of skin meconium staining in newborn dogs classified according to their birth weight; 3) Relationship between the dam's weight and the surface temperature of her puppies at different postpartum stages; 4) Relationship between the weight of the newborn pup and its thermal equilibrium. Perinatal mortality is an aspect that afflicts all species. Figures ranging between 5 and 40% have been reported in dogs. Electronic fetal and uterine monitoring has recently been used in domestic animals to assess the bitch and newborn before and during whelping. In this way, fetal heart rate and uterine dynamics can be recorded. Due to the high mortality rates in puppies, quickly detecting the causes and preventing newborns from dying is extremely important. Therefore, evaluating vitality, blood profile, and the degree of meconium staining of the skin are valuable resources in canine perinatology. The thermal stability of newborns is an essential parameter that can be recorded for neonatal evaluation. Knowing the thermal windows to evaluate and maintain a constant temperature helps reduce the metabolic rate, thereby improving survival in the first hours of life.. Hypothermia is a factor associated with neonatal mortality. It can occur immediately after birth as a protective mechanism to prevent hypoxic damage in newborns, or to reduce the metabolic rate and thereby improve the chances of survival in the first hours of life. The animals' heat exchange and surface temperature can be evaluated using infrared thermography (IRT) For the preparation of the first phase of the study, 96 bitches and their 476 puppies were grouped into four experimental groups of 24 individuals each (12 primiparous and 12 multiparous), according to their body weight: G_1 (4-8 kg), G_2 (8.1 -16 kg), G_3 (16.1 to 32 kg) and G_4 (32.1 to 39.6 kg). Fetal heart rate decelerations (DIP 2), uterine dynamics, and blood profile including glucose, lactate, pCO_2, pO_2, pH, HCO_3^-, and Ca^{++},,were assessed. This to evaluate the uterine dynamics in bitches with different weights and parity numbers. It was observed that weight is a value that can affect the vitality of newborns and the uterine dynamics of bitches, showing differences in the frequency, intensity, and duration of myometrial contractions. The expulsion interval between pups increased in lighter primiparous females and decreased in heavier multiparous females. The duration of the expulsion phase, as well as the number of stillborn, was greater in the heavier primiparous females. Newborn male pups had significantly higher weights than newborn females. For the preparation of the second phase of this study, 435 puppies from 85 bitches close to parturition were recruited and divided into four quartiles according to the birth weight of the pup: Q_1 (127–200 g) *n*=110 puppies, Q_2 (201– 269 g) *n*=108 pups, Q_3 (270–388 g) *n*=108 pups and Q_4 (389–464 g) *n*=109 pups. This is to evaluate the effect of birth weight on blood profile variables, the vitality of newborn puppies, and the degree of meconium staining, integrating these three aspects. It was concluded that the weight of the newborns was correlated with the degree of meconium staining, presenting more cases of severe meconium staining in the puppies of the highest birth weight group. The weight of newborns was correlated with a greater number of stillbirths and alterations in blood variables, finding that the most severe cases of metabolic acidosis, hypoxia, and hypoglycemia in puppies were those grouped in the Q_4 quartile. By contrast, no statistically significant correlations were found between newborn weight and vitality. The analysis of the results showed that the most vigorous pups were found in Q_1. However, at 60 minutes after birth, all pups in all four quartiles had their vitality scores standardized. For the preparation of the third phase of this study, the surface temperature was evaluated using infrared thermography in eight thermal windows and seven different moments: when the pup is wet due to

amniotic fluid immediately after birth up to 24 h of life in newborn pups of females divided into four groups according to their body weight. The above is to evaluate the surface temperature and the alterations in the different thermal windows of puppies with mothers of different weights and their repercussions. The results revealed a positive correlation between the weight of bitches and the ability to achieve thermostability in newborn puppies in all the thermal windows evaluated. Regarding the effect of the time the pups were evaluated, the lowest temperatures were recorded when the pups were still wet, and the highest temperatures were recorded 24 hours after birth. The thermal windows with the highest temperature were abdominal, thoracic, nasal, and left upper palpebral. Those with the lowest temperature were the biceps brachii thoracic limb thermal window, elbow thoracic limb, metacarpal thoracic limb, and femoral pelvic limb. Similarly, a significant temperature increase was observed in the abdominal, thoracic, and left upper palpebral thermal windows immediately after colostrum ingestion. It can be concluded that the weight of the bitches is an important factor that positively intervenes in the thermoregulatory capacity of the puppies, especially when the newborns are dry, and 24 hours have passed after birth. For the fourth phase of this study, microcirculatory alterations were evaluated in eight different thermal windows monitored through IRT at seven different moments in 289 newborn puppies assigned to different groups. Three thermograms were taken from four areas of each pup: the facial, frontal, right lateral, and left lateral regions. Newborn pups were grouped into four quartiles according to their weight: Q_1 (126–226 g) n=73, Q_2 (227–330 g) n=72, Q_3 (331–387 g) n=74 and Q_4 (388– 452g) n=70. A total of eight thermal windows and seven evaluation times from wet at birth to 24 hours after birth were considered. The results revealed a positive correlation between the pup's weight and its ability to achieve thermostability in all the thermal windows evaluated. Statistically significant differences (p< 0.0001) between the four quartiles (Q_1, Q_2, Q_3 and Q_4) were found. The lowest temperatures were recorded when the pups were still wet and the highest at 24 hours after birth. The thermal windows with the highest temperatures were the abdominal (34.234 ± 0.056°C), thoracic (33.705 ± 0.049°C), nasal (30.671 ± 0.110°C) and upper left palpebral (34.066 ± 0.052°C). In comparison, the lowest were thoracic limb biceps brachii (27.534 ± 0.051°C), thoracic limb elbow (27.141 ± 0.049°C), thoracic limb metacarpal (27.024 ± 0.062°C) and femoral pelvic limb (27.654 ± 0.055°C). It is concluded that evaluating the thermal response in newborn puppies can help to identify drastic temperature changes or deficient thermoregulatory compensation during the first hours of life and, in this way, help prevent the consequences of hypothermia.

Keywords: fetal monitoring, uterine monitoring, meconium staining, IRT, gasometry, asphyxia, birth weight, puppies, dams, intrapartum mortality, vitality score.

Dr. Daniel Mota Rojas

1. INTRODUCCIÓN

El nacimiento es un gran desafío para el recién nacido ya que debe adaptarse a la vida extrauterina y sobrevivir al período neonatal. Se trata de un proceso fisiológico mediante el cual el feto es expulsado del útero al exterior debido a las contracciones y a la dilatación del cuello uterino (Norwitz *et al*. 1999). Estas contracciones son causadas por la liberación de diversas hormonas, incluida la oxitocina (Mitchell *et al.,* 1998; Lezama-García *et al*. 2019a), y por cambios en el potencial de acción que provocan la despolarización y repolarización de las células del miometrio (Kanda y Kuriyama 1980). Aunado a ello, durante dicho periodo de transición existen diversos factores estresantes adicionales (ambientales, fisiológicos, algunos referentes al manejo, entre otros) que pueden afectar la vitalidad del recién nacido (Mota-Rojas *et al.* 2018). La vitalidad se describe como la capacidad del recién nacido para desarrollarse, sobrevivir y recuperarse de cualquier desequilibrio causado por el estrés en el proceso de nacimiento. La baja vitalidad es un problema recurrente en perinatología veterinaria y varios factores pueden culminar en la muerte del recién nacido (Mota-Rojas *et al*. 2018).

En neonatología veterinaria canina se ha observado una mortalidad perinatal mucho mayor a la observada en humanos, obteniéndose en perros cifras entre el 5 y 35% (Münnich y Küchenmeister 2014). Por ello, es indispensable establecer un protocolo aplicable a cachorros recién nacidos para la identificación de su estado de salud y de una oportuna intervención médica (Vassalo *et al.* 2015a).

La gestación en las perras dura un promedio de 64 días. Durante el parto, las tasas de mortalidad de los perros pueden alcanzar hasta el 40% (Veronesi, 2016). Aunado a esto, uno de cada diez cachorros puede morir antes de los 60 días de nacido (Chastant-Maillard *et al.* 2017). Por este motivo, estas altas tasas de mortalidad preocupan a los dueños y criadores de perros (Veronesi *et al.* 2009a; Veronesi 2016),

Existen diferentes causas que provocan la muerte del feto antes o durante el parto, dentro de las que se pueden citar la condición física y nutrición de la perra, y sobre todo las infecciones durante la preñez. La gestación prolongada conduce a partos distócicos y puede causar la muerte del cachorro al momento del parto. Otros factores que complican el parto y provocan la muerte fetal son el diámetro reducido del canal del parto, provocado por diferentes anomalías anatómicas de los huesos de la pelvis o por fallas en la dilatación de los tejidos blandos, como la hipoplasia vaginal, tumores genitales o estenosis vaginal (Martí Angulo, 2011).

Las causas de muerte en cachorros pueden ser de origen infeccioso y no infeccioso. Las enfermedades clasificadas como infecciosas son todas aquellas de origen viral, bacteriano, o parasitarias. Las enfermedades clasificadas como no infecciosas, son aquellas de influencia genética, congénitas, deficiente alimentación de la madre hacia el cachorro a través del calostro y el cuidado que se le brinda antes y/o después del parto (Santiago-García *et al.* 2008).

En perros, uno de los factores más importantes que intervienen en la mortalidad durante el parto es el bajo peso al nacer (Manani *et al*. 2013; Lezama-García *et al*. 2022a), ya que representa un factor importante en la vitalidad del recién nacido durante las primeras 48 horas de vida del neonato, debido a que se asocia con una mayor tasa de mortalidad, mayores problemas de adaptación a la vida extrauterina y con un bajo crecimiento postnatal. Otro factor importante es la presentación de hipotermia, la cual es considerada una condición que puede afectar directamente la supervivencia del recién nacido (Lezama-García *et al*. 2022b, 2022a, 2022c; Uchańska *et al*. 2022). En otras palabras, la termorregulación juega un papel esencial en la supervivencia de todas las especies altriciales y organismos poiquilotérmicos, especialmente en los recién nacidos (Nord *et al.* 2009; Terrien 2011; Mota-Rojas *et al.* 2021b; Lezama-García *et al*. 2022d; Reyes-Sotelo *et al*. 2022). Los individuos que realizan la transición del ambiente cálido del útero a un ambiente extrauterino presentan una disminución significativa de su temperatura corporal al nacer (Nowak y Poindron 2006; Vannucchi *et al.* 2012). Este cambio térmico afecta la capacidad del cachorro recién nacido para termorregularse, ya que mecanismos como el titiriteo y la vasoconstricción aún están poco desarrollados (Indrebø *et al.* 2007; Nakamura y Morrison 2011).

Para determinar las variaciones en la temperatura corporal, varios métodos han sido empleados en animales, pero la mayoría pueden ser invasivos y alterar el valor final (Fisher *et al.* 2008; Sevegnani *et al.* 2016). Esto puede deberse al estrés que les genera el hecho de ser manipulados (Travain *et al.* 2015). Por ello, en los últimos años se ha visto que una técnica no invasiva para evaluar los cambios de temperatura, tanto en granjas como en animales de compañía, es la TIR (Casas-Alvarado *et al*. 2020). Su amplia aplicación en los últimos años se debe a que la TIR también puede registrar las mediciones exactas de la temperatura de la superficie corporal de cualquier organismo desde una distancia de 30 cm o más (Bertoni *et al*. 2020). Además de que detecta cambios en el flujo sanguíneo de la microvasculatura en respuesta a eventos fisiopatológicos o ambientales como el estrés por calor o frío (Mota-Rojas *et al*. 2021a).

Por otro lado, la asfixia fetal también puede intervenir en las altas cifras de mortalidad de manera indirecta (Pereira *et al.* 2022), ya que con ella se pueden ver afectadas la viabilidad y la vitalidad de los cachorros (Lezama-García *et al*. 2023), además de que puede ser la causa directa de hipoxia con importantes repercusiones en la circulación sanguínea y alteraciones fisio metabólicas en los neonatos (Mota-Rojas *et al.* 2018).

El término hipoxia se define como un estado de deficiencia de oxígeno en el organismo, ya sea en tejidos o células. La hipoxemia es una disminución anormal de la presión parcial de oxígeno (pO_2) en sangre arterial. Los signos clínicos de la hipoxia en caninos incluyen: dificultad para respirar, respiración corta, rápida, profunda y superficial, respiración por la boca, ritmo cardiaco aumentado (taquicardia) y color de mucosas de pálidas a cianóticas. La causa principal de hipoxia en fetos es la asfixia (Alonso-Spilsbury *et al*. 2005). El hecho de presentar asfixia transitoria puede volverse normal durante el parto, generando en los cachorros acidosis transitoria e hipercapnia (Massip 1980; Wiberg *et al.* 2006; Pereira *et al.* 2022; Lezama-García *et al.* 2023a). El intercambio gaseoso podría verse alterado si estas condiciones continúan (Bleul *et al.* 2007), disminuyendo las tasas de respiración y generando acidosis metabólica en los recién nacidos (Andres *et al.* 1999). La evaluación del perfil fisio-metabólico durante las primeras horas de vida del recién nacido es de suma importancia. Lo anterior es porque durante el trabajo de parto, el neonato puede experimentar sufrimiento fetal como resultado de un aumento en la duración del parto y en el intervalo de expulsión. Cuando los neonatos presentan sufrimiento fetal, la vitalidad neonatal se reduce considerablemente, debido a que el animal experimenta alteraciones en el perfil fisio-metabólico (Indrebo *et al*. 2007; Groppetti *et al*. 2010). Los gases sanguíneos se pueden evaluar a través del cordón umbilical y de esta manera se puede obtener información valiosa sobre el estado de la relación acido-básica neonatal (Armstrong y Stenson 2007). Por lo tanto, la gasometría es actualmente una herramienta importante para evaluar la salud y el estado de los recién nacidos (Vassalo *et al.* 2015a). La evaluación de los parámetros sanguíneos umbilicales es una técnica que se ha aplicado a lechones recién nacidos para evaluar concentraciones de pH, presión parcial de oxígeno (pO_2), presión parcial de dióxido de carbono (pCO_2), glucosa, lactato, hematocrito, sodio, potasio y calcio ionizado (Rootwelt *et al.* 2012), sin embargo, su estudio en perros es limitado.

De igual manera, la tinción de meconio en la piel al nacer y la aspiración de meconio reflejan procesos de distocia con hipoxia intrauterina grave (Mota-Rojas *et al.* 2022). Los recién nacidos expuestos a la aspiración de meconio desarrollan el Síndrome de Aspiración de Meconio (SAM), el cual puede ocurrir en diversas especies, provocando importantes tasas de mortalidad. Por ejemplo, en cachorros, la mortalidad ocasionada por el SAM puede alcanzar cifras de entre 1 y 3 % al ocasionar obstrucción de las vías respiratorias e hipoxia fetal, siendo éste, uno de los factores críticos que pueden provocar pérdida de vitalidad en los recién nacidos (Swarnam *et al*. 2012; Martínez-Burnes *et al.* 2019).

Existen diferentes estudios en humanos (Apgar 2015) y lechones que evalúan de manera integral la hipoxia y sus repercusiones fisio metabólicas, neurológicas y de comportamiento. Se ha demostrado también el efecto de la edad de la madre o el número de partos, así como el peso del lechón y su asociación con la hipoxia (Mota-Rojas *et al.* 2012). Sin embargo, no existe información sobre la monitorización electrónica de la actividad uterina y fetal en perras por efecto de su peso y experiencia materna. Del mismo modo, tampoco existen evidencias en neonatos canídeos con hipoxia y sus efectos. Todo lo anterior debido a que se trata de una especie altricial, con pobre desarrollo neurológico neonatal

que requiere ser estudiado con escalas neurológicas diferentes, con una monitorización electrónica de la actividad uterina y fetal y con una evaluación clínica neonatal que incluya el grado de tinción de meconio en la piel y la capacidad de desplazamiento de los recién nacidos (Veronesi 2016; Mota-Rojas *et al.* 2018; Lezama-García *et al.* 2019b).

La monitorización electrónica fetal y uterina es uno de los principales métodos utilizados para evaluar y determinar clínicamente la vitalidad y el bienestar de los fetos y la dinámica uterina de la madre antes y durante el parto (Freeman 1990; Hasan *et al*. 2009b; Ayres-De-Campos y Nogueira-Reis 2016; Sbrollini *et al.* 2019). Este método registra los movimientos fetales, la frecuencia cardíaca fetal (FCF) (latidos por minuto, lpm) y las contracciones uterinas (mmHg) (Romagnoli *et al.* 2019). El segundo componente de un monitor fetal y uterino es el tocodinamómetro. Este dispositivo mide la intensidad, velocidad y duración de las contracciones uterinas (Ayres-De-Campos 2018). Es una técnica eficaz, no invasiva y una alternativa comercial a las técnicas Doppler tradicionales (Quevedo *et al.* 2019) que no representa riesgos para el feto ni para la madre (Reef *et al.* 1996; Maul *et al.* 2003). Según Davidson (2001), Groppetti *et al*. (2010), Ayres-De-Campos y Nogueira-Reis (2016), Lezama-García *et al*. (2023, 2023a), recientemente se ha comenzado a implementar esta técnica de seguimiento en perras porque se ha visto que en esta especie ayuda considerablemente a prevenir y reducir la mortalidad antes, durante y después del parto, además de ser fácil de realizar incluso por el criador o propietario del animal bajo un entrenamiento específico.

Por otro lado, la FCF es uno de los parámetros más importantes para determinar el estado de salud y bienestar de un feto. Monitorizándolo se ayuda a detectar fallos de oxigenación a tiempo (Alnuaimi *et al.* 2017), evitando así la hipoxia fetal (Gil *et al*. 2014; Biloborodova *et al.* 2021) y posibles daños neurológicos secundarios o incluso la muerte durante el parto (Hasan *et al.* 2021). La FCF depende del Sistema Nervioso Autónomo (SNA) y el nivel de estas respuestas depende, a su vez, de la cantidad de oxígeno a la que tiene acceso el feto (Ayres-De-Campos y Nogueira-Reis 2016). Por lo tanto, cuando los niveles de oxígeno caen bruscamente en el feto, se produce rápidamente una caída inmediata de la FCF (Warner 1962). Una desaceleración sostenida de la FCF refleja la manifestación de estrés fisiológico en el feto. Por lo que es fundamental conocer los parámetros normales de la FCF canina y felina al final de la gestación, que son 180-250 latidos/minuto o, al menos, cuatro veces la frecuencia cardíaca materna (Esquivel 2004; Gil *et al.* 2014).

Por lo descrito anteriormente, el objetivo de la presente tesis es evaluar el efecto del peso y la experiencia materna (primípara *vs*. multípara) por medio de la monitorización electrónica uterina y fetal durante el parto y su efecto en la respuesta neurológica (escala de vitalidad), intercambio gaseoso pulmonar, respuesta termoneutral, grado de tinción de meconio en piel y desajustes fisiológicos sanguíneos, en un modelo altricial de neonato canídeo.

2. MARCO TEÓRICO

Importancia de la mortalidad en cachorros

Los perros (*Canis lupus familiaris),* son una especie altricial que al nacer presenta poco desarrollo en diferentes órganos, tales como el hígado, riñones y cerebro, además de nacer con los ojos y el canal auditivo cerrados (Indrebø *et al.* 2007; Martínez-Burnes *et al*. 2019).

El período neonatal canídeo se refiere a las primeras 2 a 3 semanas de vida y representa un desafío para la supervivencia de los cachorros. Se sabe que la tasa de muerte fetal y neonatal es relativamente alta en perros (hasta del 40%) (Veronesi, 2016), ya que su requerimiento de energía al nacimiento es alto, pero las reservas energéticas son bajas, aunado a ello, el hígado inmaduro es ineficiente para generar energía. Esto hace que el neonato esté predispuesto a presentar hipoglucemia (Mila *et al.* 2015). Debido a que la función renal en cachorros es inmadura, hay un mayor riesgo de deshidratación, esto debido a que los recién nacidos tienen un alto porcentaje de agua corporal (82%) en comparación con los adultos y tienen una mayor pérdida de agua a través de los pulmones y la piel debido a una gran relación superficie / volumen, lo que aumenta aún más el riesgo de deshidratación (Mila *et al*. 2015).

La mortalidad en fetos y cachorros representa un factor sumamente importante, tanto para criadores como para tutores, ya que llega a representar de un 15% hasta un 20% de acuerdo con la etapa en la que ocurra (Vassalo *et al.* 2015a). Desde la gestación hasta el momento del parto, la mortalidad puede abarcar del 10 al 12%, dentro de las primeras dos semanas de vida representa del 13 o 14% y después del destete hasta el 15%. Existen diversos estudios que mencionan que el aumento del porcentaje de mortandad en las primeras 2 semanas de vida va del 17 al 40%, mientras que otros estudios reportan hasta un 20% dentro de las 7 a 8 semanas de edad (Vassalo *et al.* 2015b; Veronesi, 2016).

Distocia

El proceso del parto representa un evento importante en la supervivencia del neonato. La distocia se produce cuando hay algún impedimento para iniciar o completar el trabajo de parto y puede deberse tanto a causas maternas, como fetales. La distocia es una de las principales causas de muerte de cachorros, aproximadamente el 16% de las hembras gestantes sufren de distocia y es particularmente común en las razas braquicéfalas donde los valores pueden alcanzar hasta el 60% de los casos, debido a su desproporcionada conformación anatómica que se caracterizan por presentar cráneos más anchos con respecto a otras razas (Doebeli, *et al.* 2013).

Los factores maternos más comunes de distocia son la atonía uterina (pérdida de tono muscular), así como el tamaño pélvico estrecho y las anomalías congénitas o adquiridas del tracto reproductor caudal. Las causas fetales más comúnmente asociadas a distocia son los fetos desproporcionadamente grandes y las malformaciones fetales, como la artrogriposis, donde la rigidez de los miembros dada por hipoplasia muscular dificulta o impide el paso del feto a través del canal pélvico. Una mala presentación del feto también causa frecuentemente distocia (Batista *et al*. 2014).

Hipoxia fetal

El término hipoxia se define como un estado de deficiencia de oxígeno en el organismo, ya sea en tejidos o células. La hipoxemia es una disminución anormal de la presión parcial de oxígeno en sangre arterial. Los signos clínicos de la hipoxia en canídeos incluyen: dificultad para respirar, respiración corta, rápida, profunda y superficial, respiración por la boca, ritmo cardiaco aumentado (taquicardia) y color de mucosas de pálidas a cianóticas.

La causa principal de hipoxia en fetos es la asfixia (Alonso-Spilsbury *et al.* 2005). La asfixia fetal durante el parto es un evento común en muchas de las especies animales y en humanos. De acuerdo con la patogénesis, existen cuatro mecanismos básicos que pueden ocurrir en el útero o después del nacimiento y que dan lugar a la hipoxia: a) asfixia fetal por interrupción del flujo sanguíneo del cordón umbilical por torsión, compresión o ruptura de éste; b) asfixia fetal debido a una alteración de intercambio de oxígeno a través de la placenta; c) asfixia fetal debido a la insuficiencia placentaria; d) asfixia neonatal

por insuficiencia pulmonar. Los cuatro mecanismos provocan en el feto o neonato un estado de hipoxia con posibles consecuencias graves (Alonso-Spilsbury *et al.* 2005).

Síndrome de Aspiración de Meconio (SAM)

El término meconio, denomina la materia fecal que se acumula en el colon fetal durante la gestación. El meconio es una sustancia viscosa y espesa de color verde oscuro a negro, compuesta por células muertas y secreciones del estómago e hígado, que reviste el intestino del recién nacido. (Haraux *et al.* 2018; Cleary y Wiswell, 1998). Cuando las membranas placentarias se rompen, se produce una corioamnionitis y funisitis aguda, o si existe una alteración en el cordón umbilical, ya sea por torsión, compresión o ruptura del mismo, se provoca una alteración del flujo sanguíneo y disminución del transporte de oxígeno para el feto, dando lugar a la hipoxia fetal, lo que causa una redistribución del flujo sanguíneo en el feto y como consecuencia se incrementa el peristaltismo intestinal, así como el relajamiento del esfínter anal y finalmente la expulsión del primer contenido intestinal llamado meconio hacia el líquido amniótico. Una vez en el líquido amniótico, el meconio tiñe la piel del feto con un color amarillo (Kim y Kim*,* 2017).

El nacimiento de neonatos humanos teñidos con meconio es común y representa del 10 a 16% de todos los nacimientos de embarazos a término y en su mayoría no presentan problemas en el recién nacido. Sin embargo, cuando la hipoxia fetal es severa y persistente, o se provoca un reflejo de inspiración con la glotis abierta, lo que resulta es la inhalación del líquido amniótico contaminado con meconio y esto a su vez ocasiona una neumonía química. Las complicaciones clínicas de un neonato con este tipo de aspiración constituye el llamado Síndrome de Aspiración de Meconio (SAM) (Kim y Kim, 2017). La fisiopatología del SAM es compleja y multifactorial. La expulsión de meconio al líquido amniótico se debe principalmente a estrés fetal y a la oclusión del cordón umbilical. La hipoxia, estimula la actividad colónica y la expulsión de meconio, como consecuencia de un estímulo transitorio o permanente del sistema nervioso parasimpático, aumentando así el peristaltismo intestinal y la relajación del esfínter anal (Karabayir *et al.* 2015).

En contraste con la abundante información disponible en medicina humana sobre el SAM, la prevalencia y significancia de este síndrome es poco conocida en medicina veterinaria. Existen casos reportados de la presencia de fetos o neonatos teñidos con meconio y de su aspiración en diferentes especies como cerdos, bovinos, equinos y caninos (López y Bildfell, 1992).

En otro estudio realizado en perros, se recolectaron 60 cachorros, de los cuales 50 nacieron muertos o murieron durante el parto y los 10 restantes nacieron vivos, pero murieron durante las primeras semanas de vida. En este estudio, se determinó que la tinción de meconio en cachorros es un evento común como ocurre en otras especies, sobre todo predominando en cachorros nacidos por cesárea, en perras de raza pequeña y en hembras primerizas. El total de los cachorros teñidos con meconio fue de 41%. Se demostró que el 76% nacieron con tinción de meconio en piel en grado leve, 16% en forma moderada y solo 8% en forma severa. El 26.7% presentaban meconio macroscópicamente en vías aéreas. Se concluyó que la tinción de meconio en la piel no estaba aparentemente asociada a su aspiración y presencia en los pulmones y se hizo evidente la necesidad de otros estudios, en los que se pueda monitorizar el parto y determinar el efecto de la hipoxia en parámetros fisiológicos y metabólicos y su impacto en la vitalidad neonatal (Santiago-García *et al*. 2008).

Evaluación de la vitalidad neonatal

La viabilidad neonatal, es el potencial del recién nacido para sobrevivir fuera del útero después del nacimiento. La llamada escala Apgar es una evaluación rápida de la vitalidad en los bebés al momento del nacimiento desarrollada en 1953 por una pediatra anestesista llamada Virginia Apgar. Esta evaluación da un pronóstico numérico y además, permite determinar la necesidad de tratamiento por dificultad respiratoria. La vitalidad del neonato se evalúa mediante la puntuación de 5 parámetros, con una escala de 0,1 y 2 y generalmente se realiza 1 minuto después del nacimiento (Apgar 2015) **(Cuadro 1).**

En los últimos años, se ha adaptado la escala de Apgar en medicina veterinaria para evaluar de forma similar la vitalidad de los animales recién nacidos. En el caso de lechones, la escala de Apgar fue modificada inicialmente por Zaleski y Hacker en 1993 (1993) y posteriormente por Mota *et al*. (2005a). Dicha evaluación analiza variables de lechones relacionados con la probabilidad de muerte fetal y el puntaje de viabilidad en camadas, en donde se incluye: la edad de la cerda, condición de la cerda, la duración de la gestación, el número de lechones en la camada, el peso del lechón al nacer, el orden del nacimiento y la morfología del cordón umbilical.

En 2009, Veronesi *et al*. (2009a) propusieron una puntuación de Apgar adaptada para la evaluación de viabilidad neonatal en canideos utilizando los mismos cinco parámetros propuestos originalmente por la Dra. Virginia Apgar en bebés, pero con modificaciones necesarias para la fisiología neonatal canina. Con esta prueba se evalúa el color de las membranas mucosas, frecuencia cardiaca, irritabilidad, el realizar un movimiento de cabeza como respuesta a la estimulación, frecuencia respiratoria y vocalización (Veronesi 2016) (**cuadro 1**)

CUADRO 1. Escala para medir la vitalidad del cachorro recién nacido de acuerdo con Veronesi *et al.* (2009a)**.** Para realizar la evaluación se van sumando de 0 a 2 puntos por cada parámetro y se basa en un puntaje total de 1 a 10, cuanto más alto sea el puntaje obtenido, mejor será la evolución del recién nacido. Un puntaje de 7, 8 o 9 es normal y es una señal de que el cachorro está bien de salud.

Parámetros	0	1	2
Frecuencia cardiaca (lpm)	<180	180-220	>220
Latencia a intento de inspiración	Sin llanto/<6	Con llanto/ 6-15	Llanto claro/>15
Color de las mucosas	Pálido	Cianótico	Rosado
Reflejo de irritabilidad	Ausente (sin vocalización)	Mueca (retracción leve del miembro evaluado y vocalización débil)	Vigoroso (vocalización fuerte y retracción rápida)
Reflejo de motilidad	Ausente	Débil	Activo

Monitorización electrónica fetal y uterina

Uno de los principales factores que preocupa en perinatología veterinaria, es la elevada mortalidad perinatal que llegan a presentar algunas especies domésticas. La importancia de monitorizar el desarrollo del feto a lo largo de la gestación y el parto radica en que de esta manera se puede ayudar a mejorar el cuidado perinatal y a reducir la mortalidad de los recién nacidos, especialmente para animales con alto valor genético (Quevedo *et al.* 2019). Por ejemplo, en los perros (*Canis lupus familiaris)* se han observado cifras del 17-30% (Vassalo *et al*. 2015), aunque de acuerdo con Veronesi *et al.* (2009), estas cifran varían del 5-35% en perros. Comparativamente, de acuerdo con Mota-Rojas *et al*. (2002), van Rens y van der Lende (2004), Borges *et al.* (2005) y González-Lozano *et al.* (2009), la mortalidad intraparto varía desde el 3 al 30% en cerdos (*Sus scrofa domesticus*), ya sea por asfixia, problemas en el cordón umbilical, desprendimiento de placenta, distocia, entre otras causas. Por ello, la evaluación de la vitalidad y la oportuna detección de estrés fetal puede contribuir a disminuir la mortalidad al nacimiento en diversas especies (Groppetti *et al.* 2010). En el caso de los fetos de bovinos, la electrocardiografía fetomaterna permite un análisis continuo de la frecuencia cardiaca fetal (FCF) y de la madre, las cuales reflejan la actividad simpático adrenal en las últimas etapas de la gestación en vacas y terneros recién nacidos y con ello, se puede evaluar su nivel de bienestar (Reef *et al.* 1996).

Aunado a esto, la monitorización de la FCF por medio de electrocardiografía fetal transabdominal ha demostrado ser una técnica efectiva, no invasiva y una alternativa comercial a las técnicas de Doppler

tradicionales (Quevedo *et al.* 2019), que no presenta riesgos ni para el feto, ni para la madre (Quevedo *et al.* 2019; Reef *et al*. 1996; Maul *et al.* 2003). Además, a pesar de que existen otras pruebas (Van Rens y Van Lende, 2004), la monitorización fetal electrónica (MFE) o cardiotocografía, sigue siendo la manera más popular de evaluar clínicamente a los fetos (Sbrollini *et al*. 2019; Freeman, 1990; Hasan *et al.* 2009; Ayres-De-Campos *et al.* 2016; Georgieva *et al.* 2019), suplementado en ocasiones con muestreos sanguíneos del feto o recién nacido (Alfirevic *et al.* 2013). Asimismo, el monitoreo de la FCF con cardiotocografía (CTG) sigue siendo el pilar de la vigilancia fetal intraparto, ya que su objetivo es prevenir la muerte fetal y materna, mediante la identificación de hipoxia o isquemia incipiente en un feto sano, en un momento en que la intervención puede prevenir o mitigar la presentación de convulsiones (Alfirevic *et al.* 2017; ACOG, 2014) o una lesión permanente (Georgieva *et al.* 2019), además de que puede ayudar a evitar pérdidas emocionales y económicas importantes para los criadores o responsables de los animales (Siena y Milani, 2021). El estrés fetal provocado por la hipoxia puede ocurrir durante un parto distócico y se manifiesta por el descenso de la FCF y de ahí radica la importancia de su monitorización (Kutzler *et al.* 2003; Gil *et al.* 2014). En las últimas cinco décadas, la monitorización electrónica de la FCF ha sido uno de los pilares del diagnóstico del sufrimiento fetal (Copley, 2002; Parra, 2005). En humanos, el MFE, ha sido ampliamente utilizado y, de acuerdo con lo mencionado anteriormente, también se ha empezado a utilizar en la monitorización de los partos de otros mamíferos. Por ejemplo, en perras, se ha visto que ayuda de manera significativa a predecir si un parto terminará o no en cesárea (Gaudet y Kitchell, 1985), además de que es una herramienta que puede utilizarse en el hogar del responsable o del criador cuando se les ha capacitado previamente para hacerlo, aunado a esto, las perras lo toleran bien ya que no es invasivo y la ansiedad que el parto genera sobre el responsable del animal disminuye considerablemente (Davidson, 2001). La aplicación de la tecnología Doppler en la arteria uterina y fetal de las yeguas gestantes se ha usado en los últimos años para monitorear la respuesta hemodinámica fetal. Sin embargo, el uso del Doppler por sí solo en la arteria uterina no proporciona la evidencia suficiente de la viabilidad del feto, además de que el cordón umbilical del feto equino se visualiza de manera inconsistente después del día 250 de gestación (Bucca *et al*. 2020), es por ello que la implementación del monitoreo fetal y uterino de nuevo toma gran importancia en este sentido. Diversos estudios reportan la evaluación del flujo sanguíneo del ducto venoso con Doppler en corderos (Tchirikov *et al.* 1998; Panarace *et al.* 2008) y en perros (Barella *et al*. 2014; 2016) o de las contracciones uterinas con tocodinamómetros (Davidson, 2011). Del mismo modo, en un estudio realizado por Vassalo *et al*. (2015), en donde evaluaron partos eutócicos y distócicos en 7 diferentes razas de perras consideraron los cambios en la frecuencia cardiaca fetal por medio de un ultrasonido Doppler para determinar en qué momento intervenir y realizar una cesárea en caso de así requerirse. La monitorización de los parámetros de la FCF se ha realizado también en bovinos (*Bos Taurus*) (Jonker *et al.* 1994), ovejas (*Ovis orientalis aries*) (Martin, 1986) y caballos (*Equus caballus*) (Adams-Brendemuehl y Pipers, 1987), sin embargo, así como en las demás especies, es necesario tener conocimiento sobre los parámetros basales antes del parto.

Del mismo modo, con estas evaluaciones se pueden detectar a tiempo problemas, tales como la asfixia (en humanos, de cada 1000 nacimientos, de 2-5 bebés presentan asfixia), acidemia, encefalopatía isquémica hipóxica, así como desórdenes del neurodesarrollo (en humanos el 30% de los nacimientos puede presentar un problema de neurodesarrollo) (Malin *et al.* 2010) o parálisis cerebral (Hirsch, 2019; Lamb y Haezell, 2019).

Se ha observado que la muerte perinatal puede ocurrir en cualquier etapa de la gestación en mamíferos (Ginther, 1985), es decir, es posible que suceda en el útero, durante la expulsión, al momento de nacer, posterior al nacimiento, en los primeros días o semanas de vida, o después del destete. De cualquier modo, la muerte perinatal es más elevada durante el parto, inmediatamente después del parto o durante los primeros días de vida (Veronesi *et al*. 2009). Si la muerte fetal ocurre dentro del primer tercio de la gestación, se lleva a cabo una reabsorción embrionaria y cuando se presenta en el tercio medio o final, puede presentarse una momificación del feto (England y Russo, 2006) y aquí, de nuevo queda clara la invaluable ayuda que se obtiene con el MFE.

Al notar la importancia del MFE, se han realizado diversos estudios en donde se han comparado diferentes equipos para el MFE, por ejemplo, Alzayyari (2019), comparó dos equipos: Legendre neural networks (LNN) y el Volterra neural network (VNN), concluyendo que el LNN era más sencillo de aplicar, ya que utilizaba menos requerimientos computacionales, además de que la convergencia era más rápida y con un rango de error más bajo. En la actualidad, para animales existen equipos modernos, como el WhelpwiseTM Veterinary Perinatal Specialties®, el cual utiliza sensores de presión uterina especializados adecuados para su uso en el manejo del parto en perras y permite registrar objetivamente (cuantitativa y cualitativamente) la actividad uterina para reconocimiento del inicio del parto (Davidson, 2001).

Por todo lo mencionado anteriormente, el objetivo del presente capítulo es analizar y contrastar información acerca del uso, los aspectos generales, la importancia, la interpretación y las ventajas, de la implementación del monitoreo electrónico fetal y uterino en mamíferos, aumentar las crías nacidas vivas, disminuir la morbilidad y la mortalidad preparto, intraparto y postparto de las hembras y favorecer la supervivencia de los neonatos durante los primeros 7 días de vida, favoreciendo con ello el bienestar animal de las hembras y sus crías.

Antecedentes

Hace 350 años, los sonidos cardiacos fetales en humanos fueron descritos por primera vez, por el médico francés Marsac. Sin embargo, tomó casi 150 años más, para que se usaran de manera rutinaria estetoscopios mecánicos, en la práctica clínica en el Oeste de Europa y América del Norte (Ayres-De-Campos, 2018). Fue entonces cuando la descripción de la FCF para monitorizar la condición clínica del feto comenzó en el siglo XIX. Kennedy (1833) escribió acerca de la auscultación obstétrica (Hirsch, 2019); Cremer (1906) realizó la primera grabación de la FCF posteriormente Hillis (1917) comenzó a utilizar el fetoscopio para revisar la FCF durante el parto.

Fue hasta 1950 que las grabaciones y la monitorización de la FCF a lo largo de la gestación y el parto en humanos se volvieron más comunes, gracias a Edward H. Hon, quién las perfeccionó y notó que, aunque fueran mínimas las variaciones de la FCF, tenían gran importancia clínica (Modanlou *et al.* 2019). Por las mismas fechas, Álvarez y Caldeyro-Barcia (1954) y Hammacher (1962), también hicieron sus mediciones. El primer monitor fetal comercial se desarrolló en 1968 (Freeman y Garite, 1981) y no fue sino hasta el año 2000, que el primer electrocardiograma fetal (STAN), fue introducido a la práctica diaria (Amer-Wahlin y Kwee, 2015).

¿Qué es la cardiotocografía?

La cardiotocografía es una herramienta de evaluación fetal simple, no invasiva, económica y libre de riesgos (Tranquili *et al.* 2013) que registra los movimientos fetales, la FCF (latidos por minuto, lpm) y las contracciones uterinas (mmHg) (Romagnoli *et al.* 2019) (**Figuras 1 y 2**). El segundo componente de un monitor fetal es el tocodinamómetro. Este dispositivo mide la intensidad, la velocidad, y el tiempo que duran las contracciones uterinas. Esto es básicamente un transductor de presión estilo anular que está atado al abdomen materno mediante un cinturón que mantiene el contacto continuo con el abdomen. El transductor contiene un émbolo que está deprimido cuando el útero cambia su rigidez y forma con cada contracción. Esta depresión cambia el voltaje de la corriente asociada con el émbolo y es proporcional a la fuerza de la contracción (Ayres-De-Campos, 2018). De este modo, se puede valorar la calidad del latido cardiaco fetal durante parte de la gestación (en humanos a partir de la semana 27) (Reece y Hobbins, 2009), así como las reacciones fisiológicas del feto a las contracciones uterinas durante el parto (Payne, 2015). En perras, por ejemplo, se debe comenzar a utilizar al menos 7 días antes de la fecha tentativa de parto y en este tiempo se emplea dos veces al día (cada 12 horas) (Davidson, 2001). Se trata de una técnica relativamente novedosa en perras (aunque en humanos ha sido usada desde hace muchos años), que ayuda a predecir oportunamente partos distócicos (Groppetti, *et al.* 2010), permitiendo con ello, detectar a tiempo problemas que ocasionen estrés fetal y condiciones patológicas, tales como, hipoxia y acidosis metabólica (Ayres-De-Campos *et al.* 2016).

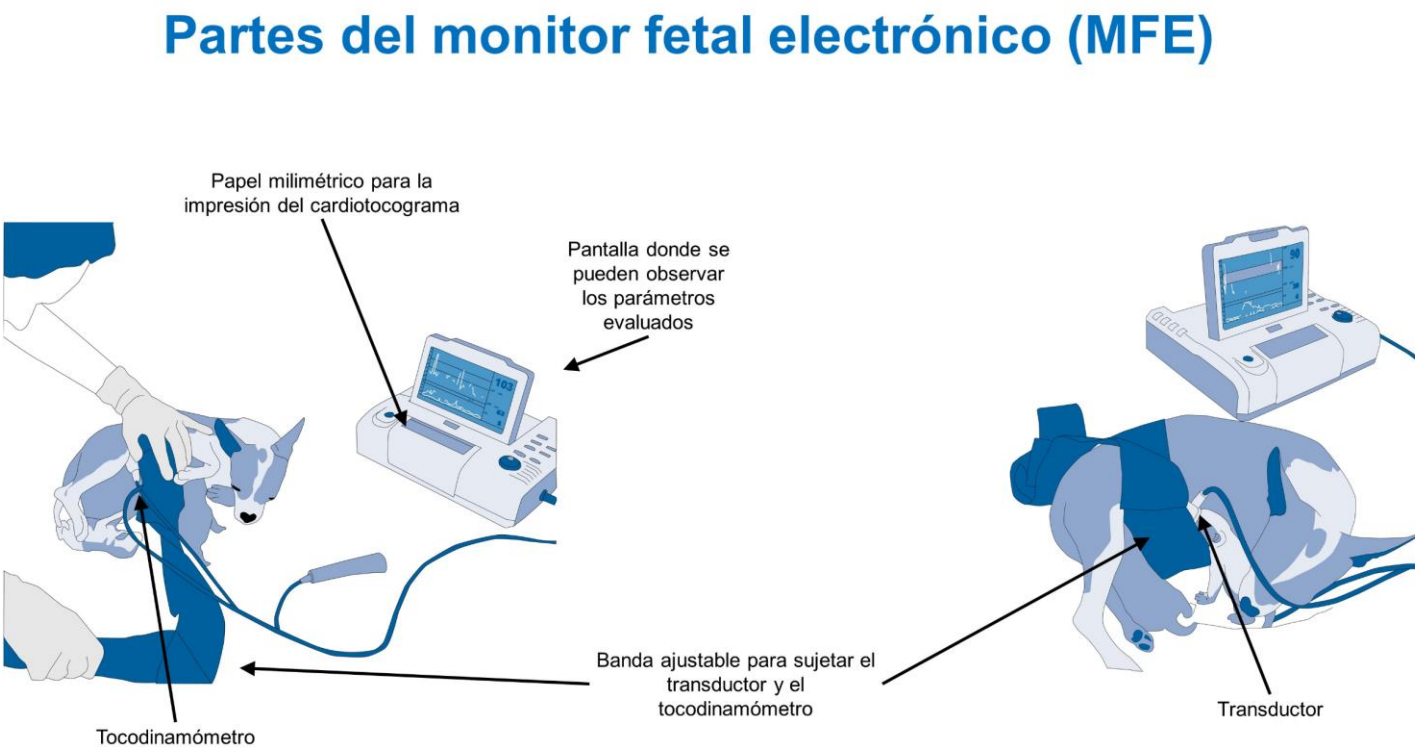

Figura 1. Partes que componen el monitor fetal electrónico.

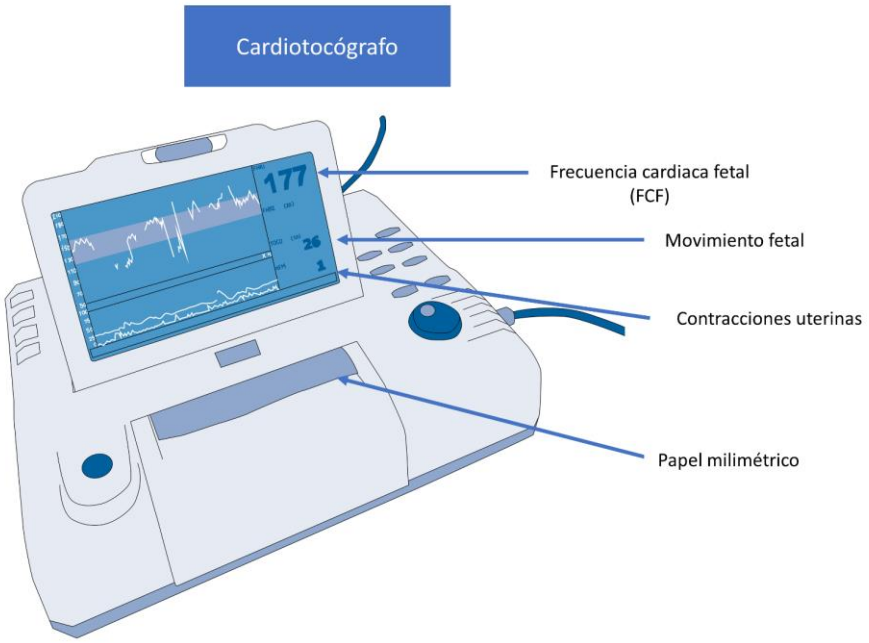

Figura 2. Cardiotocógrafo. Dentro de los parámetros que se pueden medir con este equipo se encuentran: la FCF, la duración, frecuencia y la fuerza de las contracciones uterinas.

Diversas características del monitoreo electrónico fetal, tales como la frecuencia cardiaca, la forma de las ondas y la dinámica del comportamiento fetal, son sumamente importantes para determinar la vida fetal, el desarrollo, la madurez y la existencia de estrés fetal o enfermedades cardiacas congénitas (Hasan *et al*. 2009; Alnuaimi *et al.* 2017).

El registro se realiza sobre la piel abdominal con un transductor de ultrasonidos, el cuál detecta la FCF y un transductor de presión, el cuál evalúa la actividad del útero y ambos artefactos se conectan a una pantalla en dónde se observan y posteriormente se imprimen los resultados en papel (Comino y López, 2004) (**Figura 3**).

Figura 3A, 3B y 3C. Monitoreo fetal electrónico con cardiotocógrafo antes y durante el parto en perras de talla pequeña, en donde se pueden apreciar: la FCF, el movimiento fetal y las contracciones uterinas, tanto en el monitor, como impresas en papel. Nótese que, al ser un método no invasivo, las pacientes colaboran fácilmente.

Recientemente se han desarrollado aplicaciones de software, como el CTG Analyzer, el cual interpreta el registro cardiotocográfico, haciendo dicha interpretación más objetiva e independiente de la experiencia del clínico (Sbrollini *et al*. 2017).

Además, con el uso temprano de la cardiotocografía (>40 días de gestación) en perras con antecedentes de partos prematuros, se ha podido detectar la hipercontractilidad miometrial antes del desarrollo de la insuficiencia lútea y de este modo, facilitar la terapia con fármacos tocolíticos, tales como la terbutalina, evitando con ello, las posibles complicaciones de la suplementación con progesterona durante la gestación (Davidson, 2015).

La frecuencia cardíaca fetal (FCF)

La FCF es uno de los más importantes parámetros para determinar el estado de salud y bienestar de un feto, monitorizándola se puede ayudar a detectar oportunamente fallas en la oxigenación (Alnuaimi *et al.* 2017), evitando así llegar a hipoxia fetal (Gil *et al.* 2014; Biloborodova *et al.* 2021) y del mismo modo, evitar posibles daños neurológicos secundarios o incluso la muerte durante el parto (Hasan *et al.* 2009). Se mide en latidos por minuto (lpm) y puede ser escuchada o monitoreada de diversas formas tales como: corneta de Pinard, Doppler obstétrico o monitorización fetal electrónica (Datta, 2001).

La FCF ha sido estudiada por varios investigadores durante el trabajo de parto en humanos, caballos, ovejas y bovinos entre otros (Kovacs *et al.* 2014). Para ello, se han utilizado métodos electrónicos, continuos y directos (Pantle, 1959). Del mismo modo, se han elaborado estudios cuantificando algunos

de los elementos de la FCF durante la gestación y el parto con algún método externo (Aladjem, 1977). Por ejemplo, en fetos saludables de equinos, se ha visto que la FCF basal disminuye a medida que avanza la gestación y varía en relación con la actividad anormal (Pipers y Adams-Brendemuehl, 1984; Bucca *et al.* 2005). Los patrones de FCF han sido bien documentados en fetos equinos sometidos a estrés e incluyen taquicardia persistente (Reef *et al.* 1996), bradicardia (Pipers y Adams-Brendemuehl, 1984) y arritmia cardíaca (Bucca *et al*. 2007). En el caso del ganado bovino, el corazón del feto se desarrolla de manera temprana durante la embriogénesis y comienza a producir un latido regular alrededor del día 30 de gestación (Gargiulo *et al.* 2012).

En un estudio realizado por Gil *et al*. (2014) en 15 perras gestantes evaluadas por medio de ultrasonografía, encontraron (al igual que en los reportes en humanos), que la aceleración y desaceleración de la FC ocurre en fetos caninos durante el período ante parto y estos cambios están asociados con el pico de la contracción uterina. Algunos de los fetos mostraron estas variaciones 72 horas antes del parto y otros las mostraron entre 6 y 1 hora previas al parto. Por ello, es importante conocer la FCF basal en la especie que se vaya a evaluar, ya que esta varía en las diferentes especies**.** En la **Figura 4** se puede observar cómo los fetos de los bovinos mantienen las frecuencias cardiacas basales más bajas y los fetos de canídeos mantienen las más elevadas. Cuando suceden elevaciones de la frecuencia cardiaca basal se denomina *taquicardia*, que puede ser causada por pirexia en la madre, analgesia epidural o en algunas ocasiones debido a la secreción de catecolaminas. Cuando la FCF basal se registra por abajo de los rangos normales, se denomina *bradicardia* y puede suceder cuando la madre presenta hipotermia, utiliza fármacos tales como beta bloqueadores, el feto padece arritmias (Paul *et al*. 1975), o porque el feto sufre hipoxia (Kutzler *et al.* 2003).

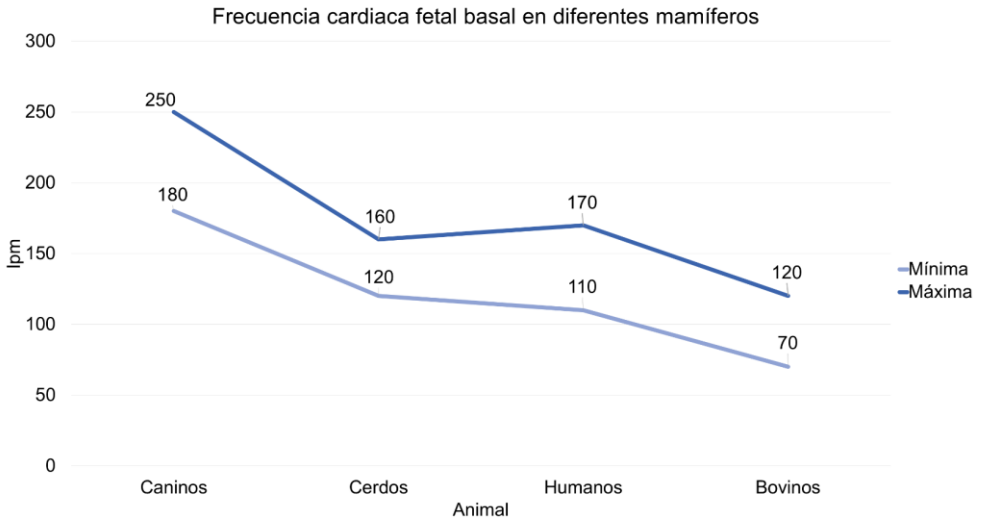

Figura 4. FCF basal en algunos mamíferos (Gil *et al.* 2014; Esquivel, 2004; Mota-Rojas *et al*. 2019; Romero-Salinas *et al*. 2003; Ayres-De-Campos *et al.* 2015; Rutter, 2004).

¿Cuál es la relación que guardan la contracción uterina y la frecuencia cardiaca?

La FCF depende del SNA y el nivel de dichas respuestas dependen a su vez de la cantidad de oxígeno al que tenga acceso el feto (Ayres-De-Campos *et al.* 2016). Por ello, cuando los niveles de oxígeno descienden bruscamente en el feto, se produce rápidamente una caída inmediata de la FCF. Sin embargo, cuando las contracciones uterinas no se presentan, se torna complicada la monitorización del feto sometido a la falta de oxígeno parcial de manera sostenida, a través del chequeo de la FC basal (Warner, 1962).

Desde el inicio del trabajo de parto, cada contracción uterina colapsa las venas que cruzan el miometrio y posteriormente, si la intensidad de la contracción es suficiente, cierra la luz de las arterias y obstruye la salida de la sangre. Al finalizar la contracción uterina, el feto se encuentra con déficit de oxígeno. Cuando el feto se encuentra con menor aporte de oxígeno dentro de su organismo, suceden cambios que lo llevan a estimular el SNA, inicialmente estimulando el Sistema Nervioso Simpático (SNS) y con ello se estimula la contracción del músculo cardíaco, ocasionando una taquicardia, vasodilatación central y vasoconstricción periférica (Escobar, 1987). Esto se pudo ejemplificar, en un estudio realizado por Romero-Salinas *et al.* (2003), en dónde encontraron que, en humanos, la frecuencia cardiaca basal iba de 110-170 lpm, con una media de 135.5 y una desviación estándar de 10.57 lpm, mientras que la amplitud de los ascensos transitorios era de 8 a 50 latidos, con un promedio de 24.81 latidos y una desviación estándar de 7.41 lpm. Ellos encontraron que las variaciones con los registros que se tenían hasta la fecha eran causadas por factores externos, que eran de esperarse, pero que no estaban registrados, como lo son las variaciones que surgen en la presión arterial por la altitud, ya que ellos hicieron su estudio en la Ciudad de México y los registros que se tenían, se habían realizado al nivel del mar.

Aspectos que predisponen a bradicardia y taquicardia

El control de la frecuencia cardiaca basal corre a cargo del SNA. El nodo sinoatrial del miocardio del feto reacciona a estímulos parasimpáticos. Por ejemplo, en el feto de oveja, sin anestesia y en reposo, la FC es similar a su valor esencial, obtenido mediante bloqueo parasimpático o betaadrenérgico simultáneo dejando en claro que, en condiciones basales, las influencias parasimpáticas o betaadrenérgicas se estabilizan unas a otras (Clapp *et al.* 1985; Escariao *et al*. 2005).

Por otro lado, la manifestación de angustia en el feto es reflejada por una desaceleración sostenida de la FC. Los parámetros normales de la FCF canina y felina al final de la gestación son de 170-230 latidos/minuto o, como mínimo, 4 veces la FC materna (Esquivel, 2004). Durante todo el tiempo que se lleva a cabo el parto, la cantidad de fluidos que puede alojar el corazón del recién nacido depende en gran medida de la FC, ya que el ventrículo derecho es poco flexible y presenta mínima capacidad para expandirse y el SNA es inmaduro (Davidson, 2001). Los descensos de la FCF relacionados con las contracciones uterinas hacen suponer que existen diferencias entre la amplitud del canal del parto y el tamaño del feto o una mala posición fetal. Las aceleraciones transitorias suceden cuando el feto presenta movimientos normales dentro del útero. Por otro lado, las FC iguales o más bajas de 150-160 latidos/minuto indican estrés, es por ello que los fetos con FCF menor o igual a 130 lpm tienen pocas probabilidades de sobrevivir si no son expulsados dentro de las 2-3 horas y los fetos con frecuencia cardíaca igual o menor a 100 lpm son una señal para la intervención médica o cesárea inmediata a fin de acelerar el parto para que no llegue a ocurrir la muerte fetal (Davidson, 2017). Por ende, cuando los niveles de oxígeno fetal bajan bruscamente, la FCF desciende de inmediato (Kutzler *et al*. 2003). Sin embargo, de no presentarse contracciones uterinas, la evaluación del feto sometido a asfixia se torna muy difícil (Castelazo, 1960).

Importancia del MEF

La importancia del MEF radica en parte en que puede ayudar a predecir la fecha del parto en una perra. Sin embargo, esto puede ser más sencillo cuando, aunado al MEF, se han realizado citologías y mediciones hormonales desde antes de la gestación. Se sabe que el parto puede ocurrir entre los días 56 a 58 posteriores al primer día de diestro o entre los días 64 y 66 posteriores al aumento inicial de la progesterona basal o cuando la hormona luteinizante es detectable. Del mismo modo, determinando cuando fue la ovulación, se puede saber que entre los 58 y los 72 días posteriores a la monta, puede dar inicio el trabajo de parto (Concannon *et al.* 1989) y estos tiempos varían dependiendo de la raza, del tamaño de la camada y de la duración del estro y del proestro en cada perra (Gil *et al*. 2014).

Por otro lado, en cerdas, las mediciones de prostaglandinas y la progesterona, también pueden ser útiles para determinar el comienzo de la fase 1 del trabajo de parto, ya que se pueden detectar elevaciones en las prostaglandinas 48 horas antes de iniciado el parto, junto con el descenso de la progesterona a niveles por debajo de 2 ng/mL y del descenso de la temperatura corporal a 37.2°C

(Taverne *et al.* 1979). Sin embargo, no siempre se tiene acceso a estas mediciones y es aquí donde de nuevo radica la importancia del MEF, para ayudar a determinar cuándo se puede llegar a presentar un parto distócico (Gaudet, 1985; Darvelid y Linde-Forsberg, 1994) y con ello, evitar dolor en la madre y sufrimiento fetal, produciéndole a las crías estrés agudo y aumentando su mortalidad y disminuyendo de este modo el vigor en el recién nacido (Martínez-Burnes *et al.* 2021). Por ejemplo, en vacas, se calcula que entre el 1.5 y el 22.6% de los partos pueden presentar distocia y pueden provocar la muerte del recién nacido, así como metritis, mastitis y retención placentaria (Zobel, 2013). En el caso de las perras, la inercia uterina es una de las principales causas de distocia derivada de partos prolongados (Darvelid y Linde-Forsberg, 1994), así como también, la estrechez pélvica de las razas braquicéfalicas (Linde-Forsberg y Persson, 2007). En los gatos, razas como los British Shorthairs están predispuestas a presentar distocia en el 2.5% de los casos (Holst *et al.* 2017). Por todo lo anterior, el MEF se torna en una herramienta muy útil para detectar y prevenir que situaciones como la distocia, pudieran presentarse en los partos de los animales domésticos.

Sufrimiento fetal

Al mejorar la calidad de las unidades de cuidados intensivos y el cuidado obstétrico, antes y durante el parto, se puede ayudar a incrementar las tasas de sobrevivencia y al mismo tiempo reducir la mortalidad y la morbilidad perinatal (Gandhi, 2018).

Pero ¿cuáles son las principales causas de mortalidad neonatal? Podemos citar, distrés respiratorio, distocia e infecciones bacterianas (Concannon, 2002). Uno de los principales indicadores de sufrimiento fetal es el SAM). El SAM se define como el estrés respiratorio en recién nacidos, los cuales, al nacer presentan cierta tinción de meconio en su piel, además, pueden aspirar líquido amniótico y con ello, desarrollar hipoxemia, acidosis y distrés respiratorio (Jensen *et al*. 2017). La aspiración de líquido amniótico está relacionada con un alto riesgo de desarrollar corioamnionitis, sepsis neonatal, parálisis cerebral, convulsiones y SAM.

El SAM es una causa importante de distrés respiratorio que conlleva a una gran morbilidad y mortalidad a nivel mundial, como resultado de la obstrucción de las vías aéreas en neonatos (Castro-Nájera *et al.* 2006; Martínez-Burnes *et al*. 2019). En bovinos (*Bos taurus*), la aspiración intrauterina de meconio es una de las principales causas que precede un aborto (López *et al.* 1984). En un estudio realizado post mortem por López et al. (López *et al*. 1994) en becerros, se encontró que en el 42.5% de los casos había restos de meconio en sus pulmones. Del mismo modo, en potros (*Equus ferus caballus*), el SAM también está considerado como uno de los factores asociados a la asfixia perinatal, ocasionándoles disfunción respiratoria (Dubielzig, 1977). En un estudio realizado en 80 yeguas preñadas se reveló que el 8.8% de los potros fueron abortos o mortinatos. Hasta el 32.5 % de los potros mostró meconio en la piel, el 20% de los potros abortados y el 30% de los que nacieron vivos y murieron tenían meconio aspirado. Los autores sugirieron usar la tinción de meconio de la piel como un indicador fiable de la hipoxia fetal en los potros y destacaron la necesidad de realizar más estudios para determinar factores de riesgo, procedimientos diagnósticos, preventivos o de tratamiento que puedan reducir el impacto de la hipoxia intraparto (Mendoza *et al.* 2008).

En un estudio realizado por García (2017) en humanos, se observaron 84 casos de sufrimiento fetal agudo (21%). Dentro de esos casos, los trastornos cardiacos fetales más comunes fueron taquicardia (10.83%), bradicardia (4.28%) y 9 fetos muertos a consecuencia de esos cambios hemodinámicos (2.27%), lo cual demuestra que gran parte de los problemas que conlleva el sufrimiento fetal son las arritmias fetales.

Durante la hipoxemia prolongada, la perfusión del tallo cerebral se mantiene e incluso puede llegar a aumentar en unas regiones cerebrales más que en otras. La actividad neurológica del tallo cerebral, como centro autónomo, es indispensable para la sobrevivencia del feto. Conforme el nivel de oxígeno sigue disminuyendo, aumentando el metabolismo anaerobio de la glucosa de manera anaerobia y la cantidad de fosfatos de alta energía disminuyen en el cerebro (Cullen-Benítez y Salgado-Ruiz, 2009). Estos cambios hacen colapsar el metabolismo cerebral, lo cual produce despolarización de las membranas de las neuronas, se abren los canales de entrada de calcio (Ca_2^+) y este fluye al interior del

citoplasma; ya dentro de la célula, el Ca_2^+ activa las proteasas, las endonucleasas y las fosfolipasas; provocando la muerte neuronal debido a todos estos cambios (Chauhan *et al.* 1995) (**Figura 5**).

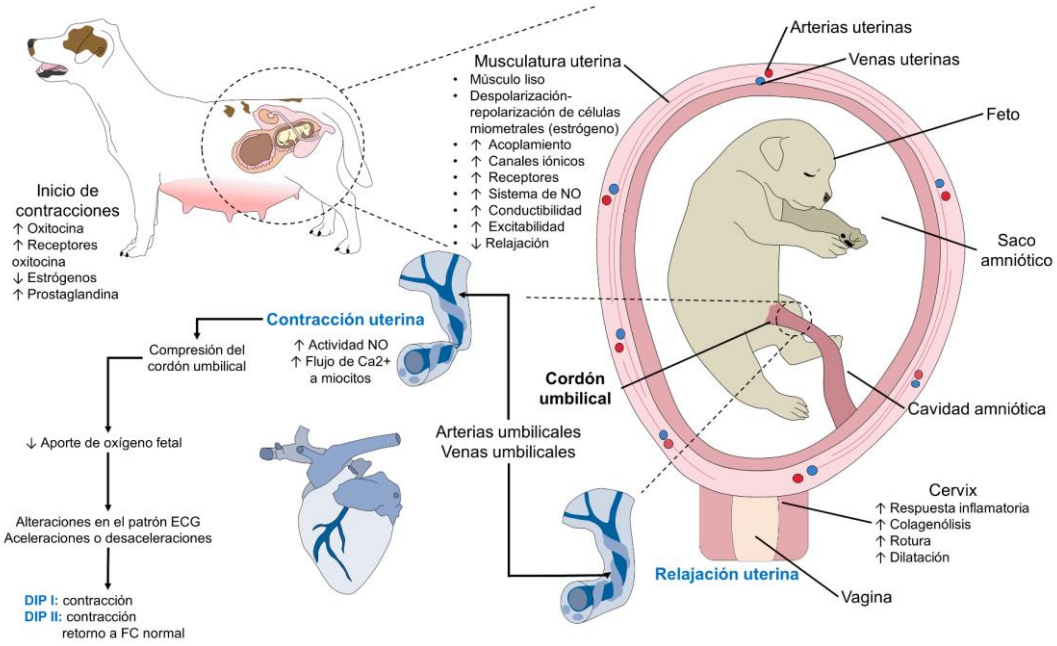

Figura 5. Modelo de contracción y relajación miometrial y su efecto en la vascularización fetal. Durante el parto, el inicio de las contracciones responde a factores endocrinos como el aumento en las concentraciones de oxitocina y prostaglandinas, los cuales generan cambios en la musculatura uterina con el fin de expulsar el feto. En estos miocitos, las hormonas mencionadas y la participación del sistema de NO genera el acoplamiento y la apertura de canales iónicos que permiten el flujo de Ca^{2+} y, con ello, cambios en el potencial de membrana y la consecuente despolarización y repolarización miometrial. Aunque las contracciones guardan una función biológica esencial durante el proceso de parto, éstas también pueden generar la compresión del cordón umbilical y las venas y arterias umbilicales, las cuales son las responsables de irrigar al feto. Si este efecto se prolonga y se desarrolla hipoxia fetal, a nivel cardiovascular se observan respuestas compensatorias que pueden ser detectadas a través del monitoreo fetal para identificar las alteraciones en el patrón del ECG o de las aceleraciones y desaceleraciones. $Ca^{2+:}$ calcio; ECG: electrocardiograma; NO: óxido nítrico.

Contracciones uterinas

Comprender la fisiología de las contracciones uterinas es vital para desarrollar métodos que ayuden a resolver cualquier complicación que pudiera surgir durante el parto (Maul *et al*. 2003). El parto es el proceso fisiológico por medio del cual el feto es expulsado del útero hacia el exterior y se lleva a cabo gracias a las contracciones uterinas y a la dilatación del cérvix (Norwitz *et al.* 1999). La sincronización de las contracciones uterinas a lo largo del parto, así como la posterior dilatación del cérvix, son debidas principalmente a la liberación de oxitocina (Lezama-García *et al.* 2019), sin embargo, la secuencia de contracción y relajación del miometrio resulta de la despolarización y repolarización cíclica de las membranas de las células musculares. Las descargas eléctricas espontáneas del miometrio consisten en ráfagas intermitentes de potenciales de acción (Kanda y Kuriyama, 1980). Del mismo modo, el volumen uterino (estiramiento crónico) y las hormonas ováricas (principalmente estrógenos) contribuyen al cambio en el potencial de acción (Osa *et al*. 1983) (**Figura 6**). En el miometrio, el proceso de preparación para el parto involucra cambios en los mecanismos de transducción y la síntesis de nuevas proteínas como la conexina, canales iónicos y receptores de uterotoninas. Al mismo tiempo, existe una disminución en la regulación de los sistemas que involucran el NO y que desencadenan la relajación uterina (Casey y MacDonald, 1997). La actividad uterina normal o anormal, se mide a lo largo de los diferentes periodos de la gestación y del parto (SEGO, 2007), de acuerdo con los siguientes parámetros:

- *Frecuencia.* Es la cantidad de contracciones que se presentan en un rango de 10 minutos, la cual en humanos es de 3-5 cada 10 minutos durante el trabajo del parto (Santamaría, 2018) y en perras es de 4-12 contracciones por hora (Groppetti *et al.* 2010).
- *Tono basal.* Es la presión intrauterina presente en el miometrio en descanso y en humanos varía entre 8 y 12 mmHg (Terré y Francés, 2006) y en perras es de 2-8 mmHg (Groppetti *et al.* 2010).
- *Intensidad.* Se registra en mmHg y compara la diferencia de presión entre el tono basal del útero y el punto de máxima contracción uterina (Maul *et al.* 2003) siendo de 30-50 mmHg en humanos (Santamaría, 2018) y de 10 o más mmHg en perras (Groppetti *et al*. 2010) (**Figura 7**).
- *Duración.* Es el tiempo que pasa desde que una contracción inicia hasta que termina y el útero recupera su tono basal. Lo normal en humanos es entre 30 y 90 segundos (SEGO, 2007) y en perras de 2 a 5 minutos (Groppetti *et al.* 2010) (**Figura 8**).

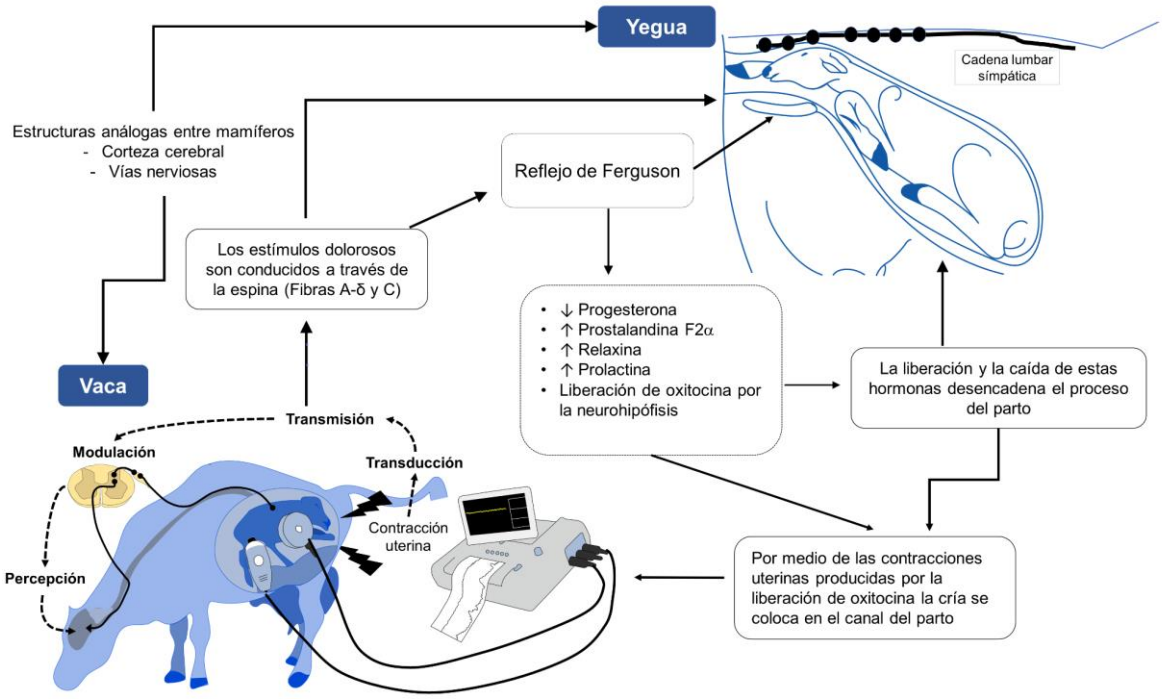

Figura 6. El repertorio conductual de la vaca y la yegua al parto requiere de la caída de la progesterona para que se desencadene, seguido del aumento en la prostaglandina F2a, el cual produce un aumento en la sensibilidad a la oxitocina en el útero de la vaca o de la yegua, aumenta la prolactina y la liberación de oxitocina por parte de la neurohipófisis y con ello, comienzan las contracciones que traen consigo la dilatación del canal del parto, se activa el reflejo de Ferguson, lo que provoca que la liberación de oxitocina continúe, persisten las contracciones uterinas, detectadas como un estímulo doloroso, conducido a lo largo de la médula espinal por medio de la transducción, transmisión, modulación y percepción. Finalmente, el feto se coloca en el canal del parto y comienza la fase de expulsión.

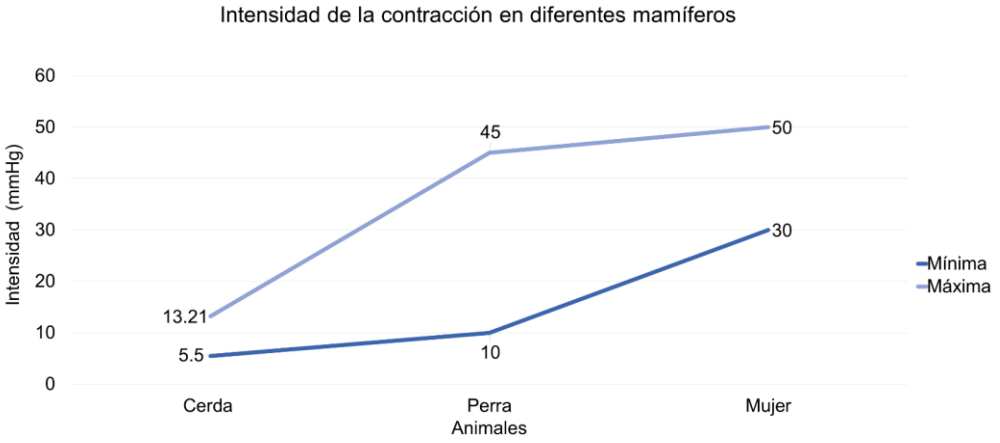

Figura 7. Intensidad de la contracción uterina durante el parto en algunos mamíferos. Se puede apreciar que las contracciones uterinas en perras y mujeres tienden a ser las de mayor intensidad y las de las cerdas son las menos intensas (SEGO, 2007; Santamaría, 2018).

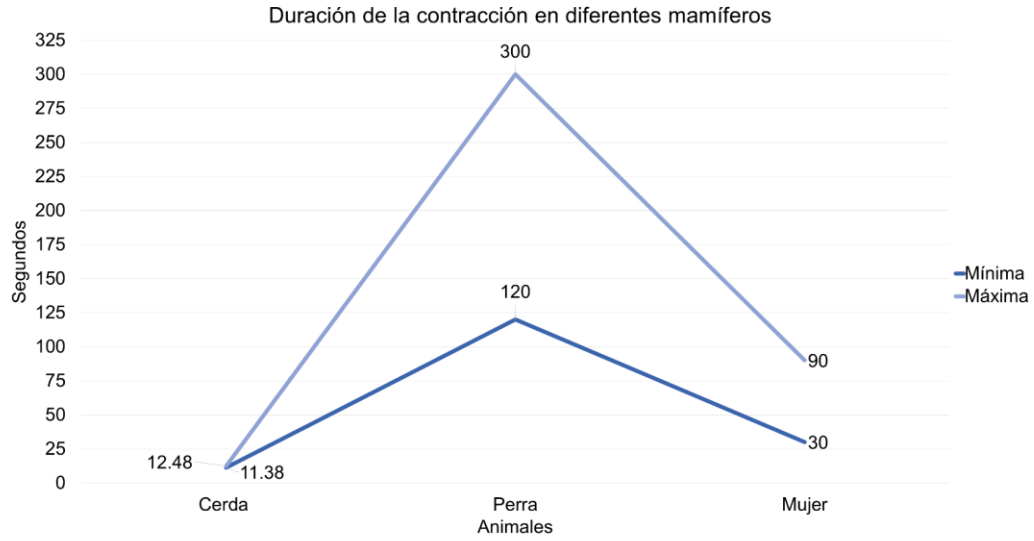

Figura 8. Duración de la contracción uterina durante el parto en algunos mamíferos. Se puede apreciar como las contracciones uterinas en perras tienden a ser las más largas y sostenidas, al compararlas con las que presentan las cerdas y las mujeres al momento del parto y por otro lado, las contracciones de las cerdas son las más cortas (Groppetti *et al.* 2010; SEGO, 2007; Mota-Rojas *et al.* 2008).

Para poder monitorizar la actividad uterina se utiliza el tocodinamómetro, el cual registra las contracciones uterinas a través de la pared abdominal (Maul *et al.* 2003). Se trata de un aparato que mide la presión externa y de este modo detecta las contracciones uterinas, permitiendo evaluar de manera objetiva (cualitativa y cuantitativamente), la actividad uterina para detectar oportunamente el momento en el que el parto comienza (Davidson, 2001).

La tocodinamometría en serie en las perras y las gatas ayuda a llevar a cabo la evaluación del progreso del trabajo de parto, se ha empleado para valorar cuantitativamente la actividad uterina, por medio del registro de las contracciones uterinas a través de la pared abdominal (Maul *et al*. 2003). Durante la fase final de la gestación y antes del inicio de la fase 1 del trabajo de parto, el miometro puede contraerse 1 a 2 veces por día. En las fases 1 y 2, las contracciones uterinas se presentan desde 0 a 12/hora y tienen una intensidad de 15 a 40 mm Hg, con elevaciones de hasta 60 mm Hg (Davidson, 2017).

Interpretación del CTG

El CTG consiste en un registro corto, usualmente de 20 minutos, en el cual se graba la FCF y la dinámica del útero cuando el parto se acerca (Devane *et al.* 2017), aunque normalmente se registra el número de contracciones que surjan en un periodo de 10 minutos (Heazell, 2013).

En el CTG normalmente se consideran los siguientes factores:

- La *línea de base o frecuencia cardiaca basal* en periodos de inactividad o contracciones, que va de 180-250 latidos/minuto en perros (*Canis lupus familairis*) (Esquivel, 2004), 120-160 latidos/minuto en cerdos (*Sus scrofa domesticus*) (Mota-Rojas *et al.* 2007), 70-120 latidos/minuto en bovinos (*Bos Taurus*) (Rutter, 2004), 110-170 latidos/minuto en humanos (*Homo sapiens*) (Romero-Salinas *et al.* 2003)**,** puede haber latidos cardiacos normales, bradicardia o taquicardia.
- Las *aceleraciones*, es decir, el aumento de la FC por arriba de 15 latidos/minuto sobre la línea basal y durante más de 15 segundos (Reece y Hobbins, 2009; Comino y López, 2004; Terré y Francés, 2006).
- La *variabilidad*, que es la irregularidad en la frecuencia cardiaca (Reece y Hobbins, 2009).
- Los *movimientos fetales*, los cuales provocan aceleraciones (saludable) y en el caso de disminución de los movimientos que pudiera indicar sufrimiento fetal (Reece y Hobbins, 2009).
- Las *desaceleraciones*, es decir, la disminución de la frecuencia cardiaca, 15 latidos por abajo de la línea de base por más de 15 segundos, las cuales pueden suceder simultáneamente o no con la contracción uterina (Reece y Hobbins, 2009; Comino y López, 2004; Kazandi *et al.* 2003).

Aceleraciones

Se trata de cambios en el ritmo cardíaco producidos por estimulación del SNS, estos cambios suceden a consecuencia de los aumentos en la actividad del SNC fetal por estímulos del SNP o debido al cierre parcial de la vena umbilical en fetos con un correcto nivel de oxígeno. Aumentan cuando se disminuye el tono vagal y disminuyen cuando el feto se encuentra en reposo o hipóxico (Sbrollini *et al.* 2017).

Sin embargo, no cualquier actividad fetal produce una aceleración, ni toda aceleración tiene que ver con movimientos fetales. Es decir, los movimientos leves del feto no modifican su frecuencia cardiaca. Todas las respuestas cardiovasculares del feto dependen de la actividad de la médula, de la corteza cerebral y del hipotálamo (Vásquez, 2013).

De acuerdo con Aladjen *et al.* (1977), las aceleraciones se pueden clasificar en:

- *Omega:* puede ser una o dos ondas de corta duración y de buen pronóstico.
- *Lambda:* incremento y disminución de la frecuencia cardiaca fetal. Se encuentra sincronizado con la presión u obliteración temporal del cordón umbilical. Es de buen pronóstico, ya que es la manera en la que el feto compensa la presión ejercida sobre el cordón umbilical (Aladjem *et al*. 1977).
- *Elíptico:* Aceleración de duración prologada. Se presenta cuando hay un estímulo hipóxico (Reece y Hobbins, 2009).
- *Periódico:* Se trata de una sucesión de ascensos transitorios tipo omega de buen pronóstico (Aladjem *et al.* 1977).

En conclusión, las aceleraciones pasajeras son alteraciones del ritmo cardíaco que se presentan en respuesta a estímulos del sistema nervioso simpático, debido a cambios en la actividad del SNC fetal, o también debido a estímulos del SNP o a la obliteración parcial de la vena umbilical en fetos con correcta saturación de oxígeno. Se presentan con mayor frecuencia en situaciones en las que el tono vagal se encuentra disminuido y reducen su presentación cuando el feto se encuentra en reposo o con baja saturación de oxígeno.

Desaceleraciones

Las variaciones de la línea basal se producen por el balance continuo del SNA). De este modo, el SNS produce aceleraciones y el Parasimpático produce desaceleraciones. Esto sucede cuando existe un control neurológico normal y la reserva fetal es adecuada. Por el contrario, la ausencia de fluctuaciones indica una depresión del Sistema Nervioso Central (SNC), asociado a hipoxia (Escobar, 1987).

Las desaceleraciones también son conocidas como DIPs o bradicardias. Se clasifican como precoces o DIP I, tardías o DIP II o de tipo umbilical, variables o DIP III (**Figura 9**).

- *DIP tipo I o precoz.* Descenso en la FCF basal de más de 15 a 20 latidos, que coincide con la contracción, tienen una morfología idéntica y son siempre mayores a 8 segundos y menores a 18 segundos (Heazell, 2013; Hon y Quiligan, 1967; Fabre, 2001). Suceden antes de la contracción y se relacionan con la presión de la cabeza fetal, por lo que no se considera que tengan significado patológico (NIHCE, 2014).

- *DIP tipo II o tardío.* Descenso de la FCF basal que surge después de que la contracción inicia y terminan después de que la contracción culmina (Lamb y Heazell, 2019). Normalmente siguen un modelo uniforme de contracción a contracción, de más de 15-20 latidos, pero tienen una duración de 18-64 segundos. Tienen un claro significado patológico (Terré y Francés, 2006; Kazandi *et al.* 2003).

- *DIP tipo III, variables o de tipo umbilical*. Disminución de la FCF basal con formas variables, aparecen por obliteración del cordón umbilical por el útero, pueden pudiendo ser breves (30-40 segundos) o prolongadas (40-60 segundos) (Lamb y Heazell, 2019; Romero-Salinas, *et al*. 2003; NIHCE, 2014; Cabero, 2003).

Cuando surgen desaceleraciones sostenidas de la frecuencia cardiaca, se manifiesta de este modo el estrés fetal. Las desaceleraciones asociadas a las contracciones uterinas sugieren que pudiera haber una mala posición, mala presentación o mala postura por parte del feto (Tutera y Newman, 1975). Del mismo modo, Gil *et al*. (2014), observaron que las aceleraciones y desaceleraciones en perras, junto con otros signos clínicos (aumento en la temperatura corporal de la perra, disminución en la progesterona), pueden ayudar a predecir la fecha probable de parto, ayudando de este modo a disminuir la mortalidad de los cachorros al poder planificar una cesárea en caso de ser necesaria. Del mismo modo, ellos encontraron que al igual que en los fetos de mujeres, en los fetos de perras se presentaron DIP I y DIP II durante el parto de las 15 perras que fueron evaluadas, tanto en parto eutócico como en los distócicos que terminaron en cesárea (Gil *et al*. 2014).

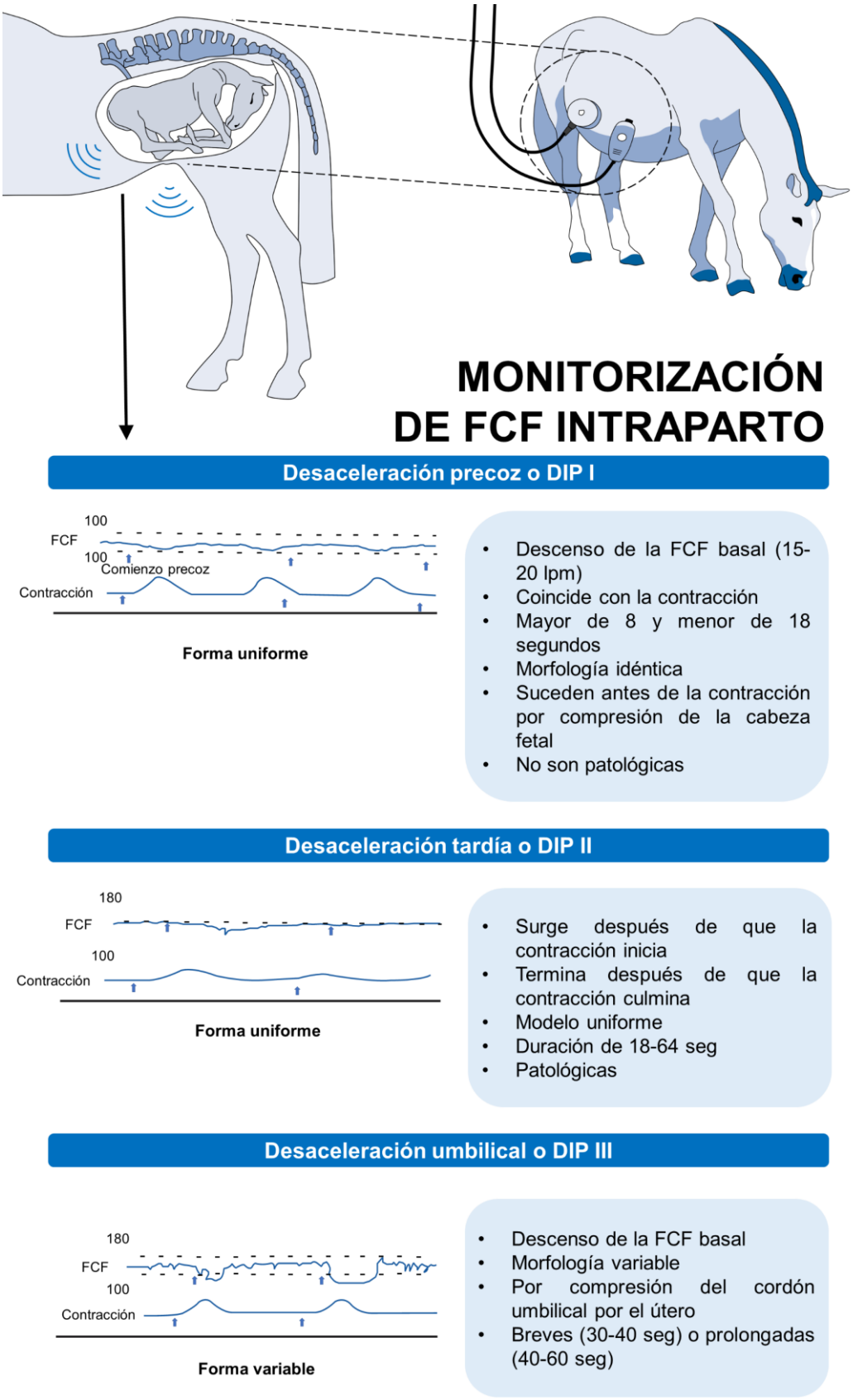

Figura 9. Desaceleraciones DIP I o precoces, DIP II o tardía y DIP III o umbilical.

En resumen, uno de los primeros y más importantes signos de compromiso fetal, es la aparición de DIP II, que surgen cuando sucede un descenso leve pero significativo del nivel de oxígeno en el feto.

Monitorización fetal electrónica en diversos mamíferos

En la actualidad, la MFE en diversas especies ha tomado mucho interés, debido a que cada vez se busca el uso de técnicas menos invasivas. Anteriormente, eran comunes las prácticas de palpación rectal para detectar la gestación de bovinos, técnicas que se caracterizaban por ser invasivas y riesgosas, tanto para el animal, como para el individuo que las realizaba (Gargiulo *et al.* 2012). Es por ello que desde hace ya algunas décadas es frecuente el uso de la monitorización fetal por medio de Doppler transabdominal en bovinos y fue por medio de los estudios realizados por Jonker *et al*. (1994) en vacas de raza Holstein-Friesian, que se obtuvieron las frecuencias cardíacas basales en fetos de bovinos, las cuales se encontraron entre 90 y 125 latidos por minuto, con aceleraciones de 7.1$\pm$1.0 por hora y desaceleraciones con un rango de 0 a 4 por hora.

Además, de acuerdo con Quevedo *et al*. (2019), la monitorización de la FCF en el ganado bovino (Holstein), no solo hace posible la detección de la salud fetal, sino que también proporciona información acerca del estado y desarrollo del SNA. Esto es bastante claro después del parto, ya que el aumento en el ritmo cardiaco alto y la disminución del ritmo cardiaco bajo indican la activación del nervio vago y de la modulación cardiaca y respiratoria.

De igual manera, se han realizado diversos estudios en yeguas gestantes para determinar el estado de bienestar de los fetos, ya sea por medio de técnicas transrectales o transabdominales (Reef *et al*. 1996; Adams-Brendemuehl y Pipers, 1987; Pipers y Adams-Brendemuehl, 1984; Bucca et al. 2005; 2006) y todos concluyen que, tanto la frecuencia cardiaca fetal, como la dinámica intrauterina de los potros son los parámetros equinos fetales más significativos.

De acuerdo con Davidson (2001), Groppetti *et al*. (2010), Ayres-De-Campos y Nogueira-Reis (2016), en perras recientemente se ha comenzado a implementar esta técnica de monitorización debido a que se ha visto que en esta especie ayuda de manera considerable a prevenir y disminuir la mortalidad antes, durante y después del parto, además, puede llegar a ser de fácil uso para el tutor del animal.

En cerdas se han realizado estudios en los que se ha empleado la MFE para detectar problemas al momento del parto o para ayudar a disminuir la mortalidad intraparto. En la mayoría de estos experimentos se han empleado inductores o aceleradores del parto, como es el caso de los estudios realizados por Mota-Rojas *et al*. (2005; 2005b) y González-Lozano *et al.* (2009). Sin embargo, en el análisis realizado por Olmos-Hernández (2006), no se emplearon dichos inductores o aceleradores y se concluye que las cerdas de primer parto mostraron el mayor índice de contracciones uterinas y el mayor número de desaceleraciones de la FCF, datos que coinciden con el elevado número de lechones muertos intraparto (20.45% de mortalidad total).

Clapp *et al*. (1985), realizaron observaciones de los patrones de la FCF en un modelo de crecimiento intrauterino experimental en borregas y observaron que había un incremento de las respuestas del SNA, con ligera taquicardia y evidencia morfológica y fisiológica de daño en el SNC, así como disminución o ausencia de las aceleraciones con el movimiento fetal y retraso en las desaceleraciones en respuesta a contracciones uterinas.

Ha sido demostrado que la monitorización de la FCF por medio de electrocardiografía fetal transabdominal es una técnica efectiva, no invasiva y una alternativa comercial a las técnicas de Doppler tradicionales, que no presenta riesgos ni para el feto, ni para la madre (Reef *et al.* 1996; Maul *et al.* 2003; Quevedo *et al*. 2019).

Por otro lado, a pesar de que existen otras pruebas (Sbrollini *et al*. 2019), la MFE) o cardiotocografía, sigue siendo la manera más popular de evaluar clínicamente a los fetos (Freeman 1990; Hasan *et al.* 2009a; Ayres-De-Campos 2018; Sbrollini *et al*. 2019), suplementado en ocasiones con muestreos sanguíneos del feto o recién nacido (Alfirevic *et al.* 2013). Aunado a esto, la monitorización de la FCF con CTG sigue siendo el pilar de la vigilancia fetal intraparto. Su objetivo es prevenir la muerte fetal y materna, mediante la identificación de hipoxia o isquemia incipiente en un feto sano, en un momento en que la intervención puede prevenir o mitigar la presentación de convulsiones o de una lesión permanente, además de que puede ayudar a evitar pérdidas emocionales y económicas importantes

para los criadores o responsables de los animales (Siena y Milani, 2021). El estrés fetal provocado por la hipoxia puede ocurrir durante un parto distócico y se manifiesta por el descenso de la FCF y de ahí radica la importancia de su monitorización (Gil *et al*. 2014).

La importancia de monitorizar el desarrollo del feto a lo largo de la gestación y el parto radica en que la mortalidad prenatal, intraparto y perinatal suele ser alta en algunas especies. Por ejemplo, en los perros (*Canis lupus familiaris)* se han observado cifras del 17-30% (Vassalo *et al*. 2015a), aunque de acuerdo con Veronesi *et al.* (2009a), estas cifran varían del 5-35% en perros, o puede llegar al 40%.

Por todo lo anterior, la evaluación integral del cachorro recién nacido que incluya la monitorización fetal y uterina, la evaluación del perfil sanguíneo, de la vitalidad, de la temperatura y del grado de tinción de meconio en piel, pueden y deben ser herramientas aplicables, tanto en clínica de pequeñas especies, como en animales de granja, para de esta manera mejorar su calidad de vida y disminuir las importantes tasas de mortalidad que esta especie presenta en el periodo perinatal.

CAPÍTULO 1.

Strategies for Hypothermia Compensation in Altricial and Precocial Newborn Mammals and Their Monitoring by Infrared Thermography

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Review

Strategies for Hypothermia Compensation in Altricial and Precocial Newborn Mammals and Their Monitoring by Infrared Thermography

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Abstract: Thermoregulation in newborn mammals is an essential species-specific mechanism of the nervous system that contributes to their survival during the first hours and days of their life. When exposed to cold weather, which is a risk factor associated with mortality in neonates, pathways such as the hypothalamic–pituitary–adrenal axis (HPA) are activated to achieve temperature control, increasing the circulating levels of catecholamine and cortisol. Consequently, alterations in blood circulation and mechanisms to produce or to retain heat (e.g., vasoconstriction, piloerection, shivering, brown adipocyte tissue activation, and huddling) begin to prevent hypothermia. This study aimed to discuss the mechanisms of thermoregulation in newborn domestic mammals, highlighting the differences between altricial and precocial species. The processes that employ brown adipocyte tissue, shivering, thermoregulatory behaviors, and dermal vasomotor control will be analyzed to understand the physiology and the importance of implementing techniques to promote thermoregulation and survival in the critical post-birth period of mammals. Also, infrared thermography as a helpful method to perform thermal measurements without animal interactions does not affect these parameters.

Keywords: thermoregulation; body temperature; brown adipose tissue; neonate welfare; shivering; vasoconstriction

1. Introduction

Neonatal mortality in domestic animals such as lambs, calves, foals, piglets, and rodents responds to several maternal and offspring factors. Within these, hypothermia caused by excessive heat loss or inhibition of thermoregulation and heat production is considered a major element that causes mortality of newborn animals [1–3]. Hypothermia is mainly the result of starvation when the offspring is unable to suckle [1]. Neonatal

survival within the first 24 to 72 hours is highly related to decreased body temperature experienced at birth. At this moment, the offspring abruptly transitions from a warm, nutritious, sterile, and controlled environment in utero to the extrauterine environment, usually at a much colder temperature (1 to 2 °C below), exposed to novel microorganisms and deprived of a nutrient supply via placenta [4–7]. These physical and physiological changes can generate hypoglycemia, hypoalbuminemia, energy alterations, or acid-base alterations leading to growth retardation or multiorgan failure [8].

The separate evolution of endothermy in mammals and birds is considered an important transition in vertebrate evolution. It is a unique case of convergence between these two groups, essential to their rapid spread across the planet and their ecological success [9,10]. Rezende et al. [10] observed that when metabolism increases, the sizes of the individuals decrease (and that is why dinosaurs gave rise to birds). In endotherm animals, thermoregulation is a homeostatic and dynamic process between the internal response of an organism and its external environment [11–13]. This internal response triggers a series of thermoregulatory mechanisms that are modulated by the preoptic area of the hypothalamus (POA) [14], and they are aimed at promoting energy conservation in the neonate, using this mechanism for growth, development, and cellular functions [15,16]. However, newborn's interspecies characteristics, such as the presence or absence of thermogenic cells, e.g., brown adipocyte tissue its acronym is BAT; the presence of fur at birth, the thickness of the dermis, behavior at birth, locomotor abilities, and general organ development, can either facilitate or hinder thermoregulation [17].

These interspecies differences respond to physiological maturity, known as the newborn's capacity to cope with the transition between the intrauterine life and the external environment. Such maturity involves activating several neuroendocrinological and behavioral changes. Nonetheless, nutrition, genetic selection, and pharmacology can also influence maturity at birth. Similarly, factors leading to the activation of the hypothalamic–pituitary–adrenal axis (HPA) [18], increasing the catecholamine and cortisol concentrations either in the fetus or the newborn animal [19], will cause changes in blood flow that will ultimately compromise the newborn's thermoregulation capacity [20].

Determined mainly by physiological maturity, mammals can be classified into altricial and precocial species (see Section 3). Although altricial and precocial newborns have several mechanisms to maintain a stable body temperature [21,22], a sudden drop in temperature experienced at birth reduces vigor and affects their feeding ability. Consequently, the acquisition of immunoglobulins and the ingestion of nutrients that fuel thermogenesis are compromised [23–28].

Thermoregulation is one of the most complex mechanisms of the organism and a critical factor for the survival of newborn non-human mammals. However, it is not always well understood due to interspecies differences. Therefore, the present review aims to discuss the mechanisms of thermoregulation in domestic mammalian newborns, highlighting the differences between altricial and precocial species. The main thermoregulation mechanisms among domestic mammals (i.e., use of BAT activation, shivering, dermal vasomotor control, and thermoregulatory behaviors) will be analyzed to understand their physiology. Additionally, the importance of implementing diverse techniques, such as infrared thermography, that evaluate and promote thermoregulation and survival in the critical post-birth period of mammals will be reviewed.

2. General Thermoregulatory Mechanisms Triggered at Birth

In many species, the drop in temperature experienced at birth induces an immediate compensatory response by the neonate to modify the physiological parameters and to avoid further heat loss. It has been described that when the neonate experiences a drop of up to 2 °C in body temperature—in the case of piglets and ruminants—the body is triggered to use thermogenesis mechanisms coordinated by the Nervous System (NS) [25,29,30]. The thermoneutral zone is the temperature range in which an animal's body temperature remains within the normal physiological range, being able to regulate either heat loss or

heat production with minimal effort [31]. Further, endothermy or homeothermy is a state associated with thermogenesis, initiated by hypothalamic activation, which aims to reduce heat loss or increase heat production [32,33].

Exposure to cold temperatures or when the environmental temperature changes from the thermoneutral zone (below the low critical temperature) induces the response of peripheral thermoreceptors located at the dermal level. Transient potential receptors (TRP) TRPM8 and TRPA1 are activated at temperatures below 27 °C and 17 °C, respectively [34,35]. These TRP transduce the thermal stimulus and transmit through the primary sensory fibers, such as the A-beta, A-delta, and C fibers, which conduct the thermal sensation to higher brain structures, such as the hypothalamus, specifically in the POA, a structure that also receives thermal signals from the solitary tract [36].

The preceding allows the understanding that the successful compensation of hypothermia largely depends on the degree of neurodevelopment of the structures that coordinate the cited response.

Heat production is the generation of heat through intensified muscular activity or shivering, or the production of metabolic heat through the breakdown of BAT, which is generated mainly in the thorax or perirenal areas [12,14,22]. The regulation of shivering involves structures that connect the POA with the lateral parabrachial nucleus, the dorsomedial hypothalamus, the raphe pallidus, and motor neurons of the spinal cord [37]. Although shivering employs thermosensitive neurons in the POA and the spinal cord, these neurons also activate mechanisms such as breaking down BAT. Banet et al. [38] determined that the POA activation threshold is higher than for shivering in the case of BAT activation, whereas the opposite case occurs in neuronal cells of the spinal cord, in which the threshold for shivering is lower than for breaking down BAT. The above means that when a newborn requires activation of BAT to produce heat, the neurons of the POA will be the first to respond; in contrast, if the required mechanism is shivering, the neurons of the spinal cord will play a primary role.

Additionally, it is mentioned that the presence of insulator fur makes animals less susceptible to thermal losses than those with glabrous skin, such as human or pig newborns. In them, there is a greater heat production capacity per unit of body weight and a smaller surface area about its weight [1]. However, BAT thermogenesis causes considerable energy consumption, and the newborn resorts to the use of glucose and oxygen reserves to produce heat, with the consequent risk of hypoxia, adynamia, hypoglycemia, and even death, emphasizing the importance of early recognition of hypothermia in the neonate [39] (Figure 1).

Another hypothalamic coordinated thermogenic response begins with reducing heat loss through peripheral vasoconstriction before catecholamine neurosecretion (adrenaline and noradrenaline). Vasoconstriction preserves the central temperature in organs with high metabolic requirements, such as the brain [1]. Furthermore, in the case of the newborn piglet, decreasing blood flow through vasoconstriction of the nearby skin capillaries helps decrease corporal heat loss during convection exchange [25,36].

The thermoregulatory capacity during the postnatal stage will primarily be dependent on the NS response to activating thermogenesis mechanisms and whether the species is altricial or precocial. In both, the compensation strategies depend on the NS maturation degree and the presence or absence of structures such as hair or fur or BAT activation, which will be discussed in detail in the following sections.

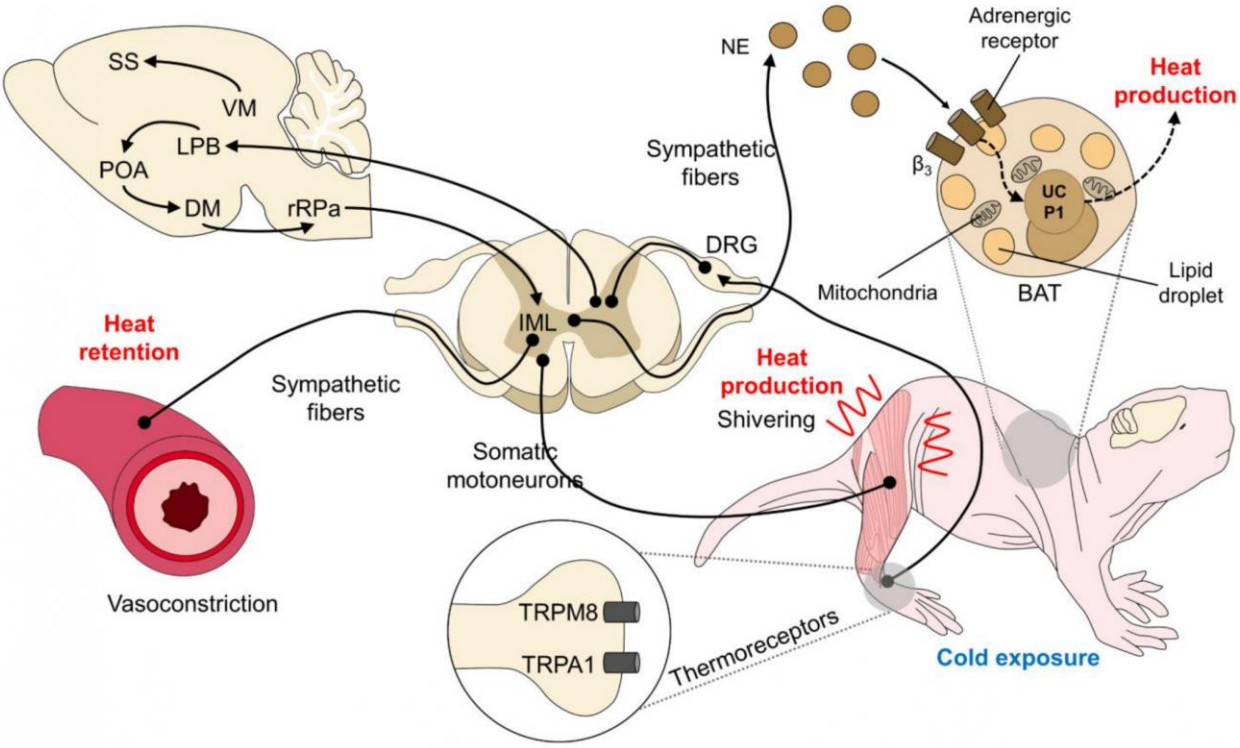

Figure 1. Neonate compensatory mechanisms in response to hypothermia. In newborns (illustrated by a naked mole (*Heterocephalus glaber*) in this figure), when the peripheral receptors responsible for the thermal sensation of cold are activated (e.g., TRPM8 and TRPA1), a neuronal response is generated that involves spinal structures (DRG) and the brain. In the brain, the thermoregulatory center (POA) receives the signal from the LPB. The POA has connections to the DMH, which, in turn, is connected to the rRPA and the IML neurons. Once in the spinal cord, two responses are produced through sympathetic efferents. Firstly, the innervation of blood vessels generates vasoconstriction and heat retention; conversely, the sympathetic release of NE acts on the BAT adrenergic receptors to produce heat. An additional response is shivering, a heat-generating process that depends on the spinal cord's somatic motoneurons and their terminals. These mechanisms promote the production or retention of heat to protect the body from the consequences of hypothermia. BAT: brown adipocyte tissue; DMH: dorsomedial hypothalamus; DRG: dorsal root ganglion; IML: intermediolateral nucleus; LPB: lateral parabrachial nucleus; NE: norepinephrine; POA: preoptic area of the hypothalamus; rRPa: rostral raphe pallidum; SS: somatosensory cortex; UCP1: uncoupling protein 1; VM: ventromedial hypothalamus.

3. Morphoanatomical Differences Associated with Thermoregulation in Precocial and Altricial Newborns

Neonates of precocial species are physically mature at birth, with fully functional ears and eyes, fur-bearing [40], and do not require constant parental care or nest rearing. The locomotion capacity of the precocial allows them to move and to immediately seek the dam's udder and start suckling after birth [41,42]. Being animals that show a greater organic development, essential vital functions such as thermoregulation are facilitated [40] since the organogenesis of structures such as the lung, liver, and brain are carried out in utero [43].

In contrast, the offspring of altricial species are small-sized, compared to precocial species, naked with little or no fur, with uncoordinated locomotion (Table 1). If they are removed from the nest or the parents, or the parents are unable to feed them, their thermoregulation is affected because they cannot produce sufficient endothermy heat, resulting

in hypothermic mortality [44]. However, enhanced postnatal plasticity is attributed to altricial animals. For example, altricial species are born with incomplete maturation of the nervous system and organs (e.g., the lungs) and a low metabolic rate, but these characteristics are completed faster than precocial animals during the first days of birth [45]. Nevertheless, because this process takes days, they lack thermoregulatory capacity immediately after birth, and they are susceptible to hypothermia [40]. Lambs have shown that their thermoregulatory ability is present within the first 60 minutes after birth, unlike other precocial species such as cattle calves or the altricial, such as puppies [5]. This difference within precocial species arises from the physiological and morphological resemblance to altricial species, when animals have reduced thermoregulatory capacity at birth, such as piglets without an adequate amount of BAT, or newborn mice and rat pups, in which the maturation of BAT coincides with the full functionality of the HPA, around seven days after birth (Table 1) [46,47]. A similar case is seen in neonatal European rabbits (*Oryctolagus cuniculus*) in which the expression of UCP1 depends on the environmental challenges to thermoregulate, not only recurring to metabolic pathways through BAT activation but behavioral changes such as huddling [48]. Figure 2 schematizes a comparison between altricial and precocial rodents and the morphological differences that influence thermoregulation.

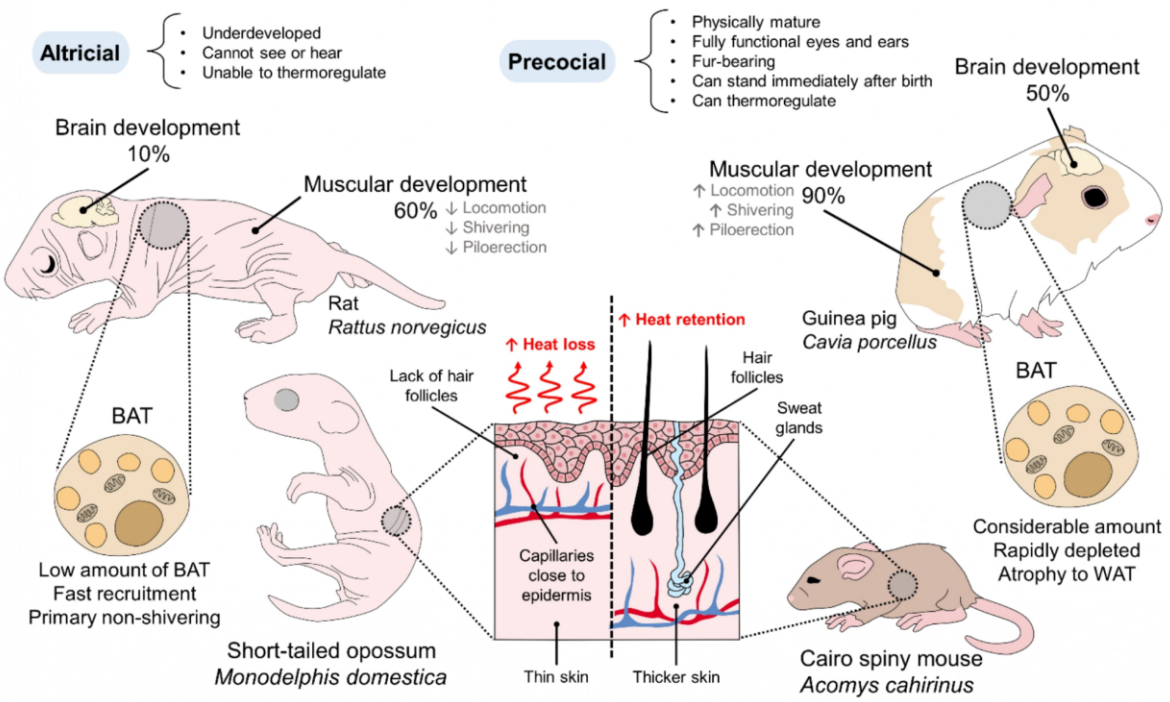

Figure 2. Morphoanatomical differences in altricial and precocial newborn rodents and their influence on thermoregulation. In altricial or underdeveloped *Monodelphis domestica* and *Rattus norvegicus*) and precocial or physically mature species (*Cavia porcellus*, *Acomys cahirinus*), morphological characteristics promote or hinder thermoregulation. The lack of fur, low amounts of BAT, uncoordinated locomotion, and thin skin with capillaries close to the epidermis contribute to heat loss and susceptibility to hypothermia in altricial species, contrarily to precocial species. BAT: brown adipose tissue; WAT: white adipose tissue.

Neurologically, differences in brain growth and development between precocial and altricial species are observed. The central nervous system maturation in altricial animals depends mainly on the postnatal period because the hypothalamic structures are the main thermoregulatory organs. During their fetal state, the brain reaches only 10% of its total growth, in contrast to precocial animals in which it reaches up to 50% of its adult size [41]. In

rats (*Rattus norvegicus*), maturation of the prefrontal cortex, with complete synaptogenesis and myelination, occurs at approximately 90 days post-birth [49]. As a result, delayed neural processing in altricial species delays the development of sensory and behavioral skills that influence their ability to thermoregulate [41].

Table 1. Main mechanism of thermoregulation and its maturation time in different species. The difference between altricial and precocial species depends on the degree of neurodevelopment which can produce variations in time to achieve optimal thermoregulation between them. Precocial species reach this capacity in the first hours after birth, while altricial species develop this ability between 20 and 45 days after birth.

	Species	Maturation Time	Main Mechanism of Thermoregulation	References
Precocial	*Bos taurus* calves	1 to 2 h after birth	Non-shivering thermogenesis Shivering thermogenesis Vasomotor control Postural changes	[18,50,51]
	Piglets	4 to 8 h after birth		[25,52–56]
	Lambs	1 to 5 h after birth	Shivering and non-shivering	[22]
Altricial	Rat pups	13 to 20 days after birth	Postural changes	[57–59]
	Rabbits	9 to 11 days after birth	Vasomotor control	[60–62]
	Kitten	45 days after birth	Non-shivering thermogenesis	[63]

The degree of muscle development, one of the thermoregulatory characteristics that generate the shivering mechanism in newborns, is another factor that presents differences between the offspring of altricial and precocial species [64]. Grand [41] has recorded the force-to-weight ratio in the altricial and the precocial as 60% and 90%, respectively, meaning greater motor and thermoregulatory independence in the latter. In the altricial, the muscular resources for heat production, such as locomotion, piloerection, and shivering are immature and do not participate until after three weeks of birth [65].

Similarly, skin thickness and the presence or absence of fur are relevant for thermoregulation, and they present differences between altricial and precocial animals. In altricial species such as gray short-tailed opossum (*Monodelphis domestica*), the skin in which the receptors in charge of perceiving thermal stimuli are located [66–68] has a thinness of 63.2 ± 2.2 µm [40], with an average range among marsupials of 36 to 186 µm [69]. In these animals, many superficial capillaries are located near the epidermis, between 38.5 ± 1.4 µm for *M. domestica* [40] and 30 to 35 µm in the case of the quokka wallaby (*Setonix brachyurus*), an extreme case of altricial species, facilitating heat loss [70]. Additionally, there is a complete absence of sebaceous and sweat glands and hair follicles [68], which, coupled with the small body size, makes them susceptible to hypothermia, relying entirely on their mother for thermoregulation. Additionally, the lack of insulator fur increases the area-to-mass ratio and susceptibility to heat loss [71]. This ratio is known as an association where, for example, small animals have a relatively larger surface area from where they can lose or dissipate heat with respect to their volume, compared to larger animals [72]. Unlike precocial species such as guinea pigs *(Cavia porcellus)* or the African spiny mouse (*Acomys cahirinus*) [43] in which the young are born with fur, achieving a maximum heat production rate of 70 ml/kg/min at ambient temperatures of 30 °C [73]. In lambs, heat production depends on shivering and non-shivering thermogenesis. The ability of lambs to cope with cooling environments has shown differences between individuals and may be related to genetic and phenotypic factors such as properties of the coat (wool), body weight, and skin. Body weight would be particularly important because of the effects and relationship between surface area and volume ratios [22].

Lastly, the primary mechanism of heat production in neonates is BAT activation; however, the perinatal development of BAT differs among species. Precocial animals such as guinea pigs, lambs, and cattle calves, are born with a well-developed BAT that

atrophies rapidly, and it is replaced by white adipose tissue (WAT) within a short time during the postpartum period [74,75]. In contrast, altricial species are born with little or no BAT, whose deposition increases when exposed to low temperatures during the first weeks of birth [75], and they are the primary source of metabolic heat [66]. In mice (*Mus musculus*), this happens at approximately two weeks of birth [76]. Analysis from days 0 to 120 after birth has reported that the interscapular BAT's weight increases from approximately 10 mg to almost 60 mg, respectively, to be replaced later by WAT [77]. This replacement or atrophy of BAT depends on the species. In rabbits (*O. cuniculus*), ovine (*Ovis aries*), bovines (*Bos taurus*, *Bos indicus*), and goats (*Capra hircus*), BAT disappears within a month, from 2 to 3 days in rabbits and ovine and from 2 to 6 days after birth in bovines [75]. A particular case among precocial species is in pigs (*Sus scrofa*). Despite having independent locomotion within a few minutes of birth [78], piglets are highly susceptible to hypothermia due to their large surface/body mass ratio [56], their little subcutaneous fat, and the fact that they are born wet and hairless [79]. In particular, the wet surface of newborns due to amniotic or placental fluids can promote hypothermia, as described in piglets by Malmkvist et al. [80], influencing or enhancing the cooling effect on the skin and heat loss by evaporation immediately after birth [81].

To sum up, the morphological characteristics of the animals at birth will dictate their thermoregulatory capacity, either facilitating or altering it. For this reason, it is necessary to understand the specific precocial and altricial mechanisms used to preserve body temperature before we introduce any intervention to prevent neonatal hypothermia.

4. Thermoregulatory Mechanisms in Altricial and Precocial Species

4.1. Brown Adipose Tissue Activation (BAT)

Among the three types of adipose cells found in mammals (brown, white, and beige), BAT represents the least abundant but the one with the greatest thermogenic capacity, especially at birth [82]. Nonetheless, its quantity depends on the fetal development during gestation, the species (altricial or precocial), and the deposited anatomical site [83].

Unlike WAT, BAT has more mitochondria, high cytochrome Cc content, a vast vascular network [22,84], and BAT activation is mediated by uncoupling protein 1 (UCP1) [8]. UCP1s respond to the secretion of norepinephrine (NE) released by the sympathetic nerves and its action on β3 adrenoreceptors, whose effect is reversible through vagal stimulation (parasympathetic) [11,85]. Among other actions, NE promotes the proliferation of preadipocytes, the differentiation of mature adipocytes, regulates the expression of genes that encode UCP1, and increases mitochondrial mass. Altogether, NE actions contribute to heat generation through ATP synthesis by UCP1 [86] and lipolysis [87]. In the case of altricial species such as laboratory rodents, the relative percentage of BAT and white adipocytes can vary, depending on environmental and nutritional conditions, sex, and age. However, its remarkable plasticity allows retroperitoneal WAT to transform to BAT when exposed to cold [88].

An example of this was reported in 27 newborn deer mice (*Peromyscus maniculatus*) kept at a temperatures of 5 °C. BAT utilization increased by 42% (measured in terms of oxygen consumption), suggesting it is the only mechanism responsible for maintaining thermal stability during the first days of life [89]. Likewise, prenatal exposure to temperatures of 15 ± 4.2 °C on a female Darwin´s leaf-eared mouse (*Phyllotis darwini*) was shown to improve the thermoregulatory capacity of neonates, achieving higher body temperatures (32.3 ± 2.41 °C) compared to animals acclimated to an ambient temperature of 30 °C, which can be attributed to higher amounts of BAT and the increased expression of UCP1 in adipocytes [45]. Nevertheless, the characteristics and the properties of BAT may differ between lines of the same species. In a comparative study between B6 and A/J mice, it was found that cold stress only induced BAT activation in A/J mice due to genetic variability in the expression of UCP1 and adipogenesis. B6 mice are an inbred strain used to study obesity, a trait associated with BAT [90], while A/J mice are another strain with susceptibility to obesity and, together with B6 mice, have shown regional differences after

adrenergic stimulation of UCP1 [91]. In B6 mice, a resistance of BAT induction has been reported by adrenergic stimulation, contrary to the A/J strain. In A/J mice, the UCP1 expression in the retroperitoneal fat at 60 days of age was higher than in B6 mice, with an induced activity of 71%, more active than interscapular BAT. In contrast, in B6 mice, the presence of BAT was lower than that found in A/J mice at one month of birth [77].

Additionally, the mother's diet and body condition have also been associated with the functionality and pre- and post-natal development of BAT. A study with female C57/BL mice of 10 to 12 weeks of age observed that obese mothers fed a high-fat diet presented a deficient activity of BAT as a thermoregulator, where the activation and the expression of UCP1 and other proteins responsible for lipolysis had a lower oxygen consumption. In contrast, a deficient BAT activation was not observed in mice from dams with balanced diets [92].

On the other hand, species that are born with a low birth weight in relation to the average birth weight of the species or breed, like canine puppies, are more exposed to hypothermia because they have less adipose tissue. Moreover, when there is competition with littermates for access to a nipple/teat or a deficiency in colostrum intake at birth, there is a higher risk of hypoglycemia, which has important repercussions on neonatal survival [93,94].

Precocial animals, such as ruminants usually present a greater development of thermoregulatory mechanisms at birth, allowing them to maintain a constant body temperature, even in cold environments [30]. In these species, non-shivering thermogenesis is the most used mechanism in neonates. For example, in lambs (*O. aries*), approximately half of the cold-induced summit metabolic rate comes from non-shivering thermogenesis. The presence of metabolic-active BAT during the early postnatal period is essential [95]. However, adipose tissue distributed in the pre-scapular, inguinal, and prerenal regions represents only 2% of the total body weight [85,96].

The thermogenic activity has been measured in perirenal adipose tissue from newborn lambs (*O. aries*) for up to 33 days. In these animals, the impact of cold acclimatization of the pregnant dams can influence the thermogenic capacity of the offspring. In lambs from mothers exposed to cold climates, they had a 21% greater perirenal fat, increased metabolic activity (40%), and higher oxygen consumption in cold temperatures (16%). Additionally, the thermogenesis responses of these lambs were due solely to non-shivering thermogenesis, in contrast to lambs from dams not climatized to the cold [97]. The activation of BAT tissue responds to an increase in blood levels of cortisol, NE, and epinephrine. These catecholamines bind to beta-3-adrenergic receptors located in BAT, activating the UCP1 in the inner mitochondrial membrane. UCP1 and thermogenin increase the H+ ion flux at the mitochondrial level without ATP production [35]. Similarly, during birth, the plasma levels of hormones such as triiodothyronine (T3) and thyroxine (T4), triggered by the release of thyroid-stimulating hormone (TSH), increase metabolic consumption of adipose tissue to produce non-shivering thermogenesis [29]. In lambs, Schermer et al. [98] studied the thermoregulatory capacity of newborn lambs with fetal thyroidectomy. According to Litten et al. [99] and Silva [100], the thyroid hormone pathway for heat production is more developed in precocial species. Thyroid hormones are critical for the generation and the maintenance of body basal temperature (BBT), and even slight changes in hormone levels can affect BBT [100]. It has been observed that minor changes in thyroxine (T4) concentrations significantly impact body temperature [100,101]. BAT contains multiple enzymes called deiodinases, essential for converting T4 to active triiodothyronine (T3). In other words, BAT can generate T3, which is crucial for producing ATP and heat [102]. When exposed to cold stimuli, the enzyme 5-deiodinase type II is activated, converting T4 to T3. However, if T3 is not produced, UCP1 synthesis is blocked, leading to hypothermia [103].

Due to the influence of thyroid hormones in thermoregulation [99,100], thyroidectomized animals presented lower colon temperatures (up to 2.35 °C) than control animals. For example, Berthon et al. [104] found that in pigs, lower plasma levels of T4 are present in animals with a lower rectal temperature after birth. Likewise, the thyroidectomized

animals had a lower oxygen consumption rate and a higher incidence of shivering thermogenesis, which coincides with a lower activity of the perirenal adipose tissue, lower levels of uncoupling protein, and a higher lipid content.

In most mammals, concurrently with non-shivering thermogenesis, colostrum intake in the first hours of life represents an energy resource that contributes to maintaining a stable temperature in neonates [2]. In particular, the nutrients present in colostrum provide water, bioactive compounds, growth factors, digestive enzymes, and immunoglobulins, and one of its main roles during the first days after birth is the supplement of energy in the form of kcal/L. Although it is said that the nutritional properties of colostrum and milk are similar during the first days of life, the energy value of colostrum can be 20–30% higher than the values registered after three days or two weeks [105]. Additionally, colostrum intake and glucose absorption prevent hypoglycemia due to the low fat and glycogen storage in newborns [106], maintaining normal glucose levels that can support thermogenesis [107]. For example, in calves, colostrum provides large amounts of glucose and amino acids, equivalent to 6.7 MJ/g, that can be used to produce heat [29,108]. Piglets (*S. scrofa*) are a species born with low amounts of BAT; their main ways of heat production rely on shivering and colostrum intake shortly after birth [23]. Furthermore, several molecular (e.g., the presence of uncoupling proteins 1, 2, or 3, the responsible for non-shivering thermogenesis), ultrastructural (e.g., number of mitochondria in *longissimus thoracis* and *rhomboideus* muscle per unit tissue area), biochemical (e.g., fat oxidation, mitochondrial processes), physiological, and metabolic adaptations in the maturation of the energy production of the musculoskeletal system in piglets are an adaptive thermoregulatory mechanism [23]. Additionally, newborn piglets use their body fat and glycogen stores to survive in the first 12 to 24 h after birth [109].

On the other hand, the fetal development of BAT, the mother's diet during gestation, and the influence of hormones such as melatonin have been shown to influence the thermoregulation capacity of the newborn. The case of 5 to 6-day-old lambs born from dams with low melatonin profiles and exposed to 4 °C showed a reduction in BAT temperature of approximately 39.8 °C compared to the control group of 40 °C and elevated NE concentrations greater than 1000 pg/ml, as a result of thermal stress [110].

Thermogenesis by BAT activation is essential in the neonate of most species, and it is also essential in hibernating animals such as American black bears (*Ursus americanus*) [111,112], which represents the first resource during the postnatal period. However, for species with limited energy reserves at birth, such as newborn piglets with low amounts of BAT or rodent pups with non-fully developed interscapular BAT, colostrum intake and other mechanisms to preserve heat are critical to prevent hypothermia.

4.2. Shivering

Shivering is the universal thermogenic mechanism through the repetitive and rapid contraction of the skeletal muscle when the body is exposed to cold environments or when there is hypothermia. The muscle fibers are from resistant aerobic muscles that can produce repeated contractions [113]. This process utilizes the oxidation of carbohydrates, lipids, and proteins obtained from muscle reserves and the circulating blood [114].

Despite being a mechanism for heat production, shivering includes consequences such as an increase in oxygen consumption of up to 20-fold, increasing the aerobic capacity of muscle fibers, and leading to fatty acid oxidation [113]. Other consequences include an increased intracranial pressure and metabolic demand, causing poor ventilation synchronization [115]. This hypoxic effect has been reported in newborns. For example, in deer mice (*P. maniculatus*), high-altitude habitats (4350 m.a.s.l.) reduce the capacity to generate heat by shivering. The capacity is reduced by 30%, and only when deer mice pups reach 27 days-old do they develop an aerobic muscle phenotype, predisposing them to hypothermia and mortality. However, this characteristic is also considered a physiological adaptation to reduce energy expenditure by thermoregulation [116].

One of the main differences in thermogenic capacity through shivering between altricial and precocial species is caused by species-specific morphological features. For example, the intensity of shivering depends on the percentage of body fat, the surface-to-volume ratio, and the ATP necessary to maintain contractions [114]. In the case of dogs, the mechanism of shivering thermogenesis is poor or absent, having a greater risk of hypothermia. Additionally, dog puppies (*Canis lupus familiaris*) have only 1.3% body fat [117]; therefore, they rely on milk intake and constant maternal care to properly thermoregulate [118,119]. A similar case is seen in cubs of polar bears (*Ursus maritimus*), where they are born in temperatures as low as −25 °C. Over time they increase the ability to shiver, improve their insulation traits and, in some cases, develop BAT [120], or resort to other methods such as vasomotor control to maintain an adequate body temperature.

As stated before, thermogenesis through rapid and oscillating muscle contractions or twitching of skeletal muscle is an involuntary mechanism that produces energy released in heat [35]. Although for most precocial species it is the most efficient method for heat production and thermal equilibrium when exposed to cold environments [50], it cannot be used as a primary method due to the immaturity of the muscle tissue observed in some species, such as ruminants) [30].

In this sense, shivering heat production in piglets has been associated with a decrease in muscle glycogen up to 47%, as well as a decrease in total lipid content, a decrease in lactate in blood, and better muscle cytochrome oxidase activity (by 20% more), indicating the increase in muscle potential with exposure to cold [121].

According to Alexander and Williams [122], in one-day-old Merino lambs and one-month-old lambs, the mechanisms of thermogenesis by shivering, in comparison with the mechanisms that use BAT, are considered the basis of its thermoregulation in the first days of life. Similarly, shivering is considered a complementary mechanism activated in the first four days of birth because newborns suffer rapid thermoregulatory alterations when the adipose tissue is insufficient to maintain thermal comfort [97].

On the other hand, in piglets exposed to low temperatures (25 °C), thermogenesis by shivering increased its activity. However, their temperatures remained slightly lower than newborns exposed to thermoneutral temperatures (34 °C) [121]. In a recent study, Schmitt et al. [123] evaluated the piglets' thermoregulation efficiency from two divergent lines for the residual feed intake (high feed efficiency and less feed efficiency). Rectal temperature, infrared thermography of ear base and tip and back were recorded. Vigor, evaluated by respiration, mobility, vocalization, and morphology, was also registered by weight, length, width, and circumference. The authors observed that both vigor and morphology did not vary between piglet lines, but it was possible to observe a greater weight gain in the efficient lines (7.1 ± 1.3 g) compared to the less efficient (3.6 ± 1.3 g). Likewise, animals with a higher efficiency had a lower temperature in the ear region (24.7 ± 0.37 °C vs. 26.3 ± 0.36 °C). All of the above allows us to establish that thermogenesis through shivering is a mechanism that depends on the type of muscle fiber of the newborn. In this process, efficient feeding is essential since neonates use their feed intake as a source of energy to maintain vital function and, consequently, survival. However, if the source of hypothermia is not addressed, continued shivering can have adverse consequences for the neonate.

4.3. Vasomotor Control

Another sympathetic-dependent response to hypothermia is vasomotor shifts in the peripheral circulation [124]. When exposed to a cold stimulus, the cold-sensitive neurons located in the POA and the activation of the HPA axis induce the secretion of catecholamines (epinephrine and NE) and other neurotransmitters such as neuropeptide and ATP [35]. Consequently, they activate receptors in the blood vessels to produce vasoconstriction [125, 126] to divert blood flow from the limbs or peripheral structures to vital organs [127–129]. Solomon et al. [130] demonstrated this in four Long Evans rats (*Rattus norvegicus*) subjected

to motility frustration (e.g., not being able to use an activity wheel). These animals showed restlessness, and the stress caused low paw temperatures due to sustained vasoconstriction.

In precocial species, some differences in thermoregulation between breeds have been reported. In pigs (*S. scrofa*), 2 to 4-hour-old Meishan piglets (*Sus domesticus*) have greater development at birth than piglets of the European breed. For example, in Meshian piglets, cardiovascular responses to cold (vasoconstriction) were observed on birth/1 day/2 days after birth. In contrast, in Pietrain, Landrace, and Large White crossbred piglets, the vasomotor response capacity was not observed until five days after birth [56]. However, these differences cannot only be associated with the vasomotor response. Renaudeau et al. [131] have studied the thermoregulatory differences between European (Large White) and Caribbean (Creole) pigs regarding breed, season, and skin histology. The authors found that the dermis of Creole pigs was thicker than Large White (3.60 vs. 3.13 mm) and they had a higher density of sweat glands (32.0 and 25.4 glands per mm2, respectively). Although these traits can be associated with enhanced adaptability of Creole pigs as a heat-tolerant breed, they may also influence the thermoregulatory efficiency in piglets during the first days of life or the growing stage, but this research needs further studies [132]. As a possible assessment of this vasoconstriction during hypothermia, infrared thermography (IRT) has been implemented, which reflects the peripheral blood flow through the emitted radiation [133].

Kammersgaard et al. [134] evaluated the thermal response in 91 newborn piglets under three different environmental temperatures (15 °C, 20 °C and 25 °C) through IRT and rectal temperature from birth to 48 h after parturition. They observed a positive correlation between the ear and rectal body temperature. For example, when the piglet's IRT indicated a temperature of 30 °C, the rectal temperature was 32 °C or less, with an IRT confidence of 91.3%. Similarly, McCoard et al. [135] evaluated the thermal response by IRT and rectal temperature after birth in 10 newborn lambs. Continuous thermograms were recorded during the evaluation of a 30-min sequential baseline (11–18 °C), 30-min cold exposure (0° C), and 30-min recovery (11–18 °C) time evaluation. They observed that the rectal temperature decreased between 0.4–1 °C from the baseline to the end of the recovery period, while there were no changes in IRT during the baseline event. Five minutes after cold exposure, a rapid decrease of 5 °C was observed. These authors attribute that the observed linear thermal response is due to the change in surface blood flow in response to cold to preserve heat. This result makes it possible to suggest IRT as a valuable tool because of its non-invasive nature and the correlation between the decrease in peripheral blood flow caused by hypothermia.

4.4. Behavior and Postural Changes

Besides the metabolic and physiological mechanisms of thermoregulation, animals perform certain behaviors and postural changes to minimize heat loss [136]. Some examples are warmth seeking, nesting, burrowing, huddling, basking, and calling for the mother [40,137].

Most of the thermoregulatory behavioral changes observed in animals are innate activities. However, there is evidence that learning is another adaptative mechanism to adverse environmental conditions, as observed in rats who have shown their capability to learn and light lamps to generate heat [138]. It is believed that these behavior and postural changes involve the activation of the POA; however, it has not yet been established [11]. In pigs (*S. scrofa*), it has been observed that adopting postures such as huddling with littermates [139] or assuming a sternal position reduces the contact surface with the ground and prevents heat loss at birth [25]. In European rabbit kits (*O. cuniculus*), the most frequent behaviors are huddling, rooting, climbing, and maintaining close contact with the rest of the littermate. These behaviors seek to retain a better position within the nest, ensuring a source of heat and food [62]. In this same sense, García-Torres et al. [140] have studied the relationship between BAT, triglyceride concentrations, and huddling of the chinchilla-strain rabbits (*O. cuniculus*, *F. domestica*). In this study, the authors determined that BAT

is the main activation mechanism of thermogenesis in newborn rabbits, and that posture changes are vital in preserving their body temperature. They reported that kits positioned on the group's periphery had lower BAT reserves and low triglyceride concentrations (101.7 ± 24.8 mg/dL), suggesting that these animals were exposed to a greater thermal and metabolic challenge than the rabbit neonates found in central positions. IRT is a tool not limited to evaluating vasomotor thermoregulation mechanisms, and there are reports that this tool can record BAT or muscle activity in laboratory animals in different settings [141]. Figure 3 shows the author's preliminary findings in concordance with those reported by García-Torres et al. [140] on the effect of huddling and animal position inside the nest. In this figure, Wistar rat pups and New Zealand rabbit (*O. cuniculus*) pups are shown to represent altricial species and the effect that central or peripheral position has on the superficial temperature of newborn rodents and lagomorphs. As stated previously, postural changes such as adopting a central position are a thermoregulatory mechanism that prevent heat loss and facilitate heat conduction by the presence of littermates and even the dam.

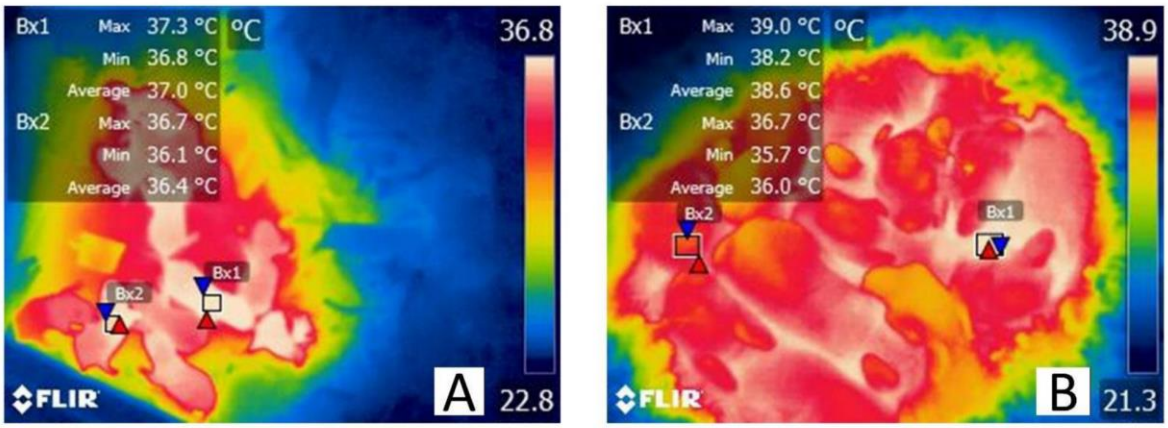

Figure 3. Huddling behavior in newborn Wistar rats (*R. norvegicus*) and New Zealand rabbit pups (*O. cuniculus*). (**A**) Thermoregulatory effect of huddling and the influence of the position within the nest in 1-day-old rat pups. The surface temperature of one of the rats in a central position, measured in the dorsal interscapular region (Bx1), shows a maximum, a minimum, and an average temperature of 37.3, 36.8, and 37.0 °C, respectively. In contrast, the temperatures of one rat pup in the nest periphery (Bx2) registered a maximum value of 36.7 °C, a minimum of 36.1 °C, and an average of 36.4 °C. (**B**) 1-day-old New Zealand rabbit pups huddling with conspecifics (Bx1) in a central position are observed as a white tone in the thermogram. A reddish-yellow tone is observed in newborn rabbits positioned in the group periphery (Bx2). Between central and peripheral positioned rabbit neonates, a difference of up to 2.3 °C in the minimum temperature (39 vs. 36.7 °C) and an average difference of up to 0.6 °C were recorded. These values measured through infrared thermography provide information on the importance of neonate rodents and leporids' behaviors to preserve heat and to maintain a stable body temperature. The authors took the thermographic images with a FLIR E80 camera with an 18 mm FOL lens at a resolution of 320 × 240 pixels and the ability to accurately measure temperatures from −20 °C to 550 °C/−4°F to 1022°F (emissivity = 0.95, distance = 30 cm).

In precocial species such as lambs, behavior at birth greatly contributes to their thermoregulation. One of its first reflexes is standing from the floor and seeking the udder to suckle and to consume colostrum. Getting up off the ground reduces heat loss, while the vitality and they speed with which the newborn finds the nipple promote early colostrum intake necessary for energy production [2]. Unlike lambs, kids (newborn goats) are considered more sensitive to hypothermia during the first hours of life. Giannetto et al. [142] have reported in Maltese kids that the circadian system is the predominant mechanism for maintaining homeostasis after birth due to the development of this system and the

genetic and phenotypic differences with lambs. In newborn piglets, vitality, and suckle capacity influence their survival rate after birth and determine their thermoregulation efficiency [143]. Together with animals' weight and size, vitality influences thermoregulation [144]. Moreover, it has been reported in newborn moose (*Alces alces*) that, in addition to the amount of BAT present at birth, newborns require feeding an average of 8 times a day in 130 second sessions to obtain nutrients and to thermoregulate [145]. Similarly, blue foxes (*Alopex lagopus*) in artic environments can reduce heat loss through postural and behavioral changes, increasing metabolic heat production to prevent heat transfer from the core to the surface [146].

4.5. Diving Air-Breathing Marine Vertebrates

Air-breathing marine vertebrates need to maintain thermal homeostasis in an oxygen-limited aquatic environment. That is why these animals, thanks to their phylogeny and thermoregulatory adaptations, have managed to survive in environments with changing temperatures using morphological, physiological, and behavioral traits [147–149]. Sirenians, for example, are the only herbivorous marine mammals with relevant thermoregulatory implications. They have a very slow metabolism, limiting their capacity for thermogenesis and making them sensitive to cold [150]. Marine mustelids and ursides are also exposed to extreme climatological challenges. Small marine mammals such as otters (*Enhydra lutris*) inhabit places with cold temperature climates to subarctic waters, while polar bears (*Ursus maritimus*) live in the arctic [151]. Another example is penguins (*Aptenodytes patagonicus*), which with their adaptations can lower their abdominal and subcutaneous temperature to −25 °C and then return to their normal temperature through subsequent rewarming [152]. We can observe that all these species have something in common: their evolutionary adaptations that have allowed them to survive in extreme temperatures.

5. Opportunity Areas and Application of IRT as an Evaluation Tool to Help the Intervention of Animals Cope with Neonatal Hypothermia

IRT has shown that it allows evaluating the thermal state of animals when exposed to extreme environmental conditions, but also in the newborn it allows monitoring each of the thermoregulatory mechanisms during hypothermia [25,134]. Its non-invasive and real-time monitoring properties make it an alternative for evaluating the body's response to hypothermia in different species [153–155]. For example, Figure 4 shows a comparison between thermograms taken 60 min after birth in a precocial (water buffalo newborn calf) and an altricial animal (newborn puppy dog). According to their characteristics and neurodevelopment, precocial species such as water buffalo can stand up almost immediately after birth [41]. This ability prevents heat loss by contact with a surface, resulting in higher superficial temperatures, as shown in the buffalo thermogram. In contrast, at the same time after birth, altricial animals such as newborn dogs are not able to incorporate or actively seek the teat until day 21 [156], and their limited thermoregulatory capacity is reflected in the lower superficial temperatures shown in the B thermogram.

IRT has been suggested as a tool that could evaluate the compensatory thermal response to hypothermia in different species such as dogs, pigs, and ruminants [25,119,135]. In this sense, IRT could help to identify hypothermia in the newborn early and assess the continuous heat loss during the postnatal period. In the same way, it has been observed that IRT helps to evaluate the organism's adaptation towards hypothermia in litters of female desert hamsters (*Phodopus roborovskii*). At birth, their surface temperature was similar to that of the environment (21 °C) due to their low body mass. In contrast, after 15 and 16 days, their body mass increased to 5.5 ± 0.2 g and 5.8 ± 0.2 g, respectively, facilitating metabolism and heat production [44]. The same effects have been observed in species that resort to a state of hibernation but cannot compensate for their temperature in environments where the cold is extreme. As a result, IRT has been suggested as a way to identify a state of severe hypothermia [157]. Some authors report the usefulness of IRT in assessing the thermal state of neonates [134,158], and some results show a positive

correlation between thermal response and rectal temperature [36,123]. However, the results are not as conclusive. For example, it has been reported that ocular temperature has no association with rectal values [159]. Soerensen and Pedersen [160] mentioned that although the temperature of the base of the ear, eyes, and udder has a high correlation with rectal temperature, factors such as age and biological status can alter its reading. Therefore, IRT may not be able to accurately assess body temperature with precision, but it is considered a valuable tool to assess changes in superficial temperature [134,159].

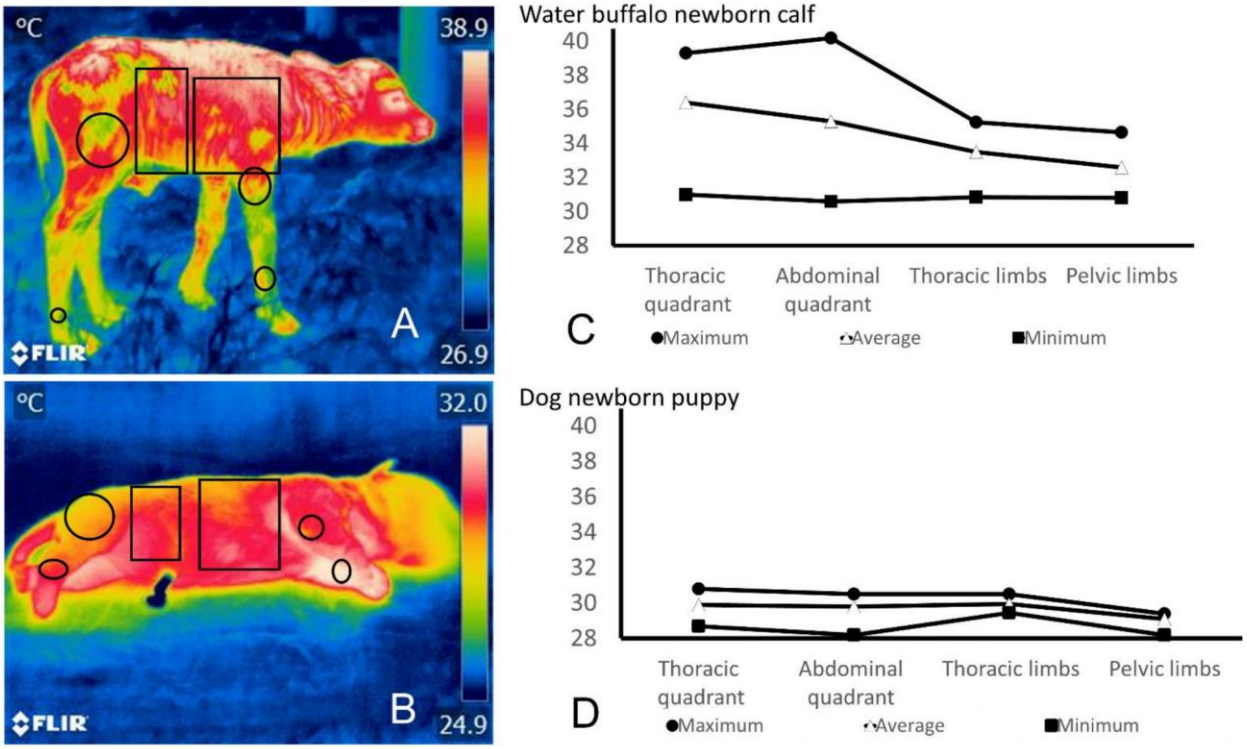

Figure 4. Thermographic evaluation in altricial and precocial neonates. (**A**) Dry newborn water buffalo calf (*Bubalus bubalis*) thermogram. The maximum temperature (shown as a white color in the thermogram) is observed in the facial and thoracic region, while the coolest temperatures (color green and yellow) are observed in the forelimbs and hindlimbs. (**B**) Dry newborn puppy (*Canis lupus familiaris*) thermogram. The maximum temperature can be seen in the cranial region of the thoracic and pelvic limbs (white color), while the back and the facial region (yellow color) and the nose (green color) of the newborn represent the coolest regions. (**C**) The graph exhibits the maximum, minimum, and average temperatures of the water buffalo newborn calf's thoracic, abdominal, forelimb, and hindlimb areas. (**D**) The graph displays the newborn puppy's maximum, minimum, and average temperature in the thoracic, abdominal, forelimbs, and hindlimb areas. A significant difference between the temperatures of both species can be recognized, where the water buffalo newborn calf has the highest values regardless of the evaluated region. Both thermograms were recorded at minute 60 post-birth to compare the thermoregulatory ability of a precocial animal (water buffalo neborn calf) and an altricial animal (puppy dog). The authors took the thermographic images with a FLIR E80 camera with an 18 mm FOL lens at a resolution of 320 × 240 pixels and the ability to accurately measure temperatures from −20 °C to 550 °C/−4 °F to 1022 °F (emissivity = 0.95, distance = 30 cm).

IRT has also been used in maternity pens to evaluate the influence of the pen characteristics (e.g., ventilation systems) on the thermal response of sows and piglets. Dela Ricci et al. [161] found that ventilation and roof sprinkles did not reduce the superficial temperature of sows during summer, meaning that the roof design and the material were

not providing an adequate heat flow. Additionally, Labeur et al. [162] have determined that the body region used to assess IRT in ewes shorn during pregnancy influences the neonate's results. In lambs, 4 hours after birth, the maximum and the average temperatures were located in the hip, and it was found that lambs born from shorn ewes maintained an adequate surface temperature when compared to the control animals, suggesting a better development of BAT in these animals.

6. Conclusions

One of the situations that can affect the survival of newborns is low temperatures or cold environments since their thermoregulatory system is limited in most cases. Neonatal animals from altricial species are usually more likely to suffer from hypothermia due to their reduced maturity/development at birth. On the contrary, precocial animals tend to adapt more quickly to thermal changes at birth, except for individuals born with a lower birth weight or difficulties during parturition that cannot have adequate access to colostrum. The ability of newborn mammals to maintain their core temperature within normal parameters is fundamental for their adaptation to the extrauterine environment and survival. That is why understanding better the specific mechanisms used by each species will allow targeted interventions to develop. Moreover, implementing thermoregulation monitoring tools, such as IRT, would help to refine and to assess the success of targeted interventions to prevent hypothermia and the consequences it can generate in neonatal mammals.

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CAPÍTULO 2.

Transient Receptor Potential (TRP) and Thermoregulation in Animals: Structural Biology and Neurophysiological Aspects

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Review

Transient Receptor Potential (TRP) and Thermoregulation in Animals: Structural Biology and Neurophysiological Aspects

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Simple Summary: In this review, recent discoveries regarding transient receptor potential are discussed and analyzed to comprehend their role in the thermoregulatory mechanisms of animals. Understanding how these receptors are activated and the pathways through which they recognize specific thermal sensations (such as cold, warm, and hot temperatures) will help researchers verify their participation in inflammatory and pathological processes. Research on transient receptor potential and their functions is ongoing, and many current studies are designed to develop therapeutic approaches that will act directly on these receptors to improve the quality of life of non-human animals.

Abstract: This review presents and analyzes recent scientific findings on the structure, physiology, and neurotransmission mechanisms of transient receptor potential (TRP) and their function in the thermoregulation of mammals. The aim is to better understand the functionality of these receptors and their role in maintaining the temperature of animals, or those susceptible to thermal stress. The majority of peripheral receptors are TRP cation channels formed from transmembrane proteins that function as transductors through changes in the membrane potential. TRP are classified into seven families and two groups. The data gathered for this review include controversial aspects because we do not fully know the mechanisms that operate the opening and closing of the TRP gates. Deductions, however, suggest the intervention of mechanisms related to G protein-coupled receptors, dephosphorylation, and ligands. Several questions emerge from the review as well. For example, the future uses of these data for controlling thermoregulatory disorders and the invitation to researchers to conduct more extensive studies to broaden our understanding of these mechanisms and achieve substantial advances in controlling fever, hyperthermia, and hypothermia.

Keywords: TRP; thermoregulation; ion channels; mammals

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1. Introduction

Thermoregulation plays a vital role in the survival of all endothermic organisms and altricial species, especially newborns [1–4]. Body temperature is a physiological and clinical parameter that provides relevant information on the individual's state [5], while variations reflect valuable biomedical data [6]. Modulations of this parameter are closely related to the stability of numerous cardiovascular, respiratory, renal, endocrine, nervous, muscular, and cellular functions [7]. One example in humans is that the integrity of some cellular processes is altered within a specific range around the ideal temperature (37 °C) [8]. This characteristic of humans and non-human animals is known as the thermoneutrality zone (TNZ), which is defined as the environmental temperature range in which an organism does not need to activate metabolic and physiological pathways to dissipate or produce heat (heat loss, heat production) [9–11]. The precise TNZ range depends mainly on the species in question, physiological status (e.g., gestation), age, sex [12], body condition scores, and other factors that affect the thermoregulatory responses of the hypothalamus and preoptic nucleus [13,14].

Identifying between-species differences and determining the thermal comfort ranges of specific animal species is mediated by neurons in the central nervous system (CNS) and peripheral receptors [15,16]. Thermosensitive receptors exist in both prokaryotic and eukaryotic organisms [17]. The best-known central route of somatosensory cutaneous thermal signaling is the spinothalamic–cortical pathway, which originates in the activation of the system made up of thermoreceptors, thermosensors, and cutaneous effectors that carry signals of thermal stimuli to the dorsal root ganglion of the spinal cord. From there, the signals travel to the thalamus and, finally, to the primary somatosensory cortex, where body temperature is consciously perceived and integrated [18,19].

The sensory thermoreceptors in mammals are nerve endings with specialized ion channels that promote transitory modifications of membrane permeability, which depend on the external stimuli perceived [20]. Most of these are made up of transient receptor potentials that perform non-selective cation diffusion [21,22]; participate in the transduction of mechanical and chemical sensory stimuli and the maintenance of the membrane resting potential; and control calcium (Ca^{2+}) and magnesium (Mg^{2+}) levels in neurons and non-excitable or cancerous cells [23,24]. TRPs are expressed in almost all tissue cell types [25], excitable or non-excitable, and in all cell membranes except the nuclear and mitochondrial membranes. Most TRPs are located in the plasmatic membrane where they contribute significantly to numerous physiological processes and homeostatic functions, and participate in vasomotor control and muscular contraction [26]. Interestingly, although their structure has been studied in great detail, we still do not fully understand the mechanism by which TRP opens and closes its gates from the moment a thermal stimulus is perceived. Against this backdrop, the goals of this review are to analyze and contrast recent scientific findings on the morphology, physiology, and neurotransmission mechanisms of TRP, and their function in thermoregulation in non-human animals, as well as enhance our understanding of their functionality and fundamental role in maintaining the body temperature of animals and those susceptible to thermal stress—as can occur during transport [27–29].

2. Classification of the Ionic TRP Channels

The first step in the transmission of thermal stimuli, which later triggers the modulatory responses necessary to maintain thermoneutrality, involves the participation of the afferent nerve fibers whose free nerve endings hold TRP channels [30]. These channels, called *gene trp* initially, were first described in late 1960 in studies with fruit flies (*Drosophila melanogaster*) [17,26]. That research found that continuous luminous stimuli induced a transformation in the membrane permeability of photoreceptors that generated a severe state of blindness—although this was reversible after one minute spent in complete darkness [31]. This response was due to a signaling cascade that contained a transient current that allowed the entry of Ca^{2+}, thereby producing a transitory change in receptor potential [32,33].

TRP are cation channels made up of transmembrane proteins [34] that function as transductors through changes in the membrane potential due to the intracellular concentrations of Ca^{2+} [35]. Q10 is the temperature coefficient of the rate of change when an organism increases its temperature by 10 °C. The normal value of Q10 is between 2–3, and in the case of biological processes or thermosensitive molecules, this value is usually more than 5. The thermosensitive TRP channels exhibit a specifically high Q10 coefficient that is higher or equal to 20 [17]. Generally, TRPs are cellular sensors that detect diverse environmental stimuli. Most respond to changes in temperature, pH, or osmolarity, but injuries, pain, pheromones, flavors, ionic imbalances (Ca^{2+}), volatile chemicals, mechanosensation, and cytokines [36,37] can also activate them. According to the differences in their amino acid sequences and typological structures, TRPs are classified into groups, families, and subfamilies. Not all types respond to thermal stimuli, though all are involved in ionic regulation. From their discovery to date, some 30 *genes trp* and over 100 TRP channels have been recognized in mammals and have been divided into their corresponding families and subfamilies.

2.1. TRP Families and Subfamilies

A total of 9 families [38] are recognized and divided into two groups depending on their degree of similarity with the *gene trp* found in *Drosophilas*. Group 1 (TRPN, TRPC, TRPV, TRPVL, TRPM, TRPS, and TRPA) includes the TRP with greater similarity to the gene, while group 2 contains those less similar (TRPP and TRPML) [23,30,32,37,39] (Figure 1).

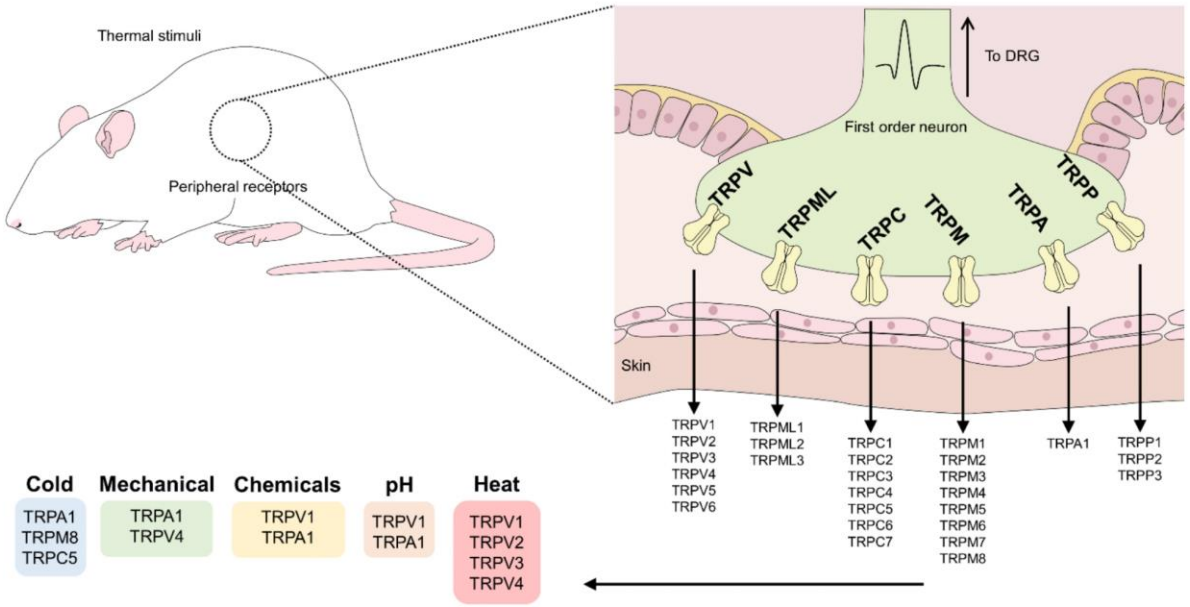

Figure 1. Families and subfamilies of TRP channels and their classification according to the type of stimulus they can perceive. DRG: dorsal root ganglion.

2.1.1. Classification of TRP

Activated in response to phospholipase C-coupled receptors and mechanical stimuli [40], TRP are divided into seven units [41]. Their characteristics include non-selective permeability to Ca^{2+} and a presence in several tissues, including the brain, gastrointestinal system, ovaries, endometrium, and ventricular myocytes. For this reason, their activation is also associated with emotional states like fear or stress [42].

2.1.2. TRPV (Vanilloid)

This group consists of six units that are considered sensory mediators activated by endogenous ligands, heat, mechanical stimuli, and osmotic changes [43]. Of the six receptors included, TRPV1, TRPV2, TRPV3, and TRPV4 are called thermosensitive [44], while TRPV5 and TRPV6 are selective channels for Ca^{2+} ions whose function is to maintain Ca^{2+} homeostasis. TRPV are channels also involved in the transduction and activation of the nociceptive arch [45].

2.1.3. TRPM (Melastatin-Related)

TRPM2, TRPM3, TRPM4, and TRPM5 are activated by heat. TRPM3 responds to harmful heat, while TRPM8 responds to cold temperatures [46]. One difference between TRPM4 and TRPM5 and the others is that they are not permeable to Ca^{2+}, though they participate in membrane depolarization [47].

2.1.4. TRPA (Ankyrin)

TRPA is the only element of this subfamily expressed in the primary somatosensory neurons, keratinocytes, astrocytes, smooth vascular muscles. and endothelium of the cardiovascular system, gastrointestinal and respiratory tracts, pancreas, inner ear, and odontoblasts [48]. It is considered a thermoreceptor sensitive to harmful cold temperatures and mechanical and sensory stimuli [49]. One of its main contributions is the transduction of nociceptive stimuli in the primary afferent nerve endings during signal transmission in the dorsal horn spinal neurons, but it also participates in episodes of neurogenic inflammation [48]. In clinical medicine, this receptor is being studied due to its activation and therapeutic effect under the administration of cannabidiol (CBD) [50].

2.1.5. TRPN (No Mechanoreceptor Potential-C)

Mechanoreceptors are found only in zebrafish and invertebrates [51]. They are in charge of proprioception in species like *Caenorhabditis elegans* [52]. In the case of NOMPC *Drosophila*, only TRPNs are related to the thermoreception of harmful cold [38,53].

2.1.6. TRPP (Polycystin)

These TRPs present in vertebrates and invertebrates respond to mechanical stimuli and the hydric balance and changes in intracellular Ca^{2+} concentrations given that non-selective channels are permeable to this compound [54].

2.1.7. TRPML (Mucolipin)

TRPML, which is present in mammals and insects [38], are considered to participate in Ca^{2+} reuptake [52] and are sensitive to temperature changes.

2.1.8. TRPVL (Vanilloid-like)

Described as a sister family to TRPV, these receptors are found only in animals belonging to the phylum Annelida (e.g., *Capitella teleta*) and Cnidaria (e.g., *Nematostella vectensis* and *Hydra magnipapillata)* [38,55]. It is suggested that this family arose as the last common ancestor of the phyla Bilateria and Cnidaria, but these receptors were lost in most bilaterians [56].

According to Peng et al. [55], TRPVL and TRPV channels share structural characteristics and, perhaps, similar functions.

2.1.9. TRPC (Canonical)

These channels are known as critical ion channels for phototransduction [32] and were first described in flies [57]. They are classified from TRPC1 to TRPC7. TRPC1 was initially found in the brain, liver, and kidneys of fetuses, and the heart tissue, testes, ovaries, and brains of adults [58]. TRPC4 and TRPC5 are non-selective cation channels that can form homomers and heteromers, and are expressed mainly in the amygdala and hippocampus.

Also, these channels are expressed in the peripheral sensory neurons of rodents [59] and humans [60]. TRPC5 is considered a cold-sensitive receptor [17], but, because TRPC5 also regulates prolactin release, TRPC5 antagonists may have greater analgesic efficacy in women since prolactin promotes pain only in them. Therefore, centrally-acting TRPC4 and TRPC5 antagonists could be an analgesic alternative to visceral and neuropathic pain [61]. On the other hand, although TRPC6 is highly expressed in the kidney, its role in kidney disease is complex and has hindered the development of some drugs aimed to improve kidney function [62].

2.1.10. TRPS (Soromelastatin)

These receptors are considered one of the most recently discovered families and the least studied (functions and structure still unknown). These receptors are not found in insects or vertebrates; however, their presence has been reported in mollusks, tardigrades, nematodes, myriapods, and chelicerates [63].

2.1.11. TRPY (Fungus-Specific TRP Channel)

Phylogenetic information has led to classifying TRPY as a different group from the previously described Group 1 and Group 2. Although the structure of this family is unknown, it is believed that these receptors may be a sister group to TRPP, which, like TRPY, has components of yeast [38,64]. TRPY has only been found in fungi and various physiological and pathological processes [65]. Likewise, it has been associated with oxidative and hyperosmotic stress, as well as in glucose-induced Ca^{2+} signaling. However, the exact function of the TRPY1-mediated Ca^{2+} release is still inconclusive [66].

2.2. Temperature-Sensitive TRP

Six subfamilies are identified as thermosensors (TRPV1-4, TRPM2-5,8, TRPA1, and TRPC5). Their firing frequency depends on the temperature they are exposed to (heat or cold) [19]. Neuronal afferents that perceive heat and cold can be differentiated by presenting specific TRP channels for each stimulus, and because their activation depends on specific thermal properties [67]. Most neurons that allow the identification of differences in the wide range of extreme temperatures (−10 to 60 °C), due to the thermosensitive receptors located in their nerve endings, are found in the trigeminal ganglion that innervates the face and head, and in the ganglia of the dorsal horn of the spinal cord [46]. According to the properties of the TRP thermosensors, these neurons recognize four basic thermal sensations: (1) cold, −10 to 15 °C; (2) cool, 16–30 °C; (3) warm, 31–42 °C; and (4) hot, 43–60 °C. Extreme cold and hot values are considered harmful stimuli [19]. The sensitive receptors to high (hot) temperatures are activated when they perceive a range of 23–52 °C [68]. They describe the thermal sensation as warm, hot, or harmful heat (pain) [30], while the ones that detect low temperatures (−10 to −42 °C) classify the thermal sensations as cool, cold, icy, or harmful cold (pain) [30,67].

Four channels of the TRPV subfamily are recognized as heat-sensitive receptors. They, in turn, are divided into those activated by harmful heat (TRPV1 at 43 °C; TRPV2 above 52 °C) [68] and those activated by harmless heat (TRPV3, 23–39 °C; TRPV4, 27–39 °C) [69–71]. Those associated with harmful cold are TRPA1 [72,73], while the ones activated by non-harmful cold are TRPM8 and TRPC5 [23,30,32,46,74] (Figure 2). TRPM2, TRPM3, TRPM4, and TRPM5 also have thermosensitive properties but are not included among the thermoreceptors because they are not located in the primary afferent axons and, therefore, require specific concentrations of Ca^{2+} for their thermal sensitivity to be activated [17].

Although, functionally, all the TRPs exhibit differences that improve our understanding of the molecular bases of thermal stimulation [17], they all share a common morphology, as will be discussed later.

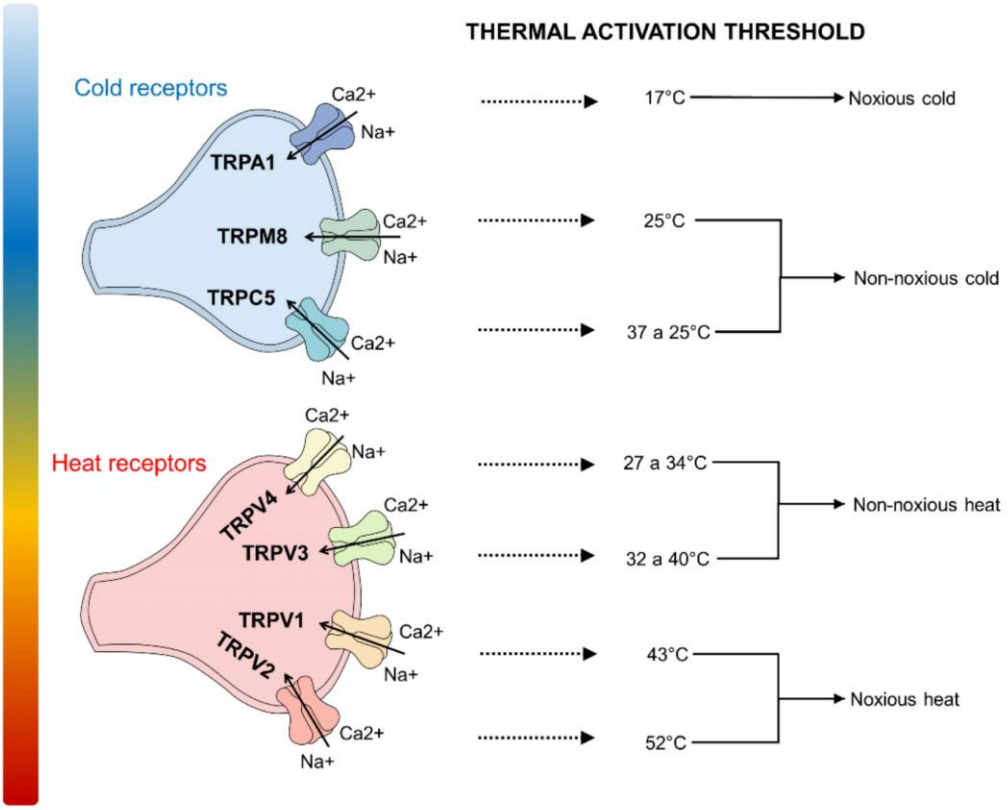

Figure 2. Thermosensitive TRP channels. Two types of thermoreceptors are known in mammals: those activated with warm or hot temperatures (range 27–52 °C) and can perceive harmful and non-harmful heat, and those whose activation threshold depends on cold stimuli (range 17–25 °C). Ca^{2+}: calcium; Na^{+}: sodium.

3. Structure

TRP channels have a common primary structure similar to that of K^{+} channels. They consist of 4 subunits surrounding an ionic permeation [75]. Each subunit has six segments, or transmembrane domains [76], of which two (S5, S6) constitute the central pore, while the others (S1–S4) form a tetramer around it [74,76]. They contain long amino (N) and carboxyl (C) groups [33], which are located intracellularly. Since each subfamily of the TRP presents particularities in its soluble domains, a functional variability in each one can be distinguished [75] (Figure 3). TRP channels also have differential domains that have led them to be classified in distinct subfamilies whose morphological variations are based on comparisons of their amino acid sequences [77].

Most of the channels responsible for modulating temperature are located in the somatic and visceral afferents. The cold receptors are small diameter, myelinated axons (A-delta) in primates, but C fibers in non-primate mammals [67]. The cold-sensitive channels include TRPC5, whose structure is similar to that of the other members of the TRPC family and consists of six transmembrane helices adhered to the ends of the N- and C-terminal domains in spiral form, a TRP domain, ankyrin repeats (around 3–4), and hydrophobic pores in the form of a loop [35]. The TRPM8 channel is also important in cold thermosensation. Its structure is similar to TRPV1 with six helicoidal segments, of which S5 and S6, which are added to the helix of pores, form the pore domain. Its cytoplasmatic region comprises the C-terminal and D-terminal domains. The latter includes four melastatin zones [78]. The TRPA1 channel is another cold sensor. It contains a transmembrane domain (composed

of six helixes with a loop between S5 and S6), an N-terminal domain that presents 14–17 ankyrin repeats, and another cytosolic C-terminal; it lacks TRP domains [35].

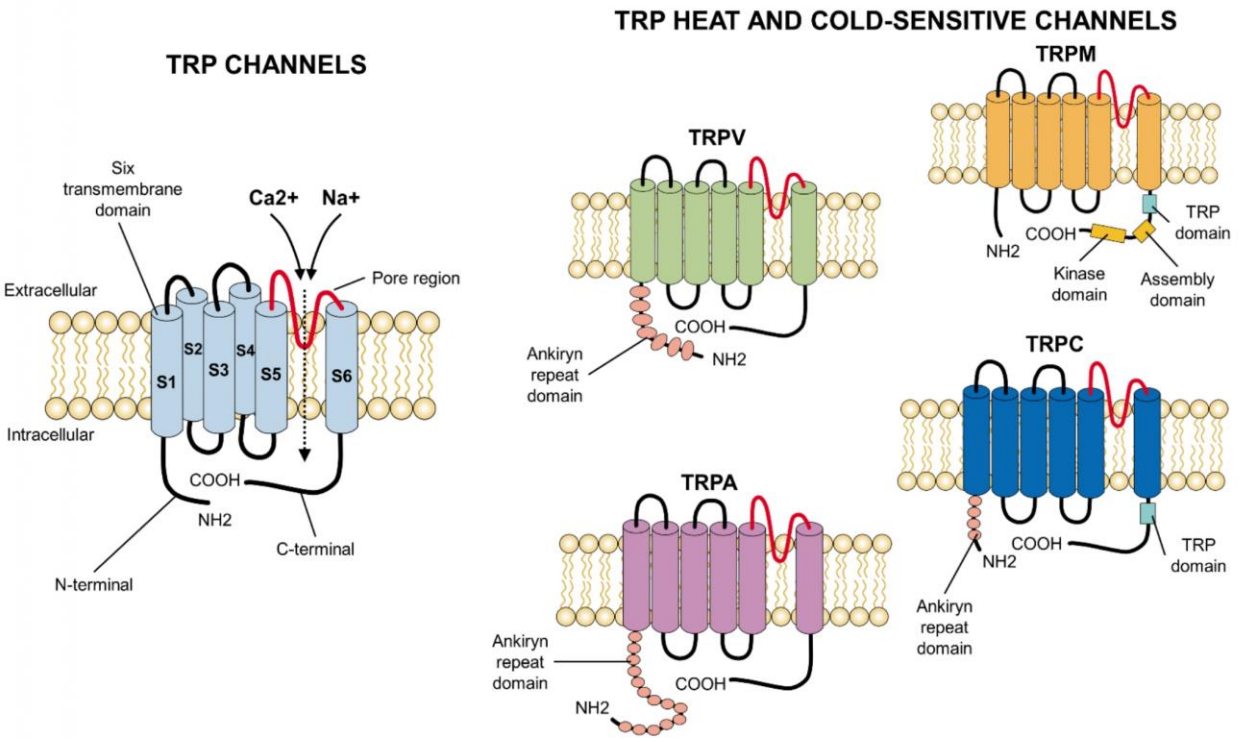

Figure 3. Primary structure of TRP ion channels. In general, all TRP channels are located in the cellular lipid bilayer and consist of six transmembrane subunits (S1–S6) with a pore region considered fundamental for the influx of cations, especially Ca^{2+} or Na^{+}. The particularities which appear at the carboxyl- (C-terminal) or amino-terminus (N-terminal) (e.g., ankyrin repeats at the latter) differ among the families of TRP channels and confer specific properties to each receptor. COOH: carboxylic acid group; NH2: amino group.

The axons that transmit heat stimuli function through unmyelinated C fibers, which are predominant in somatic and visceral tissues [67]. These channels include TRPV1, which respond to temperatures identified as harmful. TRPV1 has a quadruple structure composed of a compact zone that occupies approximately 30% of the total volume and an open domain in a somewhat basket-shaped form that makes up the other 70% [79]. TRPV2 has structural similarities to TRPV1 since both have two constriction regions—wherein one is in the S6 helix (distal zone) and the other in the selectivity filter. However, TRPV1's pore is narrower than TRPV2's, which is of complete length and, hence, capable of partially accommodating hydrated cations and other large organic ions [35].

Other important channels for heat sensitivity are TRPV3 and TRPV4, which are activated by non-harmful temperatures. The architecture of TRPV3 is similar to the TRPV channels, as it has transmembrane ionic channel domains constituted by S1–S4, amphipathic helixes, and pores added to an intracellular skirt domain that connects to the ankyrin repeats and encapsulates a cytoplasmic cavity. However, differences in its transmembrane ionic channel and ankyrin repeat domains can be observed. In addition, it presents a looped C-terminal domain that has not been seen in the other members of its subfamily [80]. TRPV4's structure differs from TRPV1 in the pore, which has only one constriction in the narrowest region (lower gate) and lacks the upper gate. In addition, its selectivity filter is

wider, and the turn that its S1–S4 domains make is in a clockwise direction and, concerning the S4 helix, at 90° [81].

4. Neurophysiology

The proteins that form the cell membrane channels regulate the influx of ions between the cell and its environment. Therefore, they have differential permeability. The main differences in the channels involve opening/closing mechanisms, conductance, dependence on membrane potential, kinetics, and other regulatory elements [82]. The mechanisms that these receptors utilize function simultaneously; that is, while the TRPV1 channels are activated by harmful temperatures above 42 °C, the TRPM8 close at temperatures over 33 °C and are activated only when temperature reductions below −15 and −30 °C are detected [46], which allow the entry of ions (Ca^{2+}, Na^{+}) that depolarize the membrane and initiate its action potential [17]. The morphology, physiological functioning, and cation permeability are similar in the nine subfamilies of the TRP channels that transmit signals to the CNS by opening gates [76]. The signaling mechanism can be direct or include the participation of second messengers [83].

The TRP channels are divided into three groups according to their permeability to Ca^{2+} or Na^{+}: (1) TRP permeable to Ca^{2+} but non-selective, where the ion flow is mediated by Na^{+} and Ca^{2+} (TRPA1, TRPV1, TRPM3); (2) TRP selective to Ca^{2+}, where the ion flow is linked only to Ca^{2+} (TRPV5, TRPV6); and (3) TRP impermeable to Ca^{2+} (TRPM4, TRPM5), where the ion flow is mediated primarily by Na^{+} [33]. Studies show that the central pore of the structure of the TRP is responsible for Ca^{2+} selectivity. The changes in the pore determine the differences in selectivity among the various types of TRP [84].

Since the TRP are non-selective cation channels and belong to the same family of voltage-gated Na^{+}, K^{+}, and Ca^{2+} channels, their activation triggers depolarization that, in turn, affects channels dependent on Ca^{2+} or those that contain it [85]. One of the principal characteristics of the TRP channels is that they function as signalers of intracellular Ca^{2+}. By extracting it from the cytosol to generate an influx of cations and the onset of electrical activity, the generation of an action potential, and, finally, the physiological thermoregulatory responses that are activated depending on the stimulus perceived [33,86] (Figure 4).

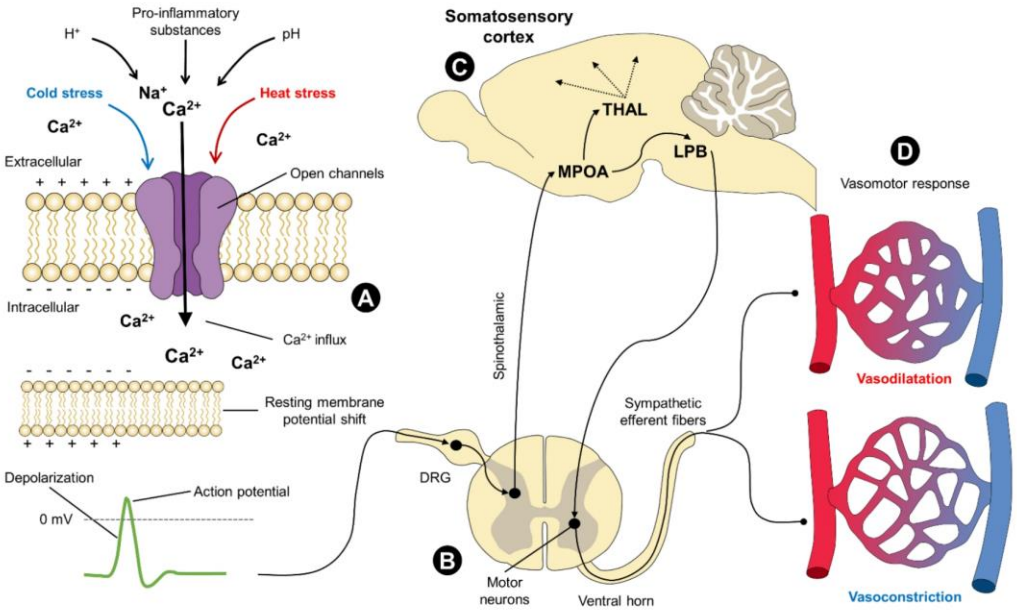

Figure 4. Activation and physiological participation of TRP channels in vasomotor thermoregulation.

TRP receptors are channels permeable to Ca^{2+} and other cations (Na^{+}). When they respond to cold or heat stress, changes in pH, the presence of H^{+}, or pro-inflammatory substances, these channels open and allow the influx of Ca^{2+} into the intracellular space. The entry of cations into the membrane induces a shift in the resting membrane potential towards a more positive value. This change creates a depolarizing effect and the subsequent generation of action potentials. Once the perceived stimulus is transduced into an electrical signal, it is transmitted by primary neurons to the DRG of the spinal cord. For example, through ascending pathways (spinothalamic) signals are conveyed to brain centers essential for thermoregulation in mammals. The hypothalamus (MPOA), its connections to the thalamus, and the somatosensory cortex constitute the pathway of thermal perception. Vasomotor responses, meanwhile, are carried out through sympathetic efferent fibers that originate in the ventral horn of the spinal cord. Vasodilation and vasoconstriction are two physiological mechanisms that act to dissipate or conserve heat under exposure to hot and cold temperatures, respectively. DRG: dorsal root ganglion; LPB: lateral parabrachial nucleus; MPOA: median preoptic area; MV: millivolts; THAL: thalamus.

4.1. The Opening/Closing Mechanism of TRP Channels

Recently, significant advances have been made to understand the structure of these channels. However, we do not yet completely understand the nature of the mechanisms through which their gates' opening and closing operations function. In general, the activity of TRP channels requires post-transcriptional modifications such as the mechanisms related to the G protein-coupled receptors, dephosphorylation, and ubiquitination. The opening of channels selective to Ca^{2+} increases the amount of cytosolic Ca^{2+} and causes depolarization of the membrane, which results in an action potential and propagation towards the CNS [87]. This contributes to impulse transmission and generates the secretion of neuropeptides involved in neurogenic inflammation and other processes that depend on the activation of these receptors through diverse mechanisms [33].

4.1.1. Membrane Voltage

A relevant number of TRP channels, most of them involved in sensory perception, have an intrinsic dependence on voltage [88,89]. Generally speaking, the influx of Ca^{2+}, or under conditions in which large amounts of Ca^{2+} exist, generates an action potential in the membrane that makes it more positive. The opposite effect occurs when the membrane returns to a negative potential, thereby causing the inactivation of most TRP channels [90]. The voltage-dependent activation of the TRP is sensitive to other triggers, such as the presence of ligands or temperature changes [91,92]. Similar to what happens with the voltage-gated potassium channels, the molecular counterparts involved in detecting voltage are likely positively charged with lysine and arginine residues in the transmembrane segment, S4, and linker, S4 and S5 [92].

4.1.2. Membrane Phospholipids

Studies mention that one route through which the TRP can be activated is mechanical stimulation, which causes a curvature in the layer and the opening of the channel due to ankyrin chains [76]. Several reports mention a direct effect of the membrane phospholipids on the regulation of TRP channel activity [93–95]. Specifically, many TRPs are highly sensitive to phosphatidylinositol 4,5-bisphosphate, which is the most abundant acid phospholipid in the plasmatic membrane [96,97].

4.1.3. Phosphorylation

Protein kinases are a type of enzyme that modifies other molecules through phosphorylation. Phosphorylation that isdependent on the protein kinase C (PKC) is likely a direct mechanism of the channels' activation, or sensitization, towards other stimuli. However, PKC reduces the function of some channels as its activation initiates dephosphorylation of TRPM8, thereby causing the inactivation of this channel [26]. Another mechanism of the activation of TRP channels involves the protein kinase A (PKA), whose activation

through stimulation of the prostaglandin E2 potentializes TRPV1's responses and counteracts the desensitization of that channel [98]. PKA and PKC present opposed mechanisms in modulating heat-activated TRPV1 and cold-activated TRPM8 [26,99].

4.1.4. Ligands

Some activities of the TRP channels are regulated by a considerable number of exogenous and endogenous ligands [89], especially the ones that perceive thermal sensations, which seem to be the ones preferred by the chemicals derived from plants. Specifically, TRPV1, TRPV3, TRPM8, and TRPM4 depend on ligands to generate an action potential [90]. An example is TRPV1, which is activated by botanical compounds like capsaicin [100], resiniferatoxin, piperine, and camphor (the latter also activates TRPV3). TRPM8 is activated by menthol and eucalyptol [101]. In addition to the components of plants that can intervene in the activation of certain TRP, diverse synthetic ligands are utilized to activate these channels. They are essential pharmacological tools used to modulate TRP channels' functions; for example, 2-aminoethyl diphenylborinate activates TRPV1, TRPV2, and TRPV3 [102], and icilin activates TRPM8 and TRPA1 [26,103].

5. Heat-Sensitive TRP

5.1. Harmful Heat

5.1.1. TRPV1

This channel is responsible for perceiving harmful heat above 43 °C [19]. A non-selective channel that is permeable to cations, TRPV1 is activated by capsaicin, low pH (<6), temperature increases, and changes in membrane polarity [100]. It thus contrasts to TRPM8, which is activated by cold and menthol [17,104]. TRPV1's activation range is 43–50 °C. Substances like ethanol also activate endogenous lipids (e.g., endocannabinoids, anandamides, and N-arachidonoyl dopamine), which are products of the lipoxygenase metabolism and topical analgesics. It is also associated with the recognition of harmful stimuli [17].

It is important to point out that diverse inflammatory mediators can reduce the temperature threshold for the activation of this thermoreceptor while increasing the magnitude of the responses of the ionic channel to produce inflammatory pain and heat hyperalgesia. For this reason, studies recommend that TRPV1 be a target for the development of analgesics [46]. In addition to its location in the sensory neurons of the dorsal root ganglia, TRPV1 is present, to a lower degree, in some cerebral structures, smooth muscle cells, adipocytes, the vascular system, and the gastrointestinal tract. Due to these circumstances, it has not yet been possible to precisely elucidate which sites TRPV1 contributes concretely to thermoregulation, for it also performs a function in regulating metabolism [46,105].

The observation that some TRPV1 knockout mice showed slight difficulties in detecting hot temperatures suggests that other mechanisms participate [106]. Reports describe the existence of a variant of the TRPV1 receptor in the sensory neurons of vampire bats that responds to a lower temperature threshold (~30 °C) and has been correlated with a delicate sensitivity that allows detection of warm-blooded prey [46,107].

5.1.2. TRPV2

TRPV2 is considered a receptor present in A-delta heat-sensitive fibers, whose activation is observed at harmful temperatures above 52 °C [19]. It is also associated with the perception of osmotic stress to mechanical stimuli and pharmacological compounds, like cannabidiol and tetrahydrocannabinol [17]. It is found in pulmonary tissue, the spleen, intestines, dorsal root ganglia, sensory ganglia, and the brain. TRPV2 is the principal receptor expressed in cerebral tissues (e.g., cerebellum, forebrain, and hippocampus). It has been suggested that in addition to its thermo-transductor functions, it may participate in the physiological processes in those cerebral structures [108]. Although TRPV2 presents 50% homology with TRPV1, it is not activated by capsaicin and is more permeable to Ca^{2+} than Na^{+} [36]. The role of this thermosensor has been studied in heat-sensitive nociceptors of

rats, where administration of drugs like gadolinium—a TRPV2 antagonist—blocked the influx of cations, thus restraining the activation [108]. In contrast, the 2-aminoethoxydiphenyl borate agonist generates action potentials in the receptors in rats and mice, but not in humans [109]. Despite these findings, doubts persist concerning the participation of this receptor in thermoregulation because studies of TRPV2-deficient mice have not reported any aberrant thermosensitivity [110].

5.2. Harmless Heat

5.2.1. TRPV3

TRPV3 is a cation channel activated at warm temperatures around 34 °C [19], and ranging from 32–40 °C [111]. It belongs to the permeable channels group that are non-selective for Ca^{2+}. It is expressed in the skin, keratinocytes, and oral and nasal mucosa, where its activation participates in other processes unrelated to thermosensitivity—such as hair growth, wound healing, itching, and pain perception [112]. Its presence in dermal tissues and the process of hair growth in mammals has led to studies of its participation in the processes of alopecia in hairless rodents. In those animals, mutations generate the absence of hairy structures due to an ionic alteration in the skin and observations of non-responsiveness to thermal stimuli and harmful chemicals [113]. Similarly, experimental results from Ferreira et al. [17] and Moqrich et al. [101] report that mice with genetic deficiencies of this receptor show altered responses to both harmless and harmful thermal stimuli. In contrast, Huang et al. [114] and Miyamoto et al. [115] mention that the TRPV3 and TRPV4 channels in mice do not play a fundamental role in heat perception or processes of heat hyperalgesia since animals deficient in this ionic channel did not manifest a deficit in their responses to heat stimuli. Variability in these results suggest that these phenotypes depend significantly on the genetic background of the mice tested [46].

5.2.2. TRPV4

This is a non-selective polymodal receptor similar to TRPV1 that is activated by moderately warm temperatures in the range of 27–34 °C [19]. In mammals, exposure to this temperature range, and the subsequent activation of TRPV4, generate the influx of Ca^{2+} and an action potential that helps them maintain thermal homeostasis concerning their environment or external stimuli [116]. Other functions of TRPV4 consist of acting as a sensor of hypotonicity [111] and as a mediator of such pathological processes as heat hyperalgesia and mechanical hyperalgesia. In both cases, excessive excitation due to direct damage, like spinal cord compression, leads to nitric oxide generation and a cascade that triggers sensitization processes like hyperalgesia and allodynia [117].

Other examples have been observed in species like mice when the TRPV4 gene is experimentally removed. This procedure revealed a deficit in perception of mechanical and chemical stimuli (tail pressure and the acetic acid test, respectively). However, those same subjects' thermal responses to harmful heat (50 °C) were not altered, suggesting that one of TRPV4's main roles is to perceive nociceptive mechanical stimuli rather than participating in thermal sensibility [118]. Like TRPV3, the fact that TRPV4 is heat-activated suggests that it participates in maintaining body temperature; however, no clear evidence exists to support this hypothesis [46].

5.2.3. TRPM2, TRPM4, and TRPM5

Since these three receptors require modulation by intermediaries (e.g., ADP-ribose, intracellular Ca^{2+}, oxidative stress), and because they are mainly located in the brain, pancreas (TRPM2 specifically in β cells), and immune system cells, they tend to be excluded from the list of primary thermosensitive receptors [17,119]. Nonetheless, some studies have demonstrated their importance as thermal sensors involved in events like fever, where they intervene to prevent hyperthermia and its organic consequences, and their participation in hypothermia development since TRPM2 activation and inhibition modulate body temperature [120]. However, no thermosensory phenotype has been described in

mice whose TRPM2 or TRPM4 receptor is inactivated. TRPM5 has been associated with the modulation of taste perception [121], but it remains unknown whether this characteristic acquires an important role in other homeostatic contexts [46].

6. Cold-Sensitive TRP

6.1. Harmful Cold

TRPA1

This receptor responds to a thermal threshold of 17 °C, which is considered harmful cold [19]. Increased intracellular Ca^{2+} concentrations mediate its activation due to the cold more than any direct action. Significantly, depolarization of TRPA1 is considered a fundamental element for inducing the cutaneous vasoconstriction responses that occur when individuals are exposed to low temperatures [122]. Some studies have found differences between species in which cold temperatures have activated TRPA1, like in rats and mice but not in humans or Rhesus monkeys [123]. Moreover, contrasting results have been reported within the same species (mice) since some authors indicate that this receptor contributes to the sensation of cold [124,125], but others rule out any such participation in detecting cold in vivo [126,127] or in mediating cold defense responses [128,129].

This receptor's responses to harmful thermal (heat) and chemical stimuli have been studied in frogs and lizards, like the green anole (*Anolis carolinensis*). Saito et al. [130], for example, found that TRPA1 is activated under exposure to both conditions. A finding related to an evolutionary effect in which those species have preserved the TRPA1 channel for the detection of temperatures that significantly alter their homeostasis and, in this way, increase their probability of survival. Similarly, TRPA1 is associated with the transduction of nociceptive stimuli, like those linked to pro-inflammatory transmitters, such as the bradykinins [131].

6.2. Harmless Cold

6.2.1. TRPM8

This ion channel was the first one to be described as a thermoreceptor that specialized in the thermal sensation of cold. Activation of TRPM8 occurs under exposure to temperatures below 25 °C [19]. However, some authors mention that its activation begins at around 33 °C in the sensory neurons and that its signaling velocity increases proportionally to the temperature decrease [17,104,120]. This receptor is present in a subset of afferent neurons in the dorsal root ganglia that innervate the skin and sensory neurons of the trigeminal ganglion that innervate the head, eyes, and cornea [46]. There are also reports that TRPM8 is expressed in adipocytes, as mentioned with regards to TRPV1 [132,133].

Scientific evidence indicates that diverse, selective TRPM8 antagonists produce dose-dependent hypothermia in rats and mice. The topical application of menthol, a TRPM8 agonist, triggers hyperthermia and shivering-like muscle activity, vasoconstriction at the level of the skin of the tail, and heat-seeking behavior [134]. These findings concluded that there is a hypothermic effect dependent on this receptor's activity [46].

6.2.2. TRPC5

TRPC5 is considered a cold-sensitive receptor that is activated at temperatures of 37–25 °C. It is present in the neurons of the dorsal ganglia and the dorsal lamina of the spinal cord [17]. Despite reports that describe the cold-induced gating of this receptor, studies of mice after ablation of the TRPC5 gene have concluded that those subjects do not present detectable defects related to cold sensitivity. Those findings call the role of this ion channel into question [46].

7. Uses of TRP in Disease Treatment

Due to the variety of functions in which the TRP channels participate, and given the diversity of mechanisms that exist for their activation, failures could occur in the permeation or entrance of a channel that would have a considerable influence on the

progress of various illnesses. The above reflects that associations have been found with affectations of the intestinal, renal, urogenital, respiratory, and cardiovascular systems, and in neurodegenerative and neuronal diseases [135]. It is also important to note that alterations of the TRP channels can affect the progression of tumors in both early and late stages, and that they have been related to diverse types of cancer, including melanoma, glioma, prostate, bladder, mammary gland, and kidney cancer [136].

The ones susceptible to thermal changes stand out because they significantly influence homeostatic processes [135]. One example of the influence of these temperature-sensitive channels can be appreciated in the study by Hoshi et al. [137], in which the authors induced edema in vitro by an ischemic stroke. Their analysis of thin slices of brain tissue under conditions of oxygen–glucose deprivation revealed that this form of deprivation-induced inflammation had been activated by the temperature increase that caused hyperthermia and generated inflammation as a consequence—but they also found that the inflammation could be blocked by administering drugs that genetically annulled TRPV4.

A study by Mustafa and Ismael [138] utilizing 20 New Zealand white rabbits isolated the carotid arteries and suspended them in organ baths, to which they added portions of ethanol, capsaicin, and capsazepine. They aimed to explore the relationship between ethanol and hyperthermia accompanied by vasoconstriction of the carotid valve. They succeeded in determining that the increase in temperature provoked by ethanol-generated vasoconstriction of the carotid valve was due to this process, which is mediated by activation of a present TRPV1 by the temperature increase and capsaicin. These findings are significant because identifying the participation of this channel and implementing antagonists that inhibit it (like capsazepine) can help prevent heat stroke or brain damage caused by hyperthermia and the decrease in cerebral perfusion induced by ethanol. A study by Tan and McNaughton [139] concluded that in addition to playing an important role in thermoregulation, TRPM2 intervenes in controlling the immune system, pain, and insulin secretion; however, its action mechanism is not clearly understood. Meanwhile, the results of Di et al.'s study [140] suggest that TRPM2 plays a negative role in the feedback of the buffering of inflammatory responses induced by oxidative stress. Beceiro et al. [141] demonstrated that in mice chronically infected with *Helicobacter pylori,* TRPM2 increased gastric inflammation and the production of macrophages and inflammatory mediators, which was accompanied by the polarization of M1 macrophages. The evidence from these studies concludes that TRPM2 has an essential role in immune system processes that are known to be closely linked to temperature, though controversy continues as to whether it intervenes favorably or unfavorably in inflammatory processes.

Other studies have shown that the activation of cold receptors in mice in environmental temperature conditions below the TNZ of the species provokes metabolic expenditures and energy demand to achieve thermogenesis. These changes entail a risk for animals, especially laboratory species, in which a disfunction of receptors increases the incidence of cardiovascular and autoimmune diseases, asthma, and cognitive diseases like Alzheimer's [14].

This discussion emphasizes the importance of determining and understanding the functioning of TRP and thermoregulation in the field of medicine [142], for this could contribute to developing new therapeutic strategies to treat specific pathologies. One example comes from work on TRPV3, a receptor mainly expressed in the skin and involved in skin health. At the experimental level, antagonists of this thermoreceptor have been used to treat dermal inflammatory pathologies because activation of TRPV3 generates secretion of proinflammatory, pro-nociceptive, and pruritic substances [143]. Due to the direct participation of TRPV3 in the process of nociceptive arch transduction, it has been proposed as an option for treating and preventing pain, though its functionality as an analgesic agent is not firmly established [144]. Finally, regulation of this receptor has been shown to affect nitric oxide production in keratinocytes that, in addition to participating in inflammation and synaptic plasticity, modulate vascular tone and, with this, blood flow and the amount of heat that a body dissipates [115].

Although currently there are analgesic options such as non-steroidal anti-inflammatory drugs [145] or opioids [146], novel therapies aimed to target specific TRP channels can contribute to modulating pain through processes such as desensitization [48]. Since most TRP channels are located in the periphery to detect noxious stimuli, drugs that target TRPs serve to block the transduction of the nociceptive stimuli [147]. For example, TRPV1 located in nociceptors is characterized by a rapid activation by noxious thermal stimuli, followed by a rapid –heat-induced desensitization [148]. This property allows the use of therapies with capsaicin patches, capsaicin analogs, and TRPV1 agonists such as resiniferatoxin (RTX). Due to its long-lasting refractory period that results in desensitization and prolonged analgesic activity [149], target therapy to TRPV1 with RTX has been suggested as a clinical alternative for severe osteoarthritic [150] or cancer pain, where its mechanism of ablation of TRPV1 channels is through calcium-induced apoptosis without generating somatosensory consequences [151].

8. Areas of Opportunity

Although the discovery of TRP channels has revolutionized our understanding of diverse physiological and sensory processes, this review highlights numerous unresolved issues. An example is the levels of participation of receptors like TRPV1, TRPV2, TRPV3, and TRPV4, since some studies report that, at least in certain mouse species, their antagonism or absence does not seem to trigger any aberrant thermosensitivity. For this reason, it is necessary to continue researching to understand these mechanisms in their totality. That knowledge could be used to control thermoregulatory disorders and develop drugs that can help improve the management of alterations caused by failures in the functioning or activation of these channels through the use of antagonists that inhibit them, given that several of them potentiate inflammatory processes. Moreover, an improved understanding of the functioning of TRP channels can contribute to adapting measures capable of mediating processes like fever, hyper-, and hypothermia.

Furthermore, TRP channels are not the only ones involved in the perception of thermal stimuli. Studies of lipid-sensitive TWIK-related potassium channels constitute another field of interest related to thermoreceptors. These channels respond to hot and cold temperatures in an activation threshold of 25–37 °C. This way, they have been associated with normal physiological maintenance and functioning through the conductance of K^+. They can be activated by mechanical stimuli, pH, unsaturated fatty acids, and general anesthetics. Upon receiving harmful thermal stimuli, they generate hyperpolarization that reduces their capacity to send signals, leading to a type of heat-pain relief [19]. Meanwhile, the non-selective cationic channels of the HCN family (HCN1) participate in firing patterns, especially during cold sensations. Mice with a deficiency in the presenting genes of these receptors show altered responses to perceptions of cold [17].

The presence of these non-TRP thermosensitive channels also influences the response of knockout animals whose TRP function is reduced. In this sense, Wang and Siemens [46] mention that the lack of a single TRP channel does not always translate into an immediate deficiency due to the synergism of other TRP and non-TRP channels that can be activated by overlapping thermal sensations (e.g., the interaction between TRPV1, TRPM3, and anoctamin 1). For example, TRPM3 is a polymodal heat thermosensor with similar functionality as TRPV1 that acts independently of the vanilloid family [152]. Likewise, Vandeuwauw et al. [153] reported that only TRPV1, TRPM3, and TRPA1 triple knockout mice completely lacked the response to noxious heat stimuli, showing that the processing of a thermal stimulus is not dependent on a single receptor but instead requires the co-expression of other thermosensitive channels. The chloride channel Anoctamin 1 (ANO1) is another receptor activated by noxious heat [154] that responds to temperatures over 44 °C [155]. In ANO1, an interaction with TRPV1 and a similar inhibitory response have been reported when substances such as 4-isopropylcyclohexanol are administered [156].

Another area in which studies of TRP channels have focused is in developing and implementing rehabilitation therapies based on cold or hot temperatures. Cold therapies

generate reductions in the degree of pain perceived in cases of skeletal muscle injury and reduced blood flow, thus decreasing local inflammation and the incidence of edemas [157]. In contrast, therapies that use ultrasound equipment with heat help reduce pain, aid in the recovery of muscular strength, and increase patients' flexibility [158]. Therefore, these approaches could constitute options for managing skeletal muscle injuries in sports medicine.

As the reader can appreciate, research has focused on analyzing the activity of these thermoreceptors in species like rats, mice, and even bats. However, the information available on birds or ectoderms is scarce, so we invite researchers to design more studies with these animals to improve our understanding of thermoregulation in more species.

9. Conclusions

The participation of some TRP channels—like TRPV1-TRPV4, TRPM8, and TRPA1—in the reception of thermal sensations is still a subject where controversy exists regarding the mechanism through which thermal stimuli are detected, and the role they perform in the thermosensitivity of diverse species. This is due to two facts: first, that research has centered primarily on mammals, and second, that even within the same species there are reports of contrasting results. While TRPV1 and TRPV2 are sensitive to harmful heat, TRPV3 and TRPV4 perceive temperatures in the range of harmless heat, and TRPM8 channels are involved in the perception of harmless cold, but TRPA1 perceives harmful cold. Another aspect of the challenges we face is that although the structures and subtypes of TRP channels are now well known, their mechanisms of activation and regulation continue to present enigmas in both the field of habitual thermoreceptor mechanisms and research on the administration of certain drugs. Consequently, we currently lack the compounds, tools, and peptide toxins that would make it possible to capture TRP channels in distinct functional states. For these reasons, pharmaceutical companies' research discoveries and developing medications must identify new molecular compounds, peptide toxins, or sensitive antibodies that can modulate the function of TRP channels.

In conclusion, it does not suffice for the pharmaceutical industry to conduct additional research in this field. It is also necessary to deepen our understanding of the action mechanisms of TRP channels to make better use of their applications in clinical medicine. Also, to prevent pain involving small animals, wild species, and farm animals, detect it opportunely, identify thermal stress situations, and even the onset of diseases that could participate in inflammatory or immune processes.

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3. JUSTIFICACIÓN

La monitorización electrónica fetal y uterina ha sido una técnica ampliamente usada en medicina humana, recientemente se ha estado aplicando en la evaluación de animales y se ha comenzado a implementar para el seguimiento en perras porque se ha visto que en esta especie ayuda considerablemente a prevenir y reducir la mortalidad antes, durante y después del parto, además de ser fácil de realizar incluso por el criador o propietario del animal y con ella se puede predecir fácilmente si un parto terminará o no en cesárea (Davidson 2001). El objetivo es prevenir la muerte fetal y materna, mediante la identificación de hipoxia o isquemia incipiente en un feto sano, en un momento en que la intervención puede prevenir o mitigar la presentación de convulsiones o de una lesión permanente, además de que puede ayudar a evitar pérdidas emocionales y económicas importantes para los criadores o responsables de los animales (Alfirevic *et al*. 2017; Siena *et al.* 2021). El estrés fetal provocado por la hipoxia puede ocurrir durante un parto distócico y se manifiesta por el descenso de la FCF y de ahí radica la importancia de su monitorización (Kutsler *et al.* 2003; Gil *et al.* 2014).

De igual forma, la gasometría es actualmente una herramienta importante para evaluar el estado de salud de los recién nacidos (Vassalo *et al.* 2015a). Sin embargo, en pequeñas especies, la evaluación de los parámetros sanguíneos umbilicales es limitado, a pesar de que con ella se pueden evaluar las concentraciones de pH, presión parcial de oxígeno (pO_2), presión parcial de dióxido de carbono (pCO_2), glucosa, lactato, hematocrito, sodio, potasio y calcio ionizado (Rootwelt *et al*. 2012). A pesar de ser una técnica que aún no es muy aplicada en perros, debido a que el costo de los equipos y consumibles no siempre es accesible, puede llegar a ser una herramienta muy útil para determinar el estado de salud y de angustia fetal y del recién nacido para disminuir de esta manera las altas tasas de mortalidad.

En perros, algunos estudios han evaluado el efecto del peso de la madre sobre el peso del cachorro al nacer, el tamaño de la camada, la vitalidad y la supervivencia del cachorro (Veronesi *et al*. 2009a; Münnich y Küchenmeister 2014; Groppetti *et al.* 2015; Mila *et al.* 2015). Además, se ha evaluado también el efecto del peso de la madre sobre la presentación de asfixia y los valores hematológicos del recién nacido (Reyes-Sotelo et al. 2021). En este sentido, en algunas especies, los recién nacidos de hembras más grandes han reportado menos problemas de termorregulación y una temperatura más alta en las zonas centrales del cuerpo (Nord *et al.* 2009; Terrien 2011; Mota-Rojas *et al*. 2021b; Lezama-García *et al.* 2022d; Reyes-Sotelo *et al.* 2022), por lo que se pueden esperar resultados similares en los perros.

Sin embargo, previo a este estudio, no existía información sobre las ventanas térmicas en cachorros (p. ej., regiones oculares o auriculares), conocidas como regiones del cuerpo del recién nacido donde se pueden evaluar las temperaturas superficiales (Casas-Alvarado *et al*. 2022). Su importancia radica en que el uso de estas regiones ayuda a comprender el proceso de termorregulación en perros recién nacidos, colaborando de esta manera a reducir las altas tasas de mortalidad en esta especie. Del mismo modo, tampoco se habían hecho estudios que reportaran el efecto del peso de la madre y de las crías sobre su capacidad termorreguladora durante las primeras 24 horas tras el nacimiento.

4. PREGUNTA GENERAL DE INVESTIGACIÓN

¿Cuál es el efecto de la experiencia materna y peso en las respuestas relacionadas con la monitorización electrónica fetal y uterina, perfil fisio-metabólico sanguíneo, cambios en la temperatura corporal del neonato hipotérmico, vitalidad y grado de tinción de meconio de la piel del recién nacido?

PREGUNTAS PARTICULARES DE INVESTIGACIÓN

1. ¿Cuáles serán las modificaciones en la respuesta uterina (intensidad, duración y número de contracciones) y episodios de bradicardia fetal por efecto del peso y experiencia materna durante la monitorización electrónica del parto en la perra?
2. ¿Cuáles serán las alteraciones neurológicas y calificación de vitalidad del canídeo recién nacido por efecto del peso y experiencia materna, durante la monitorización electrónica del parto en la perra?
3. ¿Cuáles serán las alteraciones en el grado de tinción de meconio en piel, respuestas termográficas y desajustes fisiológicos sanguíneos del neonato, por efecto de la duración del parto y tamaño de camada en perras con diferente peso y experiencia materna?

5. HIPÓTESIS

1. La dinámica uterina (intensidad, duración y número de contracciones) durante el parto se modificará de acuerdo con la experiencia materna (primípara / multípara) y talla (chica, mediana y grande) de la perra. Perras de menor peso presentarán contracciones uterinas más intensas, la duración de su parto será más corto y sus fetos tendrán menos episodios de bradicardia y sufrimiento fetal, contrario a las perras de mayor talla.

2. Los cachorros recién nacidos producto de perras multíparas y mayor peso con úteros más desarrollados tendrán menor grado de hipoxia, mayores calificaciones en la vitalidad y mejor desempeño neurológico, comparadas con las primíparas.

3. Las perras con menor experiencia materna y menor peso tendrán cachorros con mayores alteraciones morfológicas del cordón umbilical, grado severo de tinción de meconio en piel, respuestas termográficas asociadas a hipotermia y mayores desajustes fisiológicos sanguíneos.

6. OBJETIVO GENERAL

Evaluar la experiencia materna (primípara *Vs*. multípara) y peso en la monitorización electrónica uterina y fetal durante el parto y su efecto en la respuesta neurológica (escala de vitalidad), intercambio gaseoso pulmonar, morfología del cordón umbilical, respuesta termoneutral, grado de tinción de meconio en piel y desajustes fisiológicos sanguíneos, en un modelo altricial de neonato canídeo.

OBJETIVOS ESPECÍFICOS

1. Evaluar el desempeño gineco-obstétrico durante la monitorización electrónica del parto en perras con diferente peso y experiencia materna (primíparas *Vs*. multíparas) y su efecto en la intensidad, número y duración de las contracciones uterinas y número de episodios de sufrimiento fetal DIP 1 y DIP 2.
2. Evaluar el efecto de la experiencia materna y el peso, en el desempeño neurológico, vitalidad y grado de desplazamiento del neonato canídeo.
3. Evaluar la duración del parto y tamaño de camada en perras con diferente peso y experiencia materna y su efecto en el grado de vitalidad, en el grado de tinción de meconio en piel y en el perfil fisio-metabólico sanguíneo neonatal.
4. Identificar los descensos de la temperatura en las imágenes termográficas infrarrojas de las regiones rostral, apendícular y ventral de los cachorros y establecer el grado de hipotermia de los neonatos, por efecto del peso y experiencia materna.

7. MATERIAL Y MÉTODOS

Fase 1. Dinámica uterina, perfil sanguíneo y monitoreo fetal electrónico en perras primíparas y multíparas clasificadas según su peso.

7.1 Instalaciones

El presente estudio se llevó a cabo en 6 clínicas y hospitales veterinarios de la Ciudad de Campeche, Campeche, ubicada en el sureste de México, dentro de la península de Yucatán. Se solicitó autorización los propietarios para cuidar a sus perras durante toda la gestación desde el día 28 después del apareamiento hasta las 48 horas posparto y se les pidió firmar un consentimiento informado que se presenta más adelante en la sección 7.9.

7.2 Población de estudio

Se reclutaron 113 perras gestantes entre 2 y 6 años, con diferente experiencia materna (0-4 partos). Sin embargo, dado que 17 de ellas (5 de ellas con inercia uterina primaria y 12 con inercia uterina secundaria) tuvieron parto distócico que terminó en cesárea o necesitaron la utilización de oxitocina y calcio, estas perras fueron excluidas del estudio, por lo que finalmente se utilizaron un total de 96 hembras con sus 476 cachorros. Las 96 madres fueron distribuidas en cuatro grupos experimentales de 24 individuos cada uno (12 primíparas y 12 multíparas), según su peso corporal: G_1 (4 a 8 kg), G_2 (8,1 a 16 kg), G_3 (16,1 a 32 kg). y G_4 (32,1 a 39,6 kg). El peso de las perras se obtuvo al inicio de la primera contracción, cuando inició la primera etapa del parto, a través de una báscula digital (Avery Weigh-Tronix 7820-100 West Bromwich, Reino Unido). Las razas incluidas en este estudio fueron Chihuahua, Yorkshire Terrier, Cocker Spaniel, Schnauzer Estándar, Terrier Escocés, Poodle, Pastor Alemán, Labrador, Golden Retriever, Gran Danés y Pastor Belga. Los animales seleccionados fueron (a) perras clínicamente sanas con medicina preventiva (p. ej., protocolos de vacunación/desparasitación); (b) no contar con registros clínicos de problemas reproductivos; y (c) perras sometidas a estudios ultrasonográficos y radiográficos para confirmar el parto natural. Se excluyeron de este estudio los animales con las siguientes características: (a) aquellos con registros de distocia o piometra; (b) fetos malformados; c) animales que requieran la administración de inductores o aceleradores del nacimiento; (d) hembras agresivas; (e) una condición corporal superior a 8 (obesidad) según la escala WSAVA (2019); (f) razas braquicéfalas que se sabe que tienen una alta incidencia de distocia; y (g) someterse a una cesárea de emergencia. Los rangos de peso corporal se basaron en la Federación Cinológica Internacional (FCI, 2019). Se excluyeron del estudio los mortinatos tipo I (SB) y sólo se incluyeron los mortinatos tipo II, clasificados por necropsia. Según Mota-Rojas *et al.* (Mota-Rojas *et al*. 2005; 2006) los SB se pueden clasificar en: tipo I, fetos que mueren antes del final de la gestación por causas infecciosas. El aspecto de los fetos es hemorrágico, edematoso y con una coloración marrón grisácea; tipo II, muerte intraparto, es decir, aquellos que mueren durante el parto por asfixia intrauterina y rara vez por enfermedades infecciosas. Los cachorros mantienen una apariencia normal como sus compañeros de camada, pero carecen de respiración.

7.3 Historia clínica

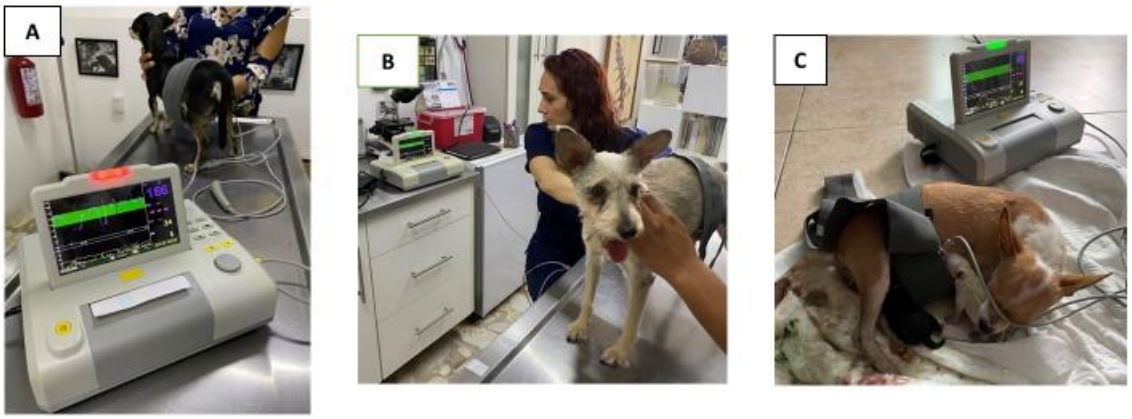
En las clínicas y hospitales donde se realizó el estudio se estandarizó el uso del software veterinario SmartZooft® LAN versión 14 K, desarrollado por SQUENDA®, Ciudad de México, México y se registró la historia clínica. Dichos datos incluían edad, raza, tipo de alimentación, número de parto, peso corporal, historial de medicina preventiva y dirección, así como los datos generales del propietario.

7.4 Procedimientos prenatales

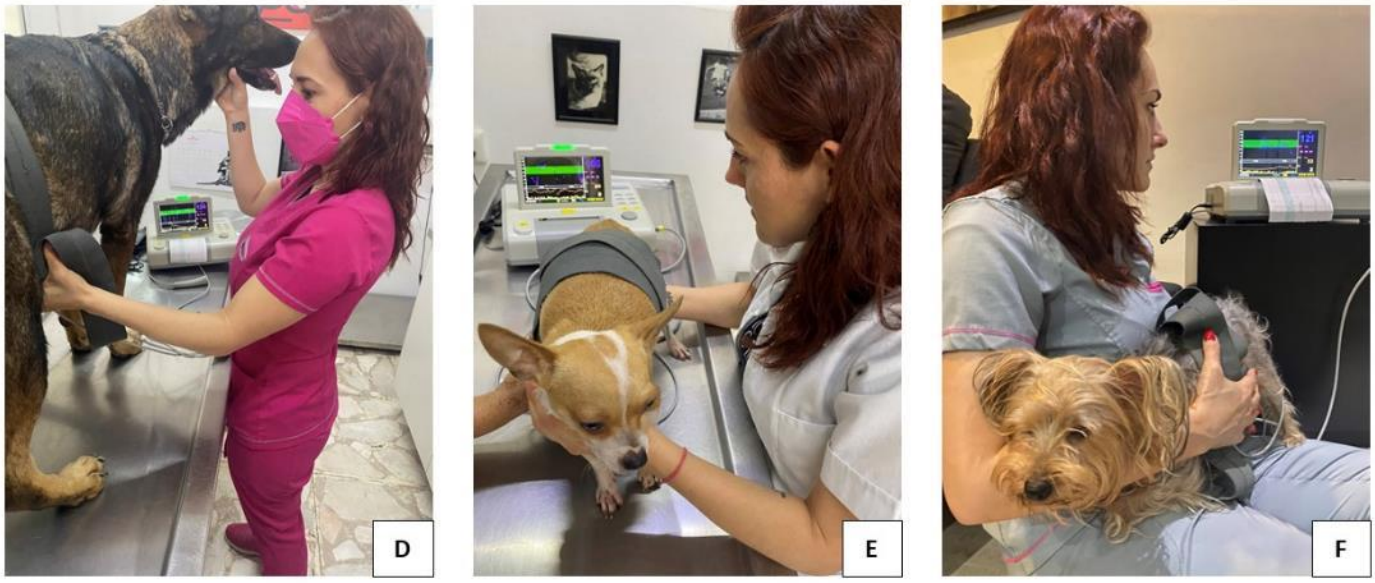
Para realizar el diagnóstico de gestación en las perras, se utilizó un equipo de ultrasonografía Mindray® modelo DP-30VetPower (Shenzhen, China) con Doppler y Doppler Pulsado (PW), utilizando un transductor convexo de 3,5 MHz, desde los días 28 al 30 post apareamiento. La gestación se confirmó visualizando los sacos gestacionales y los latidos cardiacos de los embriones. La siguiente revisión ecográfica fue realizada entre los días 40 y 43 de gestación para verificar el correcto estado de salud, crecimiento de los fetos y vitalidad. Los días 48 a 50 se realizaron radiografías para descartar posible

distocia (por desproporción cefalopélvica) (Eneroth *et al.* 1999), determinar el número de fetos y evaluar el tamaño de sus cabezas.

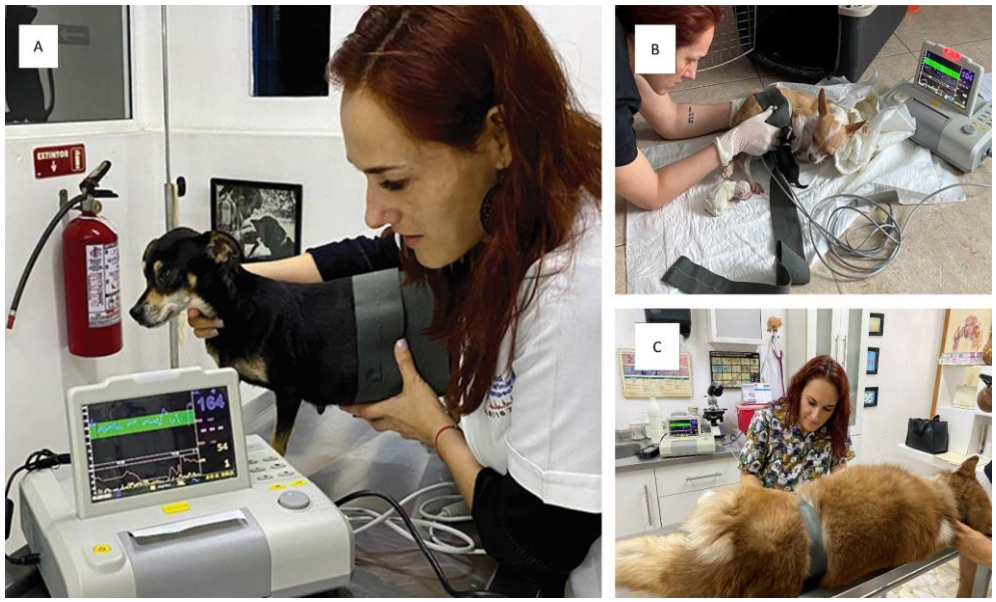
Después del apareamiento, el día 60, los fetos y las perras fueron evaluados mediante un monitor ante parto marca Sonolife® (Chihuahua, México), modelo Smart Monitor Color, con un transductor Doppler pulsado multicristal. El monitor evaluó el estado de salud tanto de la perra como de los fetos, la FCF y la actividad uterina, incluyendo el número, duración, intervalo y frecuencia de las contracciones, siguiendo una metodología previamente reportada en lechones por otros autores (Kammersgaard et al. 2013) **(Figuras 10A, 10B, 10C; Figuras 11A, 11B, 11C; Figuras 12A, 12B, 12C)**. Cabe mencionar que el inicio de la evaluación de la dinámica uterina se realizó a partir del momento en que se pudieron observar las membranas corioalantoideas en la vulva, momento que se consideró el inicio de la fase de expulsión del parto y se monitoreó por primera vez durante 60 min.



Figuras 10A. Monitorización electrónica uterina y fetal con cardiotocógrafo mediante monitor ante parto Sonolife® en una perra multípara de pequeño tamaño. **10B**. Monitorización uterina y fetal en perra de talla mediana, **10C**. Monitorización electrónica uterina y fetal durante el parto en una perra multípara de tamaño pequeño. En los tres casos se puede monitorear la FCF, el movimiento fetal y las contracciones uterinas.



Figuras 11A, 11B, 11C. Monitorización fetal y uterina electrónica con CTG en perras primíparas y multíparas de talla grande y pequeña. En estas imágenes se puede observar cómo al ser una técnica no invasiva ni dolorosa, las perras colaboran ampliamente con el veterinario para la colocación de las bandas ajustables que sujetan el tocodinamómetro y el transductor a la piel de la zona abdominal. En la figura 2A se monitorea a una perra Pastor Belga, en la figura 2B a una Chihuahua, y en la figura 2C a una Yorkshire Terrier.



Figuras 12A, 12B, 12C. Monitorización fetal electrónica con cardiotocógrafo antes y durante el parto en perras de talla pequeña y grande, en donde se puede monitorizar la FCF, el movimiento fetal y las contracciones uterinas, tanto en el monitor, como impresas en papel. Nótese que, al ser un método no invasivo, las pacientes colaboran fácilmente.

También se identificaron desaceleraciones de la FCF DIP 2 (una caída de la FCF que comienza después del inicio de una contracción uterina y regresa a la línea de base solo después de que la contracción

uterina ha finalizado, causada por insuficiencia útero placentaria) para corroborar el grado de asfixia que sufre el feto en el útero. Estos DIP 2 se atribuyen a la oclusión transitoria de los vasos umbilicales debido al útero contraído. Según Vispo *et al*. (2002), la hipoxia fetal también se desarrolla cuando la oclusión es corta y dura menos de 40 segundos. Del mismo modo que Mota-Rojas *et al*. (2005a), consideran DIP 2 desfavorables cuando estos duran más de 60 s y cuando la FCF es inferior a 70 latidos por minuto. Para detectar clínicamente estos DIP 2 desfavorables, se evaluó la FCF antes, durante e inmediatamente después de la contracción del miometrio, y se realizó una observación cuidadosa cuando coincidía con el pico de la contracción. Cuando surgió el DIP 2 se realizó cesárea de emergencia y estas perras fueron excluidas del grupo, por lo que ninguno de los cachorros utilizados para este estudio presentó líquido amniótico teñido de meconio.

La monitorización de los signos vitales de las madres se realizó mediante un monitor veterinario DESEGO® (Ciudad de México, México) modelo M8i SVGA para evaluar los trazos electrocardiográficos, frecuencia respiratoria, saturación de oxígeno, temperatura y presión arterial desde la fecha probable del parto. La FCF se evaluó antes del parto y durante el trabajo de parto utilizando el monitor electrónico uterino y fetal descrito anteriormente.

7.5 Procedimientos neonatales después del nacimiento

La frecuencia cardíaca del recién nacido se evaluó con un estetoscopio pediátrico clásico 3M™ Littmann III-5620 (Canadá). Se consideró bradicardia toda frecuencia cardíaca neonatal inferior a 100 latidos por minuto. Los cachorros se pesaron con una báscula digital (Salter Weight Tronix Ltd., West 148 Bromwich, Reino Unido). La temperatura rectal se midió con un termómetro veterinario digital Hergom-MedicalTM (Monterrey, México). Los cachorros con temperaturas rectales inferiores a 36°C se consideraron hipotérmicos.

7.6 Muestreo sanguíneo y análisis del perfil sanguíneo

Las muestras de sangre de las perras se obtuvieron mediante punción en la almohadilla plantar, por medio de tubos microcapilares impregnados con litio, justo al momento de concluir los 60 minutos de seguimiento electrónico. Las muestras se analizaron mediante GEM PremierTM 3000 (Instrumentation Laboratory Diagnostics, Lexington, KY, EE. UU./Milano, Italia) para obtener los parámetros de glucosa (mg/dL), lactato (mg/dL), pCO_2 (mmHg), pO_2 (mmHg), pH, Valores de HCO_3^- (mmol/L) y Ca^{++} (mmol/L).

7.7 Análisis estadístico

Los análisis se realizaron en R versión 4.2.2 (R Core Team, Viena, Austria) utilizando los paquetes “moments”, “ggpubr”, “stats”, “emmeans” y “multcompView”. El nivel significativo se fijó en $p<0,05$. Los supuestos de normalidad y homocedasticidad del ANOVA se verificaron mediante inspección visual de los residuos del modelo utilizando gráficos Q-Q de normalidad y gráficos de residuos versus valores predichos. Los resultados se presentan como media ± EEM

Para evaluar el desempeño reproductivo de las perras primíparas y multíparas en la fase de expulsión, se aplicó ANOVA de dos factores para el efecto del número de partos y el grupo de peso sobre la duración de la fase de expulsión, el intervalo de expulsión y el peso al nacer del cachorro. Se realizaron comparaciones post-hoc por pares utilizando pruebas Tukey HSD. Las diferencias en la proporción de cachorros nacidos muertos entre perras primíparas y multíparas clasificadas según el peso se analizaron mediante una prueba de chi-cuadrada.

Para evaluar las diferencias en intensidad, duración y número de contracciones entre perras primíparas y multíparas clasificadas según su peso se empleó ANOVA de dos factores. Como los datos para el intervalo entre la contractilidad miometrial no cumplían con los supuestos de normalidad y homocedasticidad, esta variable se analizó utilizando un modelo lineal generalizado (GLM) con distribución familiar "Gamma" y función de enlace de "Identidad". Los predictores fueron los grupos de peso (4 niveles), la paridad (2 niveles) y su interacción bidireccional. Se realizaron comparaciones post-hoc por pares utilizando pruebas Tukey HSD.

Para analizar las variaciones en el perfil fisio metabólico sanguíneo en perras primíparas y multíparas, se utilizó ANOVA de dos factores para todas las variables. Para analizarlo se utilizaron comparaciones post-hoc por pares utilizando pruebas Tukey HSD.

Para investigar el efecto del grupo de peso y la paridad en el número de DIP 2, el número de cachorros con bradicardia AB, el número de cachorros con mucosa oral cianótica y el número de cachorros hipotérmicos y adinámicos por camada (madre), se utilizó GLM con distribución de familia Poisson y función de enlace “log”. Se incluyeron como predictores los grupos de peso (4 niveles), la paridad (2 niveles) y su interacción. El número de cachorros en cada camada (tamaño de camada) se incluyó como variable de compensación para controlar este factor debido a que las camadas más grandes tienden a presentar cachorros con alguna de estas condiciones: bradicardia (menos de 100 latidos por minuto), mucosa oral cianótica, hipotermia. (menos de 36°C) y cachorros adinámicos.

Las diferencias en el peso al nacer y el intervalo de expulsión entre cachorros machos y hembras clasificados según el grupo de peso de la madre se analizaron con modelos ANOVA de dos vías separados. Los predictores fueron el grupo de peso, el sexo del cachorro y su interacción bidireccional. Se realizaron comparaciones post hoc por pares utilizando las pruebas HSD de Tukey.

Se analizó el efecto del sexo del cachorro, el peso al nacer y el intervalo de expulsión sobre la probabilidad de que un cachorro naciera muerto utilizando un modelo de regresión logística binaria. Los predictores fueron el sexo del cachorro (M, F), el peso al nacer (continuo), el intervalo de expulsión (continuo) y las interacciones bidireccionales entre el sexo y el intervalo de expulsión, y el sexo y el peso al nacer. Como la interacción entre el sexo del cachorro y el peso al nacer no fue significativa en el modelo inicial, se eliminó para aumentar el ajuste. La distribución de probabilidad fue “binomial” con una función de enlace “Logit”.

Para determinar si existieron o no correlaciones significativas se utilizaron correlaciones de Pearson para analizarlas, así como también correlaciones de rango de Spearman ya que los datos obtenidos de la dinámica uterina y los DIP2 no presentaba una distribución normal.

7.8 Declaración ética

Antes de realizar el estudio, se brindó un consentimiento informado a los dueños de los animales evaluados, quienes autorizaron la realización de los procedimientos. Todo el trabajo se realizó bajo la Norma Oficial Mexicana NOM-062-ZOO-1999 sobre especificaciones técnicas para animales de laboratorio, cuidado y uso ético en estudios etológicos aplicados (Sherwin *et al.* 2003a). Este proyecto fue aprobado por la Comisión Académica del Doctorado en Ciencias Biológicas y de la Salud con número CBS.114.19. Todas las hembras evaluadas en este estudio fueron tratadas con delicadeza, evitando al máximo el estrés que la manipulación pudiera generar, y el hecho de que el uso de un monitor fetal y uterino electrónico facilitó enormemente este aspecto al ser una técnica no dolorosa ni invasiva.

7.9 Consentimiento informado

A todos los tutores que accedieron a que sus perras y los cachorros recién nacidos fueran evaluados antes, durante y después del parto, en todas las fases experimentales, se les pidió que firmaran un consentimiento informado, el cual está anexo en la siguiente página.

Unidad Veterinaria Campeche

Consentimiento Informado

San Francisco de Campeche a __de_____de_____20__.

El/la que suscribe _____ con identificación oficial o INE no. _____ con domicilio en calle _____ colonia _____, municipio_____ teléfono _____, propietario/a de la mascota:

Nombre: _____

Especie:_____

Sexo: _____

Raza: _____ Peso: _____

Edad: _____

Expongo que he sido debidamente INFORMADO/A, por el profesional Médico Veterinario Zootecnista _____, en entrevista personal y consulta previa de mi mascota realizada con fecha _____, de que formará parte del estudio llevado a cabo para el proyecto Doctoral para la evaluación del "Efecto del peso de las madres en los cambios microcirculatorios y en la temperatura de sus cachorros a lo largo de diferentes etapas post-parto" de acuerdo con el protocolo diagnóstico descrito más adelante.

He recibido explicaciones tanto verbales como escritas, sobre la naturaleza y los propósitos del procedimiento, beneficios, riesgos, criterios de inclusión, de exclusión, alternativas y medios con los que cuenta la clínica para su realización, teniendo oportunidad de aclarar las dudas que me han surgido.

PROTOCOLO

Se realizarán los siguientes procedimientos para la madre:

- Primera consulta de acuerdo a los criterios de inclusión y registro en el programa SmartZooft® SQUENDA.

- Primer estudio ultrasonográfico para diagnóstico de gestación en los días 28-30 post servicio.

- Segundo estudio ultrasonográfico para valoración de crecimiento fetal y estimación de fecha estimada de parto (día 40-43 de gestación).

- Estudio radiográfico para valoración de diámetros cefalopélvicos materno-fetal (día 48-50 de gestación)

- Monitoreo fetal y electrónico por medio de un monitor fetal a partir del día 60 de gestación.

Se realizarán los siguientes procedimientos al recién nacido:

- Toma del peso al nacer

- Valoración de la escala Apgar para neonatos caninos (Frecuencia cardíaca, respiratoria, color de membranas mucosas, reflejo de irritabilidad y movilidad).

- El secado de los cachorros recién nacidos con toallas de trapo.

- Toma de temperatura corporal por termografía infrarroja (método no invasivo), en diferentes tiempos y zonas.

Nota: En caso de que la mascota durante el proceso de estudio sea excluida debido a los diagnósticos previos, NO se negará la atención necesaria para salvaguardar su integridad y la de sus crías. Debiendo firmar el consentimiento de Autorización de anestesia/Autorización de cirugía/Autorización de toma de muestras de laboratorio, que el medico en turno solicite para brindar atención medica necesaria.

Conociendo lo anterior, presta su conformidad y autoriza a _____, y a quien éste designe, para poder proceder con las maniobras detalladas, al animal cuyos datos han sido especificados precedentemente, para realizar todos los procedimientos destinados a procurar salvaguardar la vida del animal y/o procurar mejorar y/o recuperar la salud del mismo.

Asimismo, MANIFIESTO que he entendido y estoy satisfecho de todas las explicaciones y aclaraciones recibidas sobre el proceso médico citado y OTORGO MI CONSENTIMIENTO para que _____ participe en este estudio. Por tal motivo, dejo constancia y acepto en forma irrevocable.

Certifica con su firma que ha leído y comprendido la presente autorización, prestando su consentimiento.

_____ _____

Nombre y Firma del Propietario Nombre y firma del MVZ

Fase 2. Evaluación de la vitalidad, perfil sanguíneo y grado de tinción de meconio en la piel en cachorros recién nacidos clasificados de acuerdo con su peso al nacer

2.1. Instalaciones

Este estudio se desarrolló en las instalaciones de 10 hospitales veterinarios del municipio de Campeche, Estado de Campeche, México, donde existe un clima tropical con una temperatura entre 36 y 40°C. La región de estudio se ubica en el sureste de México, en la península de Yucatán, limitando al norte y noreste con Yucatán, al este con Quintana Roo, al sur con Guatemala y Belice, al oeste con el Golfo de México y al suroeste con Tabasco. Para realizar este estudio se solicitó a los tutores de perras gestantes su colaboración. Las perras recibieron atención médica y seguimiento desde el día 25 de gestación hasta las primeras 48 horas después del nacimiento del cachorro. Todos los nacimientos tuvieron lugar en las clínicas. Una vez que se calculó la fecha probable del parto, algunas perras permanecieron resguardadas en las clínicas, o los tutores las llevaron a las clínicas cuando empezaron a notar cambios en el comportamiento de las perras. Los tutores no participaron durante el parto; solo lo asistió y monitorizó el personal veterinario.

2.2. Población de estudio

Se reclutaron 435 cachorros de 85 perras parturientas, los cuales fueron divididos en cuatro grupos clasificados en cuartiles, siguiendo la metodología utilizada previamente por Mugnier *et al.* (Mugnier *et al.* 2019) y Tesi *et al*. (Tesi *et al.* 2020a). El primer cuartil (Q_1) representa el 25% de los valores de peso más bajos, el segundo cuartil (Q_2) representa entre el 25% y el 50%, el tercer cuartil (Q_3) representa 50-75%, y el cuarto cuartil representa 75-100% (Q_4). Los animales del grupo Q_1 fueron considerados cachorros de bajo peso, mientras que los pertenecientes al Q_4 fueron considerados cachorros de alto peso. Esta clasificación se empleó debido a la gran variedad de razas de perros existentes, que van desde los Chihuahuas que pesan 500 g en la edad adulta, hasta Mastines que pueden alcanzar hasta 100 kg (Boyko *et al.* 2010). Las razas incluidas en este estudio fueron Chihuahua, Yorkshire Terrier, Poodle, Scottish Terrier, Cocker Spaniel, Schnauzer Estándar, Pastor Alemán, Labrador, Golden Retriever, Gran Danés y Pastor Belga. Los cuartiles se calcularon con la siguiente fórmula: Qa = Li ((aN/4 + Fi-1)/Fi) Ai, donde Li es el límite inferior de la clase donde el cuartil se encuentra, N es la suma de las frecuencias absolutas, Fi-1 es la frecuencia acumulada de la clase anterior, y Ai es la amplitud de la clase, es decir, el número de valores contenida en el intervalo. Los grupos se dividieron entonces de la siguiente manera: Q_1 (127–200 g) *n*=110 cachorros, Q_2 (201–269 g) *n*=108 cachorros, Q_3 (270–388 g) *n*=108 cachorros y Q_4 (389–464 g) *n*=109 cachorros.

Los criterios de inclusión fueron los mismos utilizados en estudios previos de Reyes-Sotelo *et al.* (2021, 2022) y Lezama-García *et al.* (2022a, 2022b). Se utilizó la clasificación mortinatos en lechones tipo I y tipo II de Mota-Rojas et al. (2005b, 2006a) para definir cuáles cachorros podrían considerarse para el estudio y cuáles no. Según Mota-Rojas *et al.* (2005c, 2006a), los mortinatos se pueden clasificar en dos tipos: el tipo I, también conocido como muertos preparto o anteparto, incluye fetos que mueren antes del final de la gestación, generalmente de causas infecciosas, con un patrón hemorrágico bastante característico y apariencia edematosa con una decoloración marrón grisácea; y los mortinatos tipo II, que son también conocidos como muertos intraparto, los cuales pueden morir durante el parto, generalmente por muerte intrauterina, por asfixia y rara vez por enfermedades infecciosas, y tienen la misma apariencia de sus compañeros de camada normales, pero no respiran. Los mortinatos de tipo I fueron excluidos del estudio y solo se incluyeron mortinatos tipo II, clasificados por necropsia.

Los cachorros se pesaron utilizando una báscula digital de Salter Weight Tronix Ltd., West Bromwich, Reino Unido, inmediatamente después de que la perra dejara de lamer y limpiar los fluidos amnióticos y membranas placentarias.

2.3. Muestreo sanguíneo

Un veterinario tomó muestras de sangre cuando la perra terminó de retirar las membranas

corioalantoideas y cuando fue necesario, un asistente sostenía al cachorro en posición supina para exponer la región abdominal. Cuando los cachorros lo permitieron, se colocaban en decúbito lateral sobre un pañal de adulto para realizar el muestreo, obteniéndose sangre venosa (0.3 ml) del cordón umbilical con una jeringa de tuberculina impregnada con litio y heparina. Todas las muestras (150 µL) se procesaron utilizando un analizador GEM Premier® de variables sanguíneas críticas (Instrumentation Laboratory Diagnostics, Lexington, KY, Estados Unidos/Milán, Italia). Los metabolitos analizados fueron glucosa (mg/dL), lactato (mg/dL), gases en sangre pCO_2 (mmHg), pO_2 (mmHg) pH, HCO_3^- (mmol/L), EB (mEq/L), Ca^{++} (mmol/L) y hematocrito (Htc%).

2.4. Grado de tinción de meconio en la piel

Para evaluar el grado de tinción de meconio en la piel de los cachorros, éstos se clasificaron según la metodología de Mota-Rojas *et al.* (2006a) en ausente, leve, moderado y grave. Es importante resaltar que el proceso de reanimación del recién nacido por parte de la madre no fue interrumpido, ya que los cachorros fueron tomados justo después de que la perra terminara de retirar las membranas corioalantoideas para evitar que la perra lamiera la tinción de meconio. Para determinar el grado de tinción de meconio en los cachorros de color oscuro se utilizó un pañal de adulto color blanco para impregnarlo con los fluidos del cachorro recién nacido presionándolo sobre el cuerpo del recién nacido para observar más claramente el color impregnado **(Figura 13** y **14**) y una vez impregnada la mancha en el paño blanco, el cachorro fue devuelto a la madre para que pudiera continuar con los lamidos y cuidado materno, al igual que en el estudio realizado por Mota-Rojas *et al.* (2002a).

Los cachorros nacidos libres de tinción de meconio en la piel se consideraron ausentes de tinción, se consideró grado leve cuando el cuerpo estaba cubierto en menos del 30% de su superficie, grado moderado entre un 30 y 60%, y grado severo cuando su cuerpo estaba cubierto en más del 60%. Como se menciona anteriormente, se utilizó un pañal de cama de humano color blanco para identificar el grado de tinción de meconio en los animales de color oscuro.

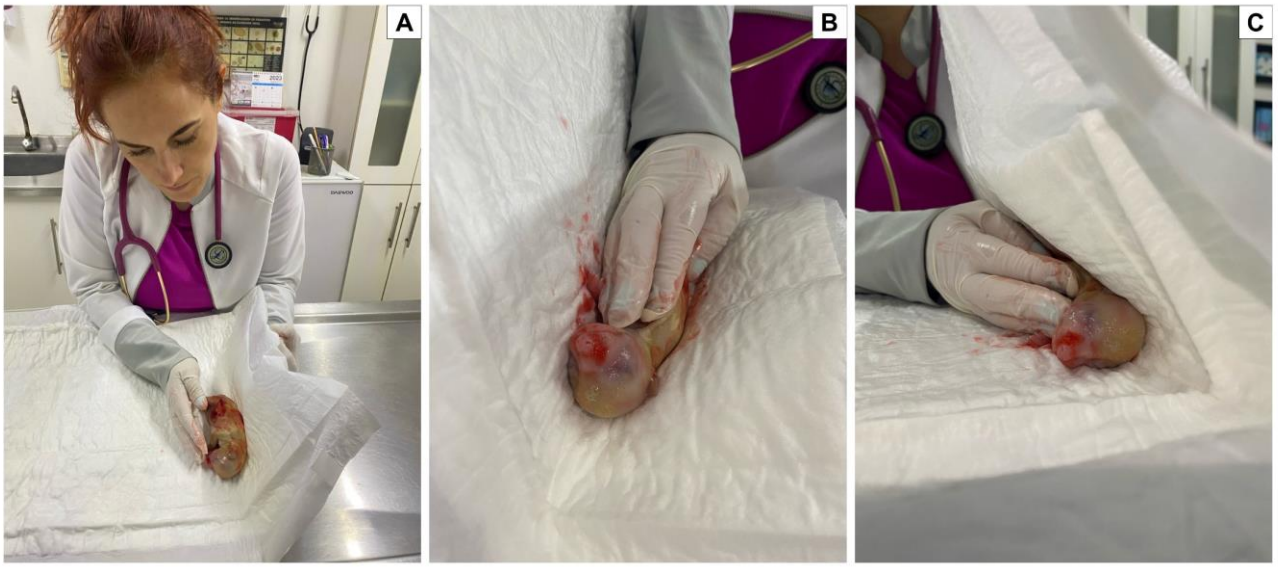


Figura 13. Metodología utilizada para evaluar el grado de tinción de meconio en cachorros. **(A):** El cachorro fue recogido suavemente antes de que la perra comenzara a lamerlo para evitar que extrajera el meconio y la tinción si estuviera presente. **(B, C):** El cachorro fue colocado en un pañal blanco y rodeado con él para impregnar la tinción de meconio.

Figura 14. Diferentes grados de tinción de meconio en la piel. (A): Ausente. (B): Ligero. (C): Moderado. (D): Severo.

2.5. Puntuación de vitalidad

Para la evaluación de la vitalidad en los recién nacidos se utilizó la escala Apgar modificada por Veronesi (2009) con la adaptación de Randall (1971) y Mota Rojas *et al*. (2005). Las variables medidas en el primer minuto después del nacimiento fueron el esfuerzo a respirar (sin llanto/< 6 frecuencias respiratorias (rpm), llanto leve/6 a 15 rpm y llanto/>15 rpm; motilidad (flácida, algunas flexiones y movimiento activo); frecuencia cardíaca (latidos por minuto): <180, entre sin llanto/< 6 frecuencias respiratorias (rpm), llanto leve/6 a 15 rpm y llanto/>15 rpm); motilidad (flácido, algunas flexiones y movimiento activo); frecuencia cardíaca (latidos por minuto): <180, entre 180 y 220, y >220; color de las mucosas (cianótico, pálido y rosado). Además, la tinción de meconio en la piel se clasificó como grave, moderada, leve o ausente según la metodología utilizada antes en cerdos por Mota-Rojas *et al.* (Mota-Rojas *et al*. 2005), esto con el fin de observar si había correlaciones positivas o negativas entre la vitalidad y el grado de tinción de meconio en la piel. La puntuación de vitalidad fue 0 (la menos favorable) 2 (la más favorable), y se obtuvo una puntuación global que oscila entre 1 y 10 para cada cachorro recién nacido **(Figura 15).** Una puntuación de vitalidad de 6 o menos se consideró reprobatoria, 180 y 220, y >220; color de las mucosas (cianótico, pálido y rosado).

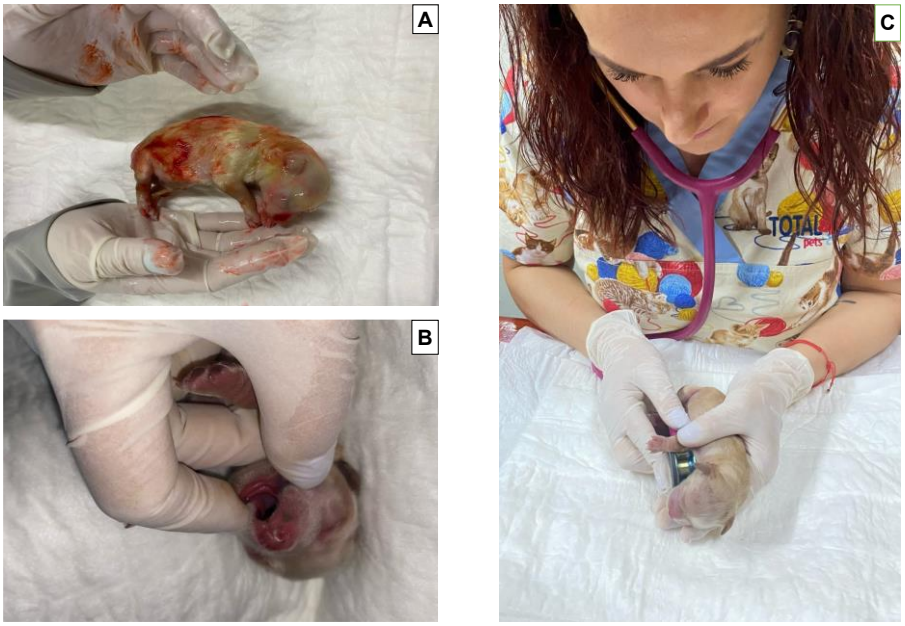


Figura 15. Evaluación de la vitalidad al primer minuto de vida del cachorro. **15A:** registro de la frecuencia respiratoria y motilidad; **15B:** evaluación del color de las mucosas; **15C**: monitorización de la frecuencia cardiaca.

2.6. Análisis estadístico

Los análisis estadísticos se realizaron en R versión 4.2.2 (R Core Team, Viena, Austria) usando los paquetes “moments”, “ggpubr”, “stats”, “emmeans” y “multcompView”. El nivel de significancia se fijó en $p < 0,05$. El efecto del peso del cachorro sobre las variables del perfil sanguíneo se analizó mediante ANOVA unidireccional independiente con los cuatro grupos de peso del cachorro (cuartiles) como predictores categóricos. La normalidad de las variables dependientes del perfil sanguíneo se evaluó mediante una inspección visual del histograma, gráficos Q-Q y la asimetría. Las comparaciones entre cuartiles se realizaron mediante pruebas Tukey HSD. El efecto del peso del cachorro en las puntuaciones de vitalidad en diferentes momentos (1 min después del nacimiento, 5 min después del nacimiento, 60 min después del nacimiento) se evaluó utilizando un modelo lineal mixto con la puntuación de la vitalidad establecida como variable. Las diferencias entre la proporción de mortinatos agrupados según el peso al nacer (cuartiles) y la presencia de tinción de meconio en la piel se evaluaron mediante una prueba de chi-cuadrada.

Fase 3. Relación entre el peso de la madre y la temperatura superficial de sus cachorros en diferentes etapas del posparto

3.1. Instalaciones

Se emplearon las mismas instalaciones mencionadas en el apartado 2.1 de la fase 2.

3.2. Población de estudio

El estudio se realizó en la ciudad de Campeche, Campeche, México, en la Península de Yucatán. Se reclutaron 72 perras multíparas jóvenes gestantes (2 a 4 partos). Sin embargo, 12 perras fueron excluidas debido a que requirieron una cesárea de emergencia durante el parto. En total, en el presente estudio se incluyeron 290 cachorros de 60 perras parturientas. Dentro de las razas incluidas en este estudio se registraron Chihuahua, Pastor Alemán, Labrador, Golden Retriever, Gran Danés, Schnauzer Estándar, Cocker Spaniel, Poodle, Terrier Escocés y Pastor Belga. Las 60 perras se dividieron en 4 grupos de 15 perras cada uno, según su peso corporal de la siguiente manera: G_1 (4 a 8 kg) *n*=47 cachorros, G_2 (8,1 a 16 kg) *n*=68 cachorros, G_3 (16,1 a 32 kg) *n*=79 cachorros, y G_4 (32,1 a 39,6 kg) *n*=96 cachorros. Se monitorizó la condición obstétrica de las perras, así como la gestación desde el inicio hasta el término (desde el día 28 al día 30 después del apareamiento), como se informa más adelante en la sección de procedimiento prenatal.

El peso corporal de las perras se obtuvo utilizando una báscula digital de Salter Weight Tronix Ltd., West Bromwich, Reino Unido, inmediatamente en la primera etapa del parto, cuando comenzaron las contracciones. Los criterios de inclusión fueron: (a) perros clínicamente sanos con registros válidos de vacunación/desparasitación; (b) no haber presentado previamente problemas reproductivos; y (c) perras con estudios ultrasonográficos y radiográficos para sustentar un parto natural. Los criterios de exclusión fueron: (a) perras con casos previos de distocia o piometra; (b) perras primíparas; (c) fetos malformados; (d) perras que hayan requerido la administración de inductores o aceleradores del parto; (e) perras agresivas; (f) perras con una condición corporal superior a 8 (obesas) según la escala WSAVA (2019); (g) cualquier raza grande y perras braquicéfalas debido a su alta incidencia de distocia; y (h) aquellas que requirieron una cesárea de emergencia. Los rangos de peso corporal se basaron en los proporcionados por la Federación Cinológica Internacional (FCI, 2019).

3.3. Historia clínica

La historia clínica de los animales muestreados se realizó mediante la recopilación de datos como edad, tipo de dieta, número de parto, peso corporal, raza, historial de medicina preventiva y descripción del lugar donde vivían. Estos datos fueron recolectados con metodologías de diagnóstico y seguimiento controladas por el software veterinario SmartZooft® LAN, versión 14 K, desarrollado por SQUENDA®, Ciudad de México, México.

3.4. Procedimientos prenatales

El diagnóstico de gestación se realizó entre los días 28 al 30 post monta en las perras, utilizando un equipo de ultrasonografía Mindray® modelo DP-30VetPower (Shenzhen, China) con tecnologías avanzadas Doppler y Doppler Pulsado (PW), equipado con un transductor convexo de 3,5 MHz. La gestación se corroboró visualizando los sacos gestacionales y la presencia de latido cardíaco en los embriones. La siguiente evaluación ultrasonográfica se realizó entre los días 40 y 43 de gestación para determinar la viabilidad de los fetos, su crecimiento y estado de salud. Posteriormente se realizaron estudios radiográficos entre los días 48 y 50 para determinar el número de fetos y el tamaño de sus cabezas, para poder realizar mediciones y predecir si podría haber un parto distócico, que incluso podría terminar en cesárea por alguna desproporción cefalopélvica (Eneroth *et al.* 1999). A partir del día 60 post monta, las perras y fetos fueron evaluados mediante un monitor fetal electrónico marca Sonolife® (Chihuahua, México), modelo Smart Monitor Color, con un transductor Doppler pulsado multicristal para evaluar el estado de salud, tanto de la madre como de los fetos, así como la actividad uterina, número, duración, intervalo y frecuencia de las contracciones, y la FCF, siguiendo una metodología previamente reportada en lechones por otros autores (Kammersgaard *et al.* 2013). En los casos en los que surgieron

desaceleraciones de la FCF tipo 2 (DIP 2) (una caída en la FCF que comienza después del inicio de una contracción uterina y regresa a la línea basal solo después de que la contracción uterina ha finalizado, causada por insuficiencia uteroplacentaria), se realizó cesárea de emergencia y estas perras fueron excluidas del grupo, por lo que ninguno de los cachorros utilizados para este estudio presentó líquido amniótico teñido de meconio. De igual manera, se realizó la monitorización de los signos vitales de las madres mediante un monitor veterinario DESEGO® (Ciudad de México, México) modelo M8i SVGA para evaluar los trazados electrocardiográficos, frecuencia respiratoria, saturación de oxígeno, temperatura y presión arterial de las madres, así como la fecha probable del parto. Sin embargo, las perras solo fueron hospitalizadas en los casos en que la atención del parto tuvo lugar en instalaciones hospitalarias/clínicas y no cuando el parto tuvo lugar en el domicilio de las perras.

3.5. Cachorros

- Una vez iniciado el parto, 290 cachorros fueron evaluados al momento de ser expulsados y cuando la perra comenzó a lamerlos para separarlos de las membranas. La temperatura de la habitación donde las perras parieron no fue controlada porque el estudio se realizó en diferentes clínicas y hospitales. Sin embargo, en la ciudad donde se realizó el estudio el clima es tropical y las temperaturas oscilan entre 36 y 40°C. En todos los casos, cuando comenzó el parto, se apagaron los aires acondicionados y ventiladores. Las mediciones de temperatura en los cachorros se registraron en 7 etapas diferentes: (1) cachorro húmedo con líquido amniótico, una vez que la madre lo liberó de las membranas y dejó de lamerlo temporalmente; (2) cachorro seco, el cual se secó frotando durante 1 min con toallas de trapo e inmediatamente después se devolvió el cachorro a la zona de la glándula mamaria de la perra, y hasta que el cachorro hizo contacto con el pezón por sí solo; (3) calostrado, inmediatamente después de que el cachorro ingirió calostro y se separó del pezón de su madre; (4) a los 30 min de nacimiento (30 min AB); (5) en la primera hora del nacimiento (1 h AB); (6) a las 4 h después del nacimiento (4 h AB); y (7) a las 24 h después del nacimiento (24 h AB). Cabe mencionar que todas las temperaturas se obtuvieron sin manipular a los cachorros excepto para secarlos, pesarlos y evaluar su vitalidad.

Finalmente, el peso de los cachorros al nacer se obtuvo mediante una balanza digital de Salter Weight Tronix Ltd., West Bromwich, Reino Unido, luego de secarlos. El peso de los cachorros se obtuvo una sola vez cuando la perra dejó de lamer las membranas placentarias. Se evaluaron todos los cachorros de cada camada. Para su identificación utilizamos un marcador de tinta indeleble de secado rápido. Cabe mencionar que los cachorros solo fueron alimentados con leche de la madre y no fueron suplementados con fórmulas lácteas adicionales, y el cachorro fue devuelto a la zona de la glándula mamaria para que comenzara a mamar por sí solo.

3.6. Termografía infrarroja

Se evaluaron un total de 16,240 datos termográficos con sus valores mínimo, medio y máximo. Estos datos resultaron de 290 cachorros, a los cuales se les tomaron 3 termogramas: uno de la zona facial, otro de la zona lateral izquierda y otro de la región lateral derecha. El termograma facial incluyó las extremidades torácicas no solo para registrar la temperatura nasal (N) y palpebral superior izquierda (PSI), sino también para tener una imagen frontal de la ventana térmica miembro torácico metacarpo (MTM). Asimismo, el termograma tomado de la región lateral derecha registró la ventana miembro torácico codo (MTC) y el miembro pélvico femoral (MPF). Los registros de temperatura del tórax y abdomen se obtuvieron de la imagen lateral izquierda. En cada cachorro se identificaron 8 ventanas térmicas en 7 momentos diferentes, las cuales se explican en detalle en la **Figura 16**; las ventanas del miembro torácico bíceps braquial (MTBB) y del miembro torácico metacarpo (MTM) se obtuvieron desde el área donde comienza la axila hasta la mitad del ancho del miembro torácico y hasta la articulación formada por los metacarpianos, cubriendo el área desde el extremo medial al lateral, respectivamente. Las ventanas del miembro torácico codo (MTC) y del miembro pélvico femoral (MPF) se delimitaron desde el área del codo que cubre el vértice formado por la articulación húmero-radio-cubital hasta el espacio delimitado por el borde del miembro pélvico en la región del bíceps femoral, respectivamente. La ventana torácica (T) quedó delimitada por la zona axilar, la posición anatómica de la última costilla y

desde la región de las vértebras espinales hasta la parte ventral de la región abdominal (A). Esta ventana estaba delimitada dos milímetros después de la última costilla hasta la zona inguinal, y desde la región de las vértebras espinales hasta la parte ventral del abdomen. La ventana nasal (N) y palpebral superior izquierda (PSI) estaban delimitadas por los bordes de la mucosa nasal y en el borde del párpado superior izquierdo, respectivamente.

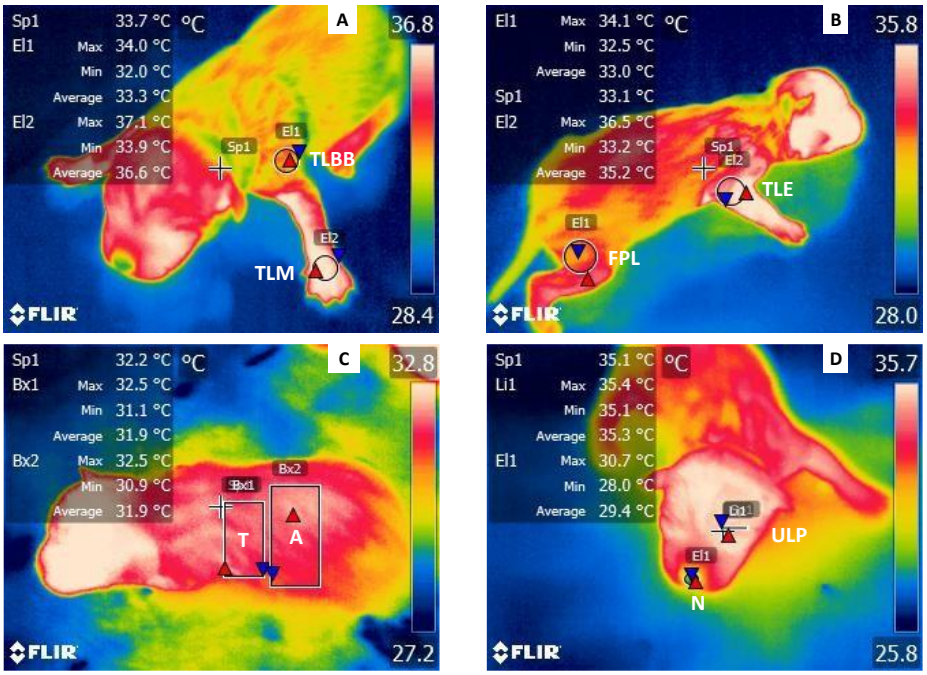


Figura 16. Ventanas térmicas en cachorros recién nacidos. (A) Las ventanas del miembro torácico bíceps braquial (MTBB) y del miembro torácico metacarpos (MTM) se obtuvieron colocando figuras circulares desde el área donde comienza la axila hasta la mitad del ancho del miembro torácico y en la articulación formada por los metacarpianos que cubren el área desde el extremo medial al lateral, respectivamente. (B) Las ventanas del codo del miembro torácico (TLE) y del miembro pélvico femoral (MPF) se obtuvieron colocando figuras circulares en la zona del codo cubriendo el vértice formado por la articulación húmero-radio-cubital y en el espacio delimitado por el borde de la pelvis extremidad en la región del bíceps femoral, respectivamente. (C) La ventana torácica (T) se realizó con figuras rectangulares delimitadas por la zona axilar, la zona de la última costilla, y desde la región de las vértebras espinales y hasta la parte ventral de la región abdominal (A) la ventana se delimitó por dos milímetros después de la última costilla, hasta la zona inguinal, y desde la región de las vértebras espinales, hasta la parte ventral del abdomen. (D) La ventana nasal (N) y palpebral superior izquierda (PSI) se realizaron con figuras circulares delimitadas por los bordes de la mucosa nasal y en la zona del borde del párpado superior izquierdo, respectivamente. Sp1: Punto 1; El1: Elipse 1; El2: Elipse 2; Bx1: Caja 1; Bx2: Caja 2; Li1: Línea 1; triángulos rojos temperatura máxima de esa zona; Temperatura mínima de los triángulos azules de esa zona.

Las imágenes termográficas se obtuvieron con una cámara infrarroja FLIR® modelo Thermal TM E80, FLIR Systems, Wilsonville, OR, EE. UU., con las siguientes especificaciones: resolución IR 320 240 píxeles, sensibilidad térmica < 0,045°C, precisión 2°C o 2 % de lectura en temperatura ambiente de 10°C a 35°C y frecuencia de imagen de 60 Hz. Todas las imágenes se recogieron con una emisividad de 0,95 a una distancia uniforme de 30 cm. Se tomaron imágenes termográficas para evaluar 8 zonas

diferentes: (1) miembro torácico bíceps braquial (MTBB); (2) miembro torácico codo (MTC); (3) miembro torácico metacarpos (MTM); (4) miembro pélvico femoral (MPF); (5) torácica (T); (6) abdominal (A); (7) nasal (N); y (8) párpado superior izquierdo (PSI). Las imágenes termográficas se guardaron en formato JPEG para analizarlas posteriormente utilizando el software especializado FLIR Tools® 6.x (FLIR Systems, Wilsonville, OR, EE. UU.). La temperatura máxima, mínima y media de cada ventana térmica se obtuvo en cada una de las 7 diferentes etapas. Es importante mencionar que para evitar el contacto directo con el piso donde fue expulsado el cachorro, se utilizó un tapete termo endurecible a base de gomaespuma (acetato de etil vinilo) con una superficie de 1 m^2, 1 cm de profundidad, con un peso de 0,032 kg, y en todos los casos se utilizó acabado mate.

3.7. Análisis estadístico

Se obtuvieron estadísticas descriptivas para todas las variables examinadas siguiendo el procedimiento descrito en el Análisis de Varianza de dos factores (ANOVA StatSoft Inc. 0.8, Tulsa, Oakland, CA, EE. UU.) para comparar el efecto de los grupos de diferente peso corporal de la perra (4 categorías: G_1, G_2, G_3 y G_4) y tiempo (7 categorías: húmedo, seco, calostrado, 30 min, 1 h, 4 h y 24 h) por ocho zonas diferentes (MTBB, MTM, MTC, MPF, T, A, N y PSI). Además, se utilizó un ANOVA de tres factores para comparar los efectos del peso corporal de la madre (4 categorías: G1, G_2, G_3 y G_4), el tiempo (7 categorías: húmedo, seco, calostrado, 30 min, 1 h, 4 h, y 24 h) y zonas (8 categorías: MTBB, MTM, MTC, MPF, T, A, N y PSI). La prueba de Tukey (p<0,05) mostró contraste de medias. Se utilizó la prueba de rangos de Spearman para establecer la correlación entre las variables, las temperaturas y los pesos de las madres.

2.8. Declaración ética

A todos los responsables de los animales de estudio se les solicitó la firma de su consentimiento informado para realizar los procedimientos. Todo el trabajo se realizó bajo los lineamientos de la Norma Oficial de México NOM-062-ZOO-1999 sobre especificaciones técnicas para la producción, cuidado y uso ético de los animales de laboratorio en estudios etológicos aplicados (Sherwin *et al.* 2003b). La Comisión Académica del Doctorado en Ciencias Biológicas y de la Salud autorizó este proyecto con el número de aprobación CBS.114.19. Los animales incluidos en el presente estudio fueron tratados con delicadeza y no fueron tocados ni estresados, ya que la termografía infrarroja es una técnica no invasiva. La única vez que se manipuló a los cachorros fue cuando terminaron de secarse y en ese momento fueron pesados y se evaluó su vitalidad, procedimiento que no tomó más de 2 min.

Fase 4. Relación entre el peso del cachorro recién nacido y su equilibrio térmico.

Para la realización de esta fase experimental los materiales y métodos fueron los mismos utilizados para la fase 3, lo único que cambió fue la población de estudio.

4.1. Población de estudio

El estudio fue realizado en la Ciudad de Campeche, municipio de Campeche, en la Península de Yucatán. En total, se reclutaron 289 cachorros de 60 perras parturientas y se dividieron en 4 grupos o cuartiles, siguiendo la metodología utilizada por Mugnier *et al.* (2019) y Tesi *et al*. (2020b). El primer cuartil (Q_1) representa el 25% más bajo de los valores registrados, el segundo (Q_2) representa entre el 25% y el 50%, el tercer cuartil (Q_3) representa entre el 50% y el 75% y el cuarto el cuartil (Q_4) representa entre el 75% y el 100%. Los animales del grupo Q_1 se consideraron de bajo peso, mientras que los pertenecientes al Q_4, fueron considerados cachorros de alto peso. Esta clasificación se debe a la gran variedad de tamaños entre las razas de perros y la mayor variabilidad morfológica dentro de cualquier especie de mamífero terrestre. En este sentido, en perros podemos encontrar pesos corporales adultos que oscilan entre los 500 g, en razas miniatura, como los Chihuahuas, hasta más de 100 kg en razas gigantes, como los Mastines (Boyko *et al*. 2012). Entre las razas incluidos en este estudio fueron Chihuahua, Pastor Alemán, Labrador, Golden Retriever, Gran Danés, Schnauzer Estándar, Cocker Spaniel, Poodle, Terrier Escocés y Pastor Belga.

Como resultado de esta considerable variación en los pesos corporales, se debe analizar el peso al nacer según el tamaño de la raza. Los cuartiles se calcularon de acuerdo con esta fórmula: Qa = Li ((aN/4 + Fi-1)/Fi) Ai, donde Li es el límite inferior de la clase donde el cuartil se encuentra, N es la suma de las frecuencias absolutas, Fi-1 es la frecuencia acumulada de la clase anterior, y Ai es la amplitud de la clase, es decir, el número de valores contenida en el intervalo. Los grupos fueron Q_1 (126–226 g) *n*=73 cachorros, Q_2 (227–330 g) *n*=72 cachorros, Q_3 (331–387 g) *n*=74 cachorros y Q_4 (388–452 g) *n*=70 cachorros.

El peso de los cachorros se obtuvo mediante una báscula digital de Salter Weight Tronix. Ltd., West Bromwich, Reino Unido, tan pronto como la madre dejó de lamer y limpiar el líquido amniótico y las membranas placentarias de ellos.

8. RESULTADOS

CAPÍTULO 3.

Uterine dynamics, blood profile, and electronic fetal monitoring in primiparous and multiparous bitches classified according to their weight.

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Uterine dynamics, blood profiles, and electronic fetal monitoring of primiparous and multiparous bitches classified according to their weight

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Perinatal mortality occurs in all species. In dogs, mortality rates have been reported to range from 5 to 35%. Electronic fetal and uterine monitoring has recently been used in domestic animals to monitor the mother and newborn before and during parturition. In this way, the fetal heart rate and uterine dynamics can be monitored. This study evaluated the uterine dynamics of bitches with different weights and parity. Ninety-six bitches and their 476 puppies were divided into four experimental groups containing 24 individuals each (12 primiparous bitches and 12 multiparous bitches), according to body weight: G_1 (4–8 kg), G_2 (8.1–16 kg), G_3 (16.1 to 32 kg), and G_4 (32.1 to 39.6 kg). The fetal heart rate decelerations (dip 2 patterns), uterine dynamics, and bitches' blood profiles were evaluated, including levels of glucose, lactate, pCO_2, pO_2, pH, HCO_3^-, and Ca^{++}. The dam weight can affect the vitality of newborns and the uterine dynamics, with differences in the frequency, intensity, and duration of myometrial contractions. The expulsion interval between puppies was longest in primiparous bitches with low weight and shortest in multiparous bitches with high weight. The expulsion interval and the number of stillborn females were higher in primiparous bitches with high weight. Newborn male puppies were significantly heavier than newborn females.

KEYWORDS

uterine contractions, bitch parturition, fetal monitoring, hypoxia, blood profile

Introduction

Birth is a physiological process in which the fetus is expelled from the uterus through uterine contractions and cervical dilation (1). These contractions are caused by the release of various hormones, including oxytocin (1), and by changes that cause depolarization and repolarization of the myometrial cells (2).

In veterinary perinatology, high prenatal, intrapartum, and perinatal mortality is observed in some domestic species. Therefore, to reduce these high rates, it is essential to monitor the development of the fetus throughout pregnancy and parturition, which can improve perinatal care and reduce newborn mortality (3, 4). For example, in dogs (*Canis lupus familiaris*), mortality rates of 17–30% have been reported (5), although, according to Veronesi et al. (6), these rates may range from 5 to 35%.

Pregnancy in dams usually lasts 63 days ±1 day (7). Parturition consists of 3 phases: Stage 1 is characterized by intermittent uterine contractions associated with cervical dilation and behavioral changes (1); Stage 2 includes intensified uterine contractions accompanied by abdominal efforts and the Ferguson reflex, which produces fetal expulsion; and finally, Stage 3 is characterized by placental expulsion (8).

Electronic fetal and uterine monitoring is one of the main methods used to clinically assess and determine the vitality and welfare of fetuses and the uterine dynamics of the mother before and during parturition (9–12). In this sense, fetal and uterine electronic monitoring records fetal movements, the fetal heart rate (in bpm, bpm), and uterine contractions (in mmHg) (13). The second component of a fetal and uterine monitor is the tocodynamometer. This device measures uterine contraction intensity, frequency, and duration (14). It is a practical, noninvasive commercial alternative to traditional Doppler techniques (3) that is safe for the fetus and the mother (15, 16). Davidson (8), Groppetti et al. (17), Ayres-De-Campos and Nogueira-Reis (11), and Lezama-García et al. (4) have recently implemented this monitoring technique in bitches because in this species, it helps prevent and reduce mortality before, during and after parturition. In bitches (*Canis lupus familiaris*), this technique can detect dystocia, facilitating the prediction of whether a birth will end in a cesarean section (18), thereby allowing the timely detection of problems that cause fetal stress and pathological conditions, such as hypoxia and metabolic acidosis (11). In addition, it is a tool that can be used at home (e.g., by a breeder) with previous training. Dogs tolerate it well since it is not invasive, and the use of this tool can considerably decrease anxiety around the birth for the person responsible for the animal (8).

Electronic fetal and uterine monitoring parameters, such as heart rate, waveform, and dynamics of fetal behavior, are essential in determining fetal life, development, and maturity and detecting fetal stress or congenital heart disease (10, 19). The recording is carried out on the dam's abdominal skin with an ultrasound transducer, which detects the fetal heart rate, and a pressure transducer, which evaluates the activity of the uterus; both devices are connected to a screen where the data can be observed. The results are printed on millimetric paper (12).

The fetal heart rate (FHR) is one of the most critical parameters used to determine the health and welfare of a fetus. By monitoring this variable, we can detect oxygenation failures in a timely manner (19), thus avoiding fetal hypoxia (20, 21) and possible secondary neurological damage or even death during birth (10). The FHR is influenced by the autonomic nervous system, and the level of these responses depends, in turn, on the amount of oxygen the fetus has access to (11). Therefore, when oxygen levels of the fetus drop sharply, an immediate FHR fall occurs (22). A sustained deceleration in the FHR reflects distress in the fetus. Therefore, it is essential to know the normal parameters of canine and feline FHR at the end of pregnancy, which are 170–230 beats/min or at least four times the maternal heart rate (20, 23).

The present study aimed to evaluate the uterine dynamics of bitches with different weights and parity. Our research questions were as follows: What is the effect of the weight of the bitch at parturition on the intensity, frequency, and duration of contractions at the expulsion phase of parturition? Are there differences in the intensity, duration, and frequency of uterine contractions between primiparous and multiparous bitches? Are the birth weight and expulsion interval of pups essential factors for predicting their survival? Finally, does the sex of the newborn influence its survival?

Materials and methods

Facilities

The present study was carried out in six veterinary clinics and hospitals in the City of Campeche, Campeche, located in southeastern Mexico, within the Yucatan peninsula. Authorization was requested from the owners to allow the veterinary clinics to care for their bitches throughout gestation, from 28 days after mating until 48 h postpartum.

Study population

One hundred thirteen pregnant bitches between 2 and 6 years of age, primiparous or multiparous (1–4 previous litters), were recruited. However, since 17 of these bitches (five with primary uterine inertia and 12 with secondary uterine inertia) had dystocic parturition that ended in cesarean section or required the supply of oxytocin and calcium, they were excluded from the study. Therefore, a total of 96 bitches were finally included, with 476 puppies. The 96 dams were divided into four experimental groups containing 24 individuals each (12 primiparous and 12 multiparous), according to their body weight: G_1 (4–8 kg), G_2 (8.1–16 kg), G_3 (16.1 to 32 kg), and G_4 (32.1 to 39.6 kg). Body weight was obtained at the onset of contraction, when the first whelping stage started, through a digital scale (Avery Weigh-Tronix 7,820–100 West Bromwich, UK). The breeds included in this study were as follows: Chihuahua, Yorkshire Terrier, Cocker Spaniel, Standard Schnauzer, Scottish Terrier, Miniature Poodle, German Shepherd, Labrador, Golden Retriever, Great Dane, and Belgian Shepherd. The inclusion criteria were as follows: (a) clinically healthy bitches receiving preventive medicine (e.g., vaccination/deworming protocols), (b) no clinical records of reproductive problems, and (c) bitches that had undergone ultrasonographic and radiographic studies to confirm natural whelping. Animals with the following characteristics were excluded from this study: (a) records of dystocia or pyometra, (b) malformed fetuses, (c) requiring the administration of birth inducers or accelerators, (d) aggressive individuals, (e) a body condition over 8 (obese: ribs not palpable under hefty fat cover, or

palpable only with significant pressure; heavy fat deposits over lumbar area and base of tail, waist absent, no abdominal tuck, apparent abdominal distention) as per the WSAVA scale (24), (f) brachycephalic breeds known to have a high incidence of dystocia, or (g) receiving an emergency C-section (25). The body weight ranges were based on those of the Federation Cynologique Internationale (FCI): small (dogs up to 30 cm in height and 15 kg in weight), medium (dogs between 30 and 40 cm in height and between 15 and 25 kg in weight), and large (dogs between 40 and 60 cm in height and between 25 and 45 kg in weight) (26). Type I stillbirths (SBs) were excluded from the study (a total of five type I stillbirths were observed), and only type II stillbirths were included, classified by necropsy. According to Mota-Rojas et al. (27, 28), SBs can be classified as type I (prepartum or antepartum deaths), which are deaths before the end of gestation due to infectious causes; fetuses appear haemorrhagic, oedematous, and have grayish-brown discolouration. Type II SBs (intrapartum deaths) refer to deaths during whelping due to intrauterine asphyxia and are rarely caused by infectious diseases; puppies maintain a normal appearance similar to their littermates but lack respiration.

FIGURE 1
Electronic uterine and fetal monitoring. **(A)** Electronic uterine and fetal monitoring during parturition in a small multiparous bitch. **(B)** Monitoring of a Chihuahua dam during parturition. **(C)** Monitoring of a Belgian Shepherd dam. **(D)** Monitoring of a Yorkshire Terrier dam at the beginning of parturition. As shown in these images, monitoring was not invasive or painful, and the bitches allowed the veterinarian to place the adjustable bands that hold the tocodynamometer and the transducer on the skin of the abdominal zone.

Clinical history

In the clinics and hospitals where the study was carried out, standardized veterinary software (SmartZooft® LAN version 14 K, developed by SQUENDA®, Mexico City, Mexico) was used, and the clinical history was recorded. Such data included age, breed, type of diet, parity, body weight, preventive medicine history, and address, as well as the general data of the owner.

Prenatal procedures

All females included in the study became pregnant through direct mating. At 28 to 30 days after mating, pregnancy was confirmed with Mindray® model DP-30VetPower ultrasonography equipment (Shenzhen, China) with Doppler and pulsed Doppler (PW) utilizing a 3.5 MHz convex transducer. Gestation was confirmed by visualization of the gestational sacs and embryo heartbeat. Another ultrasonographic assessment was performed between 40 and 43 gestational days to verify the health, growth, and vitality of fetuses. Since mating or insemination cannot be used as indications of pregnancy (29–31), gestational age was confirmed through ultrasonography (7). Gestational age was determined by applying the following formulas: $GA = DGS \times 6 + 20 \pm 3$ days for gestations shorter than 40 days or $GA = BPD \times 15 + 20 \pm 3$ days for gestations longer than 40 days, where BPD is the biparietal diameter and DGS is the diameter of the gestational sac (32).

On days 48–50 after mating, X-rays were performed to identify and exclude bitches with possible dystocia (due to cephalopelvic disproportion) (33), determine the number of fetuses, and evaluate the size of their heads.

On day 60 after mating, the fetuses and bitches were monitored using a Sonolife® (Chihuahua, Mexico) brand antepartum monitor, Smart Monitor Color model, with a multicrystal pulsed Doppler transducer. The monitor assessed the health status of both the bitch and the fetuses, the fetal heart rate, and uterine activity, including the number, duration, interval, and frequency of the contractions, following a methodology previously reported for use in piglets by other authors (34) (Figures 1A–D). It is worth mentioning that the assessment of uterine dynamics was carried out from the moment that the chorioallantois membranes could be observed in the vulva; this time point was considered the beginning of the expulsion phase of whelping, which was monitored for the first 60 min. The expulsion phase duration was defined as the period from when the chorioallantois membranes were observed in the vulva until the puppy was entirely expelled.

Decelerations of FHR, specifically dip 2 patterns (a drop in the FHR beginning after the onset of a uterine contraction and returning to baseline after the uterine contraction has ended, caused by uteroplacental insufficiency) were also identified to determine the degree of fetal asphyxia *in utero*. These changes (dip 2) are attributed to the transitory occlusion of umbilical vessels due to the contracted uterus. According to Vispo et al. (35), fetal hypoxia develops when the occlusion is short and lasts less than 40 s. Following Mota-Rojas et al. (36), unfavorable dip 2 patterns were considered if they lasted more than 60 s and when the FHR was less than 70 bpm. To clinically detect these unfavorable dip 2 patterns, the FHR was evaluated before, during, and immediately after the myometrial contraction, and observations were carefully recorded when this coincided with the contraction's peak. When dip 2 patterns arose, an emergency cesarean section was performed, and these bitches were excluded from the analysis, which is why none of the puppies included in this study presented meconium-stained amniotic fluid.

The monitoring of the vital signs of the dams was carried out using a veterinary monitor DESEGO® (Mexico City, Mexico) Model

M8i SVGA to evaluate the electrocardiographic tracings, respiratory rate, oxygen saturation, temperature, and blood pressure from the probable date of whelping. Fetal heart rate was monitored before and during labor using the uterine and fetal electronic monitor described above.

Neonatal procedures

The newborn's heart rate was evaluated with a 3M™ Littmann classic pediatric III-5620 stethoscope (Canada). Neonatal heart rates below 100 bpm were considered to indicate bradycardia. Puppies were weighed with a digital scale (Salter Weight Tronix Ltd., West 148 Bromwich, UK). Rectal temperature was measured with a Hergom-Medical™ digital veterinary thermometer (Monterrey, Mexico). Puppies with rectal temperatures below 36°C were considered hypothermic.

Blood sampling and blood profile analysis

Blood samples were collected from bitches via a puncture in the plantar pad, using microcapillary tubes impregnated with lithium, at the end of the 60 min of electronic monitoring. This time point was selected because it was easier to obtain the sample after dams had given birth and minimized disturbance. Samples were analyzed with GEM Premier™ 3,000 (Instrumentation Laboratory Diagnostics, Lexington, KY, USA/Milano, Italy) to obtain glucose (mg/dL), lactate (mg/dL), pCO_2 (mmHg), pO_2 (mmHg), pH, HCO_3^- (mmol/L), and Ca^{++} (mmol/L) values.

Statistical analysis

Analyses were performed in R version 4.2.2 (R Core Team, Vienna, Austria) using the packages "moments," "ggpubr," "stats," "emmeans" and "multcompView." The significance threshold was set at $p < 0.05$. ANOVA assumptions of normality and homoscedasticity were checked by visual inspection of model residuals using Q-Q plots and plots of residuals vs. predicted values. The results are presented as the mean ± SEM.

As shown in Table 1, two-way ANOVA was applied to assess the effect of parity and weight group on the expulsion phase duration, expulsion interval, and puppy birth weight. *Post hoc* pairwise comparisons were conducted with Tukey HSD tests. Differences in the proportion of stillborn puppies between primiparous and multiparous bitches classified according to their weight were detected with a chi-square test.

As shown in Table 2, differences in the intensity, duration, and number of contractions between primiparous and multiparous bitches classified according to their weight were detected with two-way ANOVAs. As the interval between myometrial contractions did not meet the normality or homoscedasticity assumptions, this variable was analyzed using a generalized linear model (GLM) with a "gamma" family distribution and "identity" link function. The predictors were weight group (4 levels), parity (2 levels), and their two-way interaction. *Post hoc* pairwise comparisons were conducted with Tukey HSD tests.

As shown in Table 3, a GLM with a Poisson family distribution and "log" link function was used to investigate the effect of weight group and parity on the number of dip 2 patterns, number of puppies with after birth (AB) bradycardia, number of puppies with cyanotic oral mucosa, and number of hypothermic and adynamic pups per litter (dam). Weight group (4 levels), parity (2 levels), and their interaction were included as predictors. The number of puppies in each litter (litter size) was included as a covariate because larger litters tend to contain puppies with any of the following conditions: bradycardia (less than 100 bpm), cyanotic oral mucosa, hypothermia (less than 36°C) or adynamia.

As shown in Table 4, differences in the birth weight and expulsion interval between male and female puppies classified according to dam weight group were analyzed with separate two-way ANOVAs. The predictors were the weight group, sex of the puppy, and their two-way interaction. *Post hoc* pairwise comparisons were performed using Tukey's HSD tests.

TABLE 1 Reproductive performance of primiparous and multiparous bitches at the expulsion phase of whelping classified according to their weight (mean ± SEM).

	G_1		G_2		G_3		G_4	
	P	*M*	*P*	*M*	*P*	*M*	*P*	*M*
Expulsion phase duration (min)	$268.0 \pm 31.3^{b,c}$	226.0 ± 18.3^{c}	$288.0 \pm 10.2^{b,c}$	$326 \pm 20.1^{a,b,c}$	$303.0 \pm 32.0^{b,c}$	$300.0 \pm 17.8^{b,c}$	426 ± 36.0^{a}	$335.0 \pm 18.9^{a,b}$
Expulsion interval between puppy	82.2 ± 4.8^{a}	$66.9 \pm 3.2^{b,c}$	$75.1 \pm 3.01^{a,b}$	$62.5 \pm 2.5^{b,c}$	$69.1 \pm 3.9^{a,b}$	$53.9 \pm 2.4^{c,d}$	$64.6 \pm 2.8^{b,c}$	46.3 ± 1.3^{d}
Stillborn puppies	7 (9.72%)	5 (6.94%)	9 (12.5%)	6 (8.33%)	10 (13.88%)	7 (9.72%)	17 (23.61%)	11 (15.27%)
Birth weight (g)	186.0 ± 4.8^{d}	198.0 ± 2.8^{d}	257.0 ± 6.6^{c}	273.0 ± 2.9^{c}	354.0 ± 3.4^{b}	$376.0 \pm 6.8^{a,b}$	390.0 ± 10.6^{a}	387.0 ± 4.5^{a}

Two-way ANOVA/Chi-square test for the rate of stillbirths. Dams were divided into groups according to weight: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg. P, primiparous; M, multiparous. ^{a,b,c,d}Different superscripts across columns indicate statistically significant differences between groups ($p < 0.05$).

TABLE 2 Number, intensity, duration, and interval of myometrial contractions in primiparous and multiparous dams classified according to their weight (mean ± SEM).

	G_1		G_2		G_3		G_4	
	P	*M*	*P*	*M*	*P*	*M*	*P*	*M*
Intensity (mm/Hg)	35.1 ± 0.9^{a}	30.1 ± 0.6^{b}	30.2 ± 0.3^{b}	$24.8 \pm 0.6^{c,d}$	28.6 ± 0.6^{b}	23.8 ± 0.4^{d}	$27.7 \pm 0.9^{b,c}$	23.2 ± 0.8^{d}
Duration (sec)	175.0 ± 9.4^{a}	122.0 ± 4.8^{b}	165.0 ± 7.7^{a}	120.0 ± 5.1^{b}	171.0 ± 7.2^{a}	116.0 ± 4.01^{b}	172.0 ± 7.8^{a}	118.0 ± 4.3^{b}
Contractions number	12.1 ± 0.4^{e}	9.0 ± 0.2^{f}	14.8 ± 0.4^{d}	11.2 ± 0.4^{e}	18.7 ± 0.3^{b}	$14.9 \pm 0.3^{c,d}$	20.7 ± 0.3^{a}	16.7 ± 0.5^{c}
Interval between contractions (min)	5.81 ± 0.1^{b}	7.75 ± 0.2^{a}	4.70 ± 0.1^{c}	6.24 ± 0.1^{b}	$3.71 \pm 0.1^{d,e}$	4.66 ± 0.1^{c}	3.35 ± 0.1^{d}	$4.18 \pm 0.1^{c,e}$

Two-way ANOVA/GLM with a gamma family distribution for the interval between contractions. Dams were divided into groups according to their weight: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; P: primiparous; M: multiparous. ^{a,b,c,d,e}Different superscripts across columns indicate statistically significant differences between groups ($p < 0.05$).

TABLE 3 Fetal heart rate decelerations (dip 2) in fetuses and newborn puppies, number and percentage of bradycardic, cyanotic, hypothermic and adynamic puppies of primiparous and multiparous bitches classified according to their weight.

	G_1		G_2		G_3		G_4	
	P *n* = 39	M *n* = 41	P *n* = 47	M *n* = 63	P *n* = 53	M *n* = 67	P *n* = 79	M *n* = 87
DIP 2	11 (1.307 ± 0.3)	5 (0.565 ± 0.2)	8 (0.789 ± 2)	4 (0.294 ± 0.1)	11 (0.962 ± 0.2)	7 (0.484 ± 0.1)	14 (0.821 ± 0.2)	7 (0.373 ± 0.1)
Number of puppies with AB bradycardia	11 (28.2%) (1.307 ± 0.3)	7 (17%) (0.791 ± 0.2)	11 (23.4%) (1.084 ± 0.3)	6 (9.5%) (0.441 ± 0.1)	10 (18.8%) (0.874 ± 0.2)	6 (8.9%) (0.415 ± 0.1)	18 (22.7%) (1.056 ± 0.2)	10 (11.4%) (0.533 ± 0.1)
Number of puppies with cyanotic oral mucosa	12 (30.7%) (1.426 ± 0.4)	6 (14.6%) (0.678 ± 0.2)	13 (27.6%) (1.281 ± 0.3)	8 (12.6%) (0.588 ± 0.2)	13 (24.5%) (1.136 ± 0.3)	9 (13.4%) (0.622 ± 0.2)	24 (30.3%) (1.408 ± 0.2)	14 (16%) (0.746 ± 0.1)
Number of hypothermic and adynamic pups	14 (35.8%) (1.663 ± 0.4)	9 (21.9%) (1.017 ± 0.3)	14 (29.7%) (1.380 ± 0.3)	9 (14.2%) (0.662 ± 0.2)	18 (33.9%) (1.573 ± 0.3)	13 (19.4%) (0.899 ± 0.2)	23 (29.1%) (1.349 ± 0.2)	16 (18.3%) (0.852 ± 0.2)

The results are presented as the number (proportion ± SE). Dams were divided into groups according to their weight: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg. P, primiparous; M, multiparous. n, number of puppies. AB, after birth.

TABLE 4 Mean and standard error of birth weight and expulsion interval between females and males born from dams classified according to their weight.

	G_1		G_2		G_3		G_4	
	F	*M*	*F*	*M*	*F*	*M*	*F*	*M*
Birth weigth (g)	186.0 ± 3.4^{a}	198.0 ± 4.4^{a}	263.0 ± 3.02^{b}	269.0 ± 3.3^{b}	363.0 ± 3.01^{c}	368.0 ± 3.9^{c}	386.0 ± 3.8^{d}	393.0 ± 5.2^{d}
Expulsion interval (min)	$67.2 \pm 4.07^{a,b}$	81.1 ± 5.4^{a}	$65.3 \pm 3.7^{a,b}$	$68.5 \pm 3.9^{a,b}$	61.3 ± 3.8^{b}	59.4 ± 3.6^{b}	55.4 ± 3.1^{b}	54.7 ± 3.1^{b}

Dams were divided into groups according to their weight: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg. F, female; M, male. ^{a,b,c,d}Different superscripts across columns indicate statistically significant differences between groups and according to sex ($p < 0.05$).

As shown in Table 5, two-way ANOVAs were used to assess all variables. *Post hoc* pairwise comparisons using Tukey HSD tests were used for analysis.

As shown in Table 6, the effect of the sex of the puppy, birth weight, and expulsion interval on the likelihood of a puppy being stillborn was analyzed using a binary logistic regression model. The predictors were the sex of the puppy (male or female), birth weight (continuous), expulsion interval (continuous), and the interactions between sex and expulsion interval and between sex and birth weight. As the interaction between puppy sex and birth weight was not significant in the initial model, this was removed to increase fit. The probability distribution was "binomial" with a "logit" link function.

As shown in Tables 7, 8, Pearson correlations were used to analyze correlations; as shown in Table 9, Spearman rank correlations were used because the variables did not have a normal distribution.

TABLE 5 Mean and standard error of the blood profile parameters of primiparous and multiparous dams classified according to weight.

Metabolites	G_1		G_2		G_3		G_4	
	P	*M*	*P*	*M*	*P*	*M*	*P*	*M*
Lactate (mg/dL)	7.02 ± 0.4^{d}	5.46 ± 0.2^{e}	7.80 ± 0.2^{e}	$5.24 \pm 0.3^{b,c,d}$	$8.74 \pm 0.2^{a,b}$	$7.22 \pm 0.3^{c,d}$	9.83 ± 0.2^{a}	$8.44 \pm 0.2^{b,c}$
Glucose (mm/dL)	$74.8 \pm 4.2^{a,b}$	88.0 ± 5.1^{a}	$73.6 \pm 1.5^{a,b}$	85.6 ± 8.6^{a}	$72.3 \pm 1.5^{a,b}$	$82.3 \pm 5.3^{a,b}$	63.9 ± 2.9^{b}	$70.3 \pm 1.7^{a,b}$
Ca^{2+} (mmol/L)	$1.97 \pm 0.08^{b,c}$	$1.76 \pm 0.06^{c,d}$	$2.05 \pm 0.08^{b,c}$	1.64 ± 0.05^{d}	$2.12 \pm 0.05^{a,b}$	$1.92 \pm 0.06^{b,c,d}$	2.42 ± 0.09^{a}	$2.11 \pm 0.05^{a,b}$
pH	$7.30 \pm 0.01^{a,b}$	7.39 ± 0.01^{a}	7.18 ± 0.04^{c}	$7.29 \pm 0.01^{a,b}$	$7.23 \pm 0.015^{b,c}$	$7.30 \pm 0.01^{a,b}$	$7.23 \pm 0.02^{b,c}$	7.28 ± 0.01^{b}
pO_2 (mm/Hg)	$21.1 \pm 0.87^{b,c}$	26.1 ± 1.2^{a}	18.6 ± 0.9^{c}	$24.0 \pm 0.8^{a,b}$	$20.9 \pm 1.2^{b,c}$	$24.3 \pm 0.7^{a,b}$	14.0 ± 0.8^{d}	$17.3 \pm 0.7^{c,d}$
pCO_2 (mm/Hg)	52.7 ± 2.9^{b}	47.0 ± 1.5^{b}	$57.9 \pm 4.7^{a,b}$	47.8 ± 2.2^{b}	$56.8 \pm 4.4^{a,b}$	48.4 ± 1.4^{b}	69.4 ± 3.1^{a}	$60.0 \pm 3.04^{a,b}$
HCO_3^- (mmol/L)	$19.5 \pm 0.9^{a,b,c}$	22.7 ± 0.9^{a}	$17.8 \pm 1.2^{b,c}$	$20.8 \pm 1.1^{a,b}$	16.3 ± 0.9^{c}	$18.7 \pm 0.8^{a,b,c}$	15.6 ± 0.5^{c}	$18.2 \pm 0.6^{b,c}$

Two-way ANOVA. Dams were divided into groups according to their weight: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg. P, primiparous; M, multiparous. ^{a,b,c,d}Different superscripts across columns indicate statistically significant differences between groups ($p < 0.05$).

TABLE 6 Results of the final binary logistic regression model for factors affecting the likelihood of stillbirth.

Predictor	Estimate	Std. error	*z* value	Odd's ratio	*p*-value
Intercept	−6.078996	1.064111	−5.713	0.0023	< 0.001
Birth weight	0.010371	0.002153	4.817	1.0104	**< 0.001**
Expulsion interval	−0.003644	0.011027	−0.330	0.9964	0.741
Sex male	−0.235426	0.780870	−0.301	0.7902	0.763
Expulsion interval: Sex Male	0.027298	0.012089	2.258	1.0277	**0.024**

Significant differences are indicated in bold letters.

TABLE 7 Correlations between dam weight and uterine dynamics.

Variables	Correlation coefficient *(r)*	*p*-value
G_1		
Contractions intensity (mmHg)	0.0261	0.904
Contractions duration (sec)	−0.265	0.210
Contractions number	−0.142	0.509
Interval between contractions (min)	0.164	0.443
G_2		
Contractions intensity (mmHg)	−0.165	0.442
Contractions duration (sec)	−0.157	0.463
Contractions number	−0.234	0.270
Interval between contractions (min)	0.199	0.352
G_3		
Contractions intensity (mmHg)	0.142	0.507
Contractions duration (sec)	0.122	0.570
Contractions number	0.147	0.493
Interval between contractions (min)	−0.162	0.450
G_4		
Contractions intensity(mmHg)	−0.137	0.524
Contractions duration (sec)	−0.378	0.068
Contractions number	−0.140	0.514
Interval between contractions (min)	0.083	0.697

Pearson correlations. Dams were divided into groups according to their weight: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg.

TABLE 8 Pearson correlations between uterine dynamics and blood profile.

Variables	Correlation coefficient *(r)*	*P*-value
Contractions intensity		
pH	0.007	0.949
PCO_2 (mmHg)	0.006	0.954
PO_2 (mmHg)	0.002	0.983
Glucose (mg/dL)	0.007	0.943
Ca^{++} (mmol/L)	0.047	0.646
Lactate (mmol/L)	−0.066	0.520
HCO_3^-	−0.007	0.946
Contraction duration		
pH	**−0.250***	**0.014**
PCO_2(mmHg)	**0.347***	**<0.001**
PO_2(mmHg)	**−0.230***	**0.024**
Glucose (mg/dL)	**−0.301***	**0.003**
Ca^{++}(mmol/L)	**0.365***	**<0.001**
Lactate (mmol/L)	**0.380***	**<0.001**
HCO_3^-	**−0.322***	**0.001**
Contraction number		
pH	**−0.393***	**<0.001**
PCO_2(mmHg)	**0.440****	**<0.001**
PO_2(mmHg)	**−0.536****	**<0.001**
Glucose (mg/dL)	**−0.362***	**<0.001**
Ca^{++}(mmol/L)	**0.619****	**<0.001**
Lactate (mmol/L)	**0.741*****	**<0.001**
HCO_3^-	**−0.556****	**<0.001**

*Weak correlation; **moderate correlation; ***strong correlation. Values in bold represent significant correlations.

TABLE 9 Spearman correlations between uterine dynamics and dip 2.

Variables	Correlation coefficient *(r)*	*P*-value
Contractions intensity (mmHg)	**0.119***	0.248
Contractions duration (sec)	**0.179***	0.081
Contractions number	**0.248***	**0.015**
Interval between contractions (min)	**−0.248***	**0.015**

*Weak correlation. Values in bold represent significant correlations.

Ethical statement

Before carrying out the study, informed consent was obtained from the animals' owners, authorizing the procedures. All work was performed under Mexico's Official Norm NOM-062-ZOO-1999 guidelines on the technical specifications for animal production, care, and ethical use in applied ethological studies (37). This project was approved by the Ph.D. Program in the Biological and Health Science Academic Committee (number CBS.114.19). All the female dogs evaluated in this study were treated gently, avoiding stress due to handling as much as possible; the use of an electronic fetal and uterine monitor greatly facilitated this aspect because it is not a painful or invasive technique.

Results

As expected, larger bitches had a higher number of puppies per litter: G_1 had an average of 3.3 puppies per litter, G_2 had an average of 4.5 puppies per litter, G_3 had an average of 5 puppies per litter, and G_4 had an average of 8.9 puppies per litter. These numbers affected the other parameters.

It is important to mention that the FHR could not be evaluated in all fetuses because the fetal monitor could only perceive the heartbeats of some fetuses at random. Therefore, there was no way to know which frequency belonged to which fetus. However, all the puppies' heart rates were evaluated after birth.

Expulsion phase duration

There was no significant difference between primiparous and multiparous dams in the expulsion phase duration ($F_1 = 2.018$, $p = 0.159$). However, there was a significant difference among weight groups ($F_3 = 9.963$, $p < 0.001$). Tukey's HSD tests showed that the expulsion phase duration was, on average, longer in dams from G_4 (381.0 ± 22.0 min) compared to dams from the three other groups (G_1: 247.0 ± 18.2 min, $p < 0.001$; G_2: 307.0 ± 11.7 min, $p = 0.02$; G_3: 302.0 ± 17.9, $p = 0.009$). There was a nonsignificant trend toward an interaction between weight group and parity ($F_3 = 2.515$, $p = 0.063$) (Table 1).

Expulsion interval between puppies

The average expulsion interval between puppies was significantly longer in primiparous dams (72.7 ± 2.06 min) than in multiparous dams (57.4 ± 1.67 min; $F_1 = 46.166$, $p < 0.001$). The expulsion interval also differed significantly among weight groups ($F_3 = 13.673$, $p < 0.001$). *Post hoc* comparisons showed that the expulsion interval was significantly longer in G_1 (74.6 ± 3.27 min) than in G_3 (61.5 ± 2.78 min; $p < 0.001$) and significantly shorter in G_4 (55.5 ± 2.47 min) than in G_1 (74.6 ± 3.27 min; $p < 0.001$) and G_2 (68.8 ± 2.32 min; $p < 0.001$), as shown in Table 1.

Stillborn puppies

The proportion of stillborn puppies was higher in primiparous dams (G_4: 23.61%, G_3: 13.88%, G_2: 12.5, G_1: 9.72%) than in multiparous dams (G_4: 15.27%, G_3: 9.72%, G_2: 8.33%, G_1: 6.94%; $\chi^2 = 5.9811$, df = 1, $p = 0.014$). There was no significant difference in the proportion of stillborn puppies among weight groups ($\chi^2 = 0.66928$, df = 3, $p = 0.880$) or primiparous and multiparous mothers classified according to weight groups ($\chi^2 = 7.3018$, df = 7, $p = 0.398$). The number of stillborn

puppies from primiparous and multiparous dams classified according to their weight is shown in Table 1.

Birth weight

The average birth weight was significantly higher in puppies from multiparous dams (308.0 ± 11.6 g) compared to primiparous dams (296.0 ± 12.2 g; $F_1 = 8.438$, $p = 0.005$; Table 1).

Intensity of contractions

The intensity of contractions was significantly higher in primiparous dams (30.4 ± 0.555 mmHg) than in multiparous dams (25.5 ± 0.507 mmHg; $F_1 = 96.360$, $p < 0.001$). Contraction intensity differed significantly among weight groups ($F_3 = 41.073$, $p < 0.001$). *Post hoc* comparisons showed that the intensity of contractions was significantly higher in G_1 (32.6 ± 0.771 mmHg) than in G_2 (27.5 ± 0.673 mmHg; $p < 0.001$), G_3 (26.2 ± 0.620 mmHg; $p < 0.001$) and G_4 (25.5 ± 0.766 mmHg; $p < 0.001$). Contraction intensity was also higher in G_2 than in G_4 ($p = 0.025$). The number, intensity, duration, and interval of contractions are shown in Table 2 for primiparous and multiparous bitches classified according to their weight.

Duration of contractions

Contractions were significantly longer in primiparous dams (171.0 ± 3.97 s) than in multiparous dams (119.0 ± 2.24 s; $F_1 = 123.147$, $p < 0.001$). There were no significant differences among weight groups ($F_3 = 0.255$, $p = 0.857$) (Table 2).

Number of contractions

The number of contractions was significantly higher in primiparous dams (16.6 ± 0.524) than in multiparous dams (13.0 ± 0.481; $F_1 = 156.10$, $p < 0.001$). Likewise, the number of contractions differed significantly among weight groups ($F_3 = 160.78$, $p < 0.001$). *Post hoc* tests revealed that G_4 (18.7 ± 0.524) had significantly more contractions than the three other groups (G_1: 10.5 ± 0.417, $p < 0.001$; G_2: 13.0 ± 0. 483, $p < 0.001$; G_3: 16.8 ± 0.454, $p < 0.001$). The number of contractions in G_3 was significantly higher than that in G_1 ($p < 0.001$) and G_2 ($p < 0.001$). The number of contractions in G_2 was significantly higher than that in G_1 ($p < 0.001$) (Table 2).

Interval between contractions

The general linear model (GLM) revealed that the interval between contractions was significantly longer in multiparous dams (5.71 ± 0.090 min) than in primiparous dams (4.39 ± 0.069; $\chi^2 = 43.749$, df = 1, $p < 0.001$). The interval between myometrial contractions also differed among weight groups ($\chi^2 = 253.901$, df = 3, $p < 0.001$); the interval was longer in lighter bitches and was significantly affected by the interaction between parity and weight group ($\chi^2 = 14.760$, df = 3, $p = 0.002$). *Post hoc* Tukey HSD test results are reported in Table 2.

Late deceleration of fetal rate (dip 2)

The results of the GLM revealed that the proportion of fetuses showing dip 2 was significantly higher in primiparous dams (0.950 ± 0.146) than in multiparous dams (0.416 ± 0.089; $\chi^2 = 10.433$, df = 1, $p = 0.001$). No significant differences were observed among weight groups ($\chi^2 = 2.658$, df = 3, $p = 0.447$). dip 2 was not significantly affected by the interaction between weight group and parity ($\chi 2 = 0.154$, df = 3, $p = 0.985$). The number (and rate ± SE) of fetuses showing dip 2 in primiparous and multiparous bitches classified according to their weight is shown in Table 3.

Bradycardia

All puppies were auscultated to determine whether they had bradycardia. The rate of puppies with AB bradycardia was significantly higher in litters from primiparous dams (1.069 ± 0.155) than in those from multiparous dams (0.527 ± 0.100; $\chi^2 = 9.420$, df = 1, $p = 0.002$). No significant differences were observed among weight groups ($\chi^2 = 2.104$, df = 3, $p = 0.551$). The interaction between weight group and parity did not significantly affect the rate of puppies with AB bradycardia ($\chi^2 = 0.331$, df = 3, $p = 0.954$). The number (and rate ± SE) of puppies with AB bradycardia in litters from primiparous and multiparous dams classified according to their weight is shown in Table 3.

Cyanosis

The rate of puppies with cyanotic oral mucosa was significantly higher in litters from primiparous dams (1.307 ± 0.173) than in those from multiparous dams (0.656 ± 0.113; $\chi^2 = 11.038$, df = 1, $p < 0.001$). No significant differences were observed among weight groups ($\chi^2 = 0.714$, df = 3, $p = 0.870$). The rate of puppies with cyanotic oral mucosa was not significantly affected by the interaction between weight group and parity ($\chi^2 = 0.113$, df = 3, $p = 0.990$). The number (and rate ± SE) of puppies with cyanotic oral mucosa from primiparous and multiparous dams classified according to their weight is shown in Table 3.

Hypothermia and adynamia

The number of hypothermic and adynamic puppies was significantly higher in litters from primiparous dams (1.486 ± 0.183) than in those from multiparous dams (0.847 ± 0.127; $\chi^2 = 8.563$, df = 1, $p = 0.003$). No significant differences were observed among weight groups ($\chi^2 = 1.157$, df = 3, $p = 0.763$). The proportion of hypothermic and adynamic puppies was not significantly affected by the interaction between weight group and parity ($\chi^2 = 0.285$, df = 3, $p = 0.963$). The number (and proportion ± SE) of bradycardic, cyanotic, hypothermic, and adynamic puppies from primiparous and multiparous bitches classified according to their weight is shown in Table 3.

Comparison of birth weight between females and males

Newborn male puppies (329.0 ± 5.19 g) were significantly heavier than newborn females (314.0 ± 5.46 g; $F = 5.232$, df = 1, $p = 0.023$). Pairwise comparisons are reported in Table 4.

Comparison of the expulsion interval between female and male puppies and dam weight

The expulsion interval significantly differed according to dam weight ($F = 9.095$, df = 3, $p < 0.001$). Expulsion intervals were significantly longer for puppies from G_1 (74.2 ± 3.47 min) than for puppies from G_3 (60.3 ± 2.63 min, $p = 0.005$) and G_4 (55.0 ± 2.22, $p < 0.001$). Likewise, puppies from G_2 (67.0 ± 2.70 min) had significantly longer expulsion intervals than those from G_4 (55.0 ± 2.22 min, $p = 0.005$). No significant difference was found between male (63.1 ± 1.99 min) and female puppies (61.5 ± 1.84 min, $p = 0.373$).

Table 5 shows the differences in blood profiles between primiparous and multiparous bitches. The metabolites evaluated were lactate, glucose, Ca^{++}, pH, pO_2, pCO_2, and HCO_3^-.

Lactate

Primiparous dams had significantly higher lactate levels (8.35 ± 0.213 mg/dL) than multiparous dams (6.59 ± 0.237 mg/dL; $F_1 = 70.67$, $p < 0.001$). Lactate levels significantly differed among weight groups ($F_3 = 41.59$, $p < 0.001$). G_4 (9.14 ± 0.229 mg/dL) had significantly higher lactate levels than the three other weight groups (G_1: 6.24 ± 0.279 mg/dL, $p < 0.001$; G_2: 6.52 ± 0.332 mg/dL, $p < 0.001$; G_3: 7.98 ± 0.265 mg/dL, $p < 0.001$). Similarly, lactate was significantly higher in G_3 than in G_1 ($p < 0.001$) and G_2 ($p < 0.001$).

Glucose

Multiparous dams (81.6 ± 2.95 mg/dL) had significantly higher glucose levels than primiparous dams (71.2 ± 1.50 mg/dL; $F_1 = 10.536$, $p = 0.002$). Glucose levels differed significantly among weight groups ($F_3 = 3.990$, $p = 0.01$). G_4 (67.1 ± 1.83 mg/dL) had significantly lower glucose levels than G_1 (81.4 ± 3.54 mg/dL, $p = 0.01$) and G_2 (79.6 ± 4.46 mg/dL, $p = 0.03$).

Ca^{++}

Ca levels were significantly higher in primiparous dams (2.14 ± 0.046 mmol/L) than in multiparous dams (1.86 ± 0.0385 mmol/L; $F_1 = 32.277$, $p < 0.001$). ANOVA results revealed significant differences in Ca levels among weight groups ($F_3 = 15.170$, $p < 0.001$). Ca levels were significantly higher in G_4 (2.26 ± 0.061 mmol/L) than in G_1 (1.86 ± 0.054 mmol/L, $p < 0.001$), G_2 (1.85 ± 0.065 mmol/L, $p < 0.001$) and G_3 (2.02 ± 0.047 mmol/L, $p = 0.006$).

pH

Multiparous dams (7.32 ± 0.010) had significantly higher pH levels than primiparous dams (7.24 ± 0.015; $F_1 = 24.323$, $p < 0.001$). Two-way ANOVA showed that pH levels differed significantly among weight groups ($F_3 = 24.323$, $p < 0.001$). G_1 (7.35 ± 0.015) had significantly higher pH levels than the three other groups (G_2: 7.23 ± 0.0267, $p < 0.001$; G_3: 7.27 ± 0.014, $p = 0.005$; G_4: 7.25 ± 0.012, $p < 0.001$).

pO_2

Primiparous dams (18.7 ± 0.645 mm/Hg) had significantly lower pO_2 levels than multiparous dams (22.9 ± 0.664 mm/Hg; $F_1 = 38.466$, $p < 0.001$). pO_2 levels also differed significantly among weight groups ($F_3 = 15.170$, $p < 0.001$). Specifically, *post hoc* tests showed that G_4 (15.6 ± 0.664 mm/Hg) had lower pO_2 levels than G_1 (23.6 ± 0.892 mm/Hg, $p < 0.001$), G_2 (21.3 ± 0.857 mm/Hg, $p < 0.001$) and G_3 (22.6 ± 0.819 mm/Hg, $p < 0.001$).

pCO_2

pCO_2 levels were significantly higher in primiparous dams (59.2 ± 2.10 mm/Hg) than in multiparous dams (50.8 ± 1.31 mm/Hg; $F_1 = 14.054$, $p < 0.001$). There was a significant difference in pCO_2 levels among weight groups ($F_3 = 8.775$, $p < 0.001$). G_4 (64.7 ± 2.36 mm/Hg) had significantly higher pCO_2 levels than the three other groups (G_1: 49.8 ± 1.73 mm/Hg, $p < 0.001$; G_2: 52.9 ± 2.77 mm/Hg, $p = 0.002$; G_3: 52.6 ± 2.47 mm/Hg, $p = 0.001$).

HCO_3^-

Multiparous dams (20.1 ± 0.506 mmol/L) had significantly higher levels of HCO_3^- than primiparous dams (17.3 ± 0.514 mmol/L; $F_1 = 18.132$, $p < 0.001$). HCO3- levels also differed significantly among weight groups ($F_3 = 18.132$, $p < 0.001$). G_1 (21.1 ± 0.744 mmol/L) had significantly higher HCO_3^- levels than G_3 (17.5 ± 0.658 mmol/L, $p = 0.001$) and G_4 (16.9 ± 0.486 mmol/L, $p < 0.001$).

Stillborn puppies

The likelihood of a stillbirth was significantly affected by birth weight ($\chi^2 = 29.224$, df = 1, $p < 0.001$). As birth weight increased, puppies were more likely to be stillborn. For every increment of 1 g in birth weight, puppies had 1.04% higher odds of being stillborn. Likewise, as the duration of the expulsion interval increased, male puppies were significantly more likely to be stillborn than female puppies ($\chi^2 = 5.943$, df = 1, $p = 0.015$). For every additional minute, male puppies had 2.77% higher odds of being stillborn than female puppies. The results of the final binary logistic regression model are reported in Table 6.

Pearson correlation analysis between uterine dynamic variables and dam weight was used to calculate the correlation coefficient in Tables 7, 8. Table 7 shows correlations between dam weight and uterine dynamics (there was no significant correlation when dividing

the bitches into groups). Table 8 shows correlations between uterine dynamics and blood profiles. Significant correlations are shown in bold. Regarding the duration and number of contractions, there were negative correlations with pH, PO_2, glucose, and HCO_3, and there were positive correlations with PCO_2, Ca^{++}, and lactate. In both tables, weak correlations are marked with an asterisk, moderate correlations are marked with two asterisks, and strong correlations are marked with three asterisks following the classification of Schober et al. (38).

Table 9 shows the correlations between uterine dynamics and dip 2. Spearman rank correlations were used for analysis. Significant correlations are indicated in bold. There was a positive correlation of dip 2 with the number of contractions and a negative correlation of dip 2 with the interval between contractions ($p = 0.015$).

Discussion

The results showed significant differences between primiparous and multiparous dams that not only affected their health but also affected the overall status of their newborn puppies. Although the dogs gave birth under similar conditions, this variable was not completely standardized because the births occurred in different clinics and hospitals. However, all the dogs were placed on foam mats to keep them comfortable and maintain similar temperatures on the floor where they gave birth.

Regarding fetal and uterine monitoring, in some cases, when the dams were nervous or moved too much, the tocodynamometer and transducer had to be repositioned, which created small pauses in the recording. However, most of the bitches allowed the monitor and bands to be placed without discomfort, and only a few (three bitches in G_1) initially showed nervousness. However, after a few minutes, they calmed down and allowed monitoring. During the 60 min that the monitoring was carried out, an average of 5.3 myometrial contractions and two puppies were expelled, which is similar to previous records made by Davidson (8).

Expulsion phase duration

Although no significant differences in the duration of the expulsion phase were found between primiparous and multiparous dams, this variable was influenced by the dam's weight, with heavier bitches (from G_4) exhibiting longer expulsion phases. A retrospective study on dystocia compared dogs according to their size and weight range: small (<12.7 kg), medium (12.7–20.5 kg), and large dogs (>20.5 kg) (18). Contrary to the results of the current study, no differences among dog weights were recorded. Similarly, Zonturlu and Kacar (39) did not find significant differences in the length of expulsion between German Shepherd (7.49 ± 2.44 h) and Labrador Retriever bitches (7.38 ± 1 h). However, the expulsion interval between puppies differed, with ranges of 20–415 min and 5–405 min, respectively, while Baqueiro-Espinosa et al. (40) found that the most extended whelping duration (369.73 min) was observed in dams of different breeds and parity (ranging from 0 to 4).

This study demonstrated that expulsion phase duration is positively associated with litter size since the average number of puppies born in each weight group is as follows: G_1, 3.3 puppies per bitch; G_2, 4.3 puppies; G_3, five puppies; and G_4, 6.9 puppies. Specifically, the larger the litter size was, the longer the expulsion phase duration. Another point that has been considered in some studies is the dysfunction of myometrial contractions in animals with higher weights, as observed in overweight animal models. In rats, females with high fat and high cholesterol levels exhibited asynchronous myometrial contractions and increased parturition duration (41). However, in this study, obese dams were not included. It is also important to consider that the number of contractions could influence these results, as the lighter-weight females in this study had a lower number of contractions, which could shorten the expulsion phase duration. However, these contractions were significantly more intense than those in G_4. The fact that larger bitches (G_4) tend to have larger litters and larger puppies (42, 43) than smaller bitches could be another essential factor to consider.

Expulsion interval between puppies

Primiparous dams and those in G_1 had the longest expulsion interval between puppies (82.2 ± 4.86 min). The average interval length is between 5 min and 2 h (44), while intervals of 12–16 h between the first and the last fetus are considered dystocia (45). Several studies have reported similar findings, and the increase in the interpup interval has been related to physiological exhaustion of the bitch, ineffective myometrial contractions (46), and the size of the litter (41, 42). In G_1, the average expulsion interval between puppies was 74.16 min; in G_2, it was 66.96 min; in G_3, it was 60.30 min; and in G_4, it was 55.01 min. Thus, the larger the size of the dog was, the shorter the expulsion interval between puppies, probably because these dogs had a higher number of contractions and a higher number of puppies (i.e., larger litter size). However, although the lighter primiparous bitches had the longest expulsion intervals, whelping was shorter due to the number of puppies, which is lower in small-sized dams than in large-sized ones. For example, in G_1, the interval between puppies was 74.16 min. If the bitch was carrying an average of 3 (3.3) fetuses, whelping would take 148.3 min. In G_4, the interval between puppies was 55.01 min. If the bitch was carrying an average of 7 (6.9) fetuses, whelping would take 330 min.

Stillborn puppies

The risk of stillbirth is associated with parity, as shown in Münnich and Küchenmeister's (47) study, which concluded that primiparous bitches more than 6 years old had the highest frequency of stillbirths (66.1%) and delayed whelping (3.8%). A similar result was obtained in the present study, where the proportion of stillbirths was higher in primiparous bitches in G_4. Apart from the higher frequency of stillbirths, primiparous bitches have an increased risk of requiring C-sections ($p = 0.004$), and this is directly related to the presence of stillborn puppies (40). Some authors attribute this effect to the longer parturition duration and the lack of experience in primiparous bitches (48). Nonetheless, other reports indicate that parity is not related to stillbirths (49, 50), while other authors mention that dams only exhibit a constant rate of stillbirths after the fourth litter (51, 52).

Regarding the higher proportion of stillbirths in G_4 bitches, maternal overweight is a risk factor for stillbirth in mammals (53). This is due to impaired placental function, which increases the

stillborn risk (54). However, this factor was not related to the results of the present study, as overweight females were excluded.

Birth weight

The positive relationship between the weight of the dam and the birth weight of the puppies observed in the current study has been recognized as a factor that might affect puppies' development (55), and similar results have been reported in livestock. In lambs, the weight of the ewes significantly affected the birth weight of lambs due to maternal nutrition. In this sense, the quality and amount of nutrients obtained during gestation influence fetal growth (56). Another study assessing the influence of breed and average weight found that puppies of medium-sized breeds (10–20 kg) had 0.99 times lower perinatal mortality rates than large breeds (> 20 kg) (40). Regarding parity, the higher weight recorded in puppies born from multiparous dams in this study is different from the results of Tesi et al. (57) in toy and small-sized dogs, as parity did not affect puppies' birth weight or neonatal mortality in that study. In contrast, lambs from primiparous ewes had the lowest weight (58).

Intensity of contractions

In rats (41), bovines (59), and humans (60), obesity is related to the presentation of more intense uterine contractions, and this may be associated with the regulation of connexin-43 in myometrial myocytes. In the present study, we did not include obese bitches; however, the most intense uterine contractions occurred in primiparous bitches in G_1 and G_2 (smaller bitches) as well as multiparous bitches in G_3 and G_4 (larger bitches). Therefore, more intense uterine contractions could be associated not only with the weight of the dams, as in the primiparous and lighter bitches, but also with the weight of the newborns at birth and the size of the litter, with larger-sized bitches having larger litters (43, 61) and therefore having less space *in utero* as it is fully occupied by fetuses, as well as uterine fatigue in very prolonged parturitions (46).

Duration of the contractions

In primiparous dams, the duration of contractions was more prolonged than that in multiparous dams, but their weight did not significantly affect the myometrial contraction time. The stress response is triggered in the first parturition, increasing circulating epinephrine levels, reducing the uterus's contractile activity and increasing its duration (56). Likewise, when comparing multiparous and nulliparous women, primiparous patients had longer active labor and pushing phases (62); oxytocin and its action on uterine oxytocin receptors are required to promote strong and effective contractions (63).

Number of contractions

Some breeds, such as Boxers, Border Collies, Labrador Retrievers, and Golden Retrievers, are predisposed to uterine inertia. Other predisposing factors include the dam's age, disproportionately large or small litters, obesity, and hormonal or nutritional imbalances (42). In contrast to these findings regarding predisposed breeds, in this study, the heaviest (G_4) primiparous bitches exhibited more uterine activity (more contractions) than multiparous bitches, and this group mainly consisted of Labradors and Golder Retrievers. This finding could explain why the expulsion interval between puppies was lower (55.01 min) on these bitches.

Interval between contractions

The interval between contractions was greater for the lighter multiparous bitches, and primiparous bitches had more contractions; thus, the intervals between contractions in primiparous bitches are shorter than those in multiparous bitches. According to Olsson (64), the expulsion phase is triggered by the increase in plasma vasopressin concentration; in multiparous bitches, this hormone may decrease as the time of parturition increases.

Blood profile

In general, the heaviest primiparous bitches presented the most critical changes in blood profiles. The greater the dam weight was, the larger the litter, the longer the labor, and the higher the incidence rates of uterine inertia and whelping complications. These results are associated with the longer whelping duration, which impairs uterine activity, and the consequent physiological ischaemia, hypoxia, and acidification (65) observed, with increased levels of lactate and pCO_2 and a decrease in pO_2 observed in G_4. The elevations in glucose levels registered in heavier bitches are similar to those reported in bitches and puppies, where fetal dystocia induced an hyperglycaemic state, along with an increase in cortisol, a hormone known to mobilize glucose through glycogenolysis and gluconeogenesis (66). Therefore, the whelping complications reported in G_4 bitches are consistent with the biochemical profile of dystocia cases.

dip 2

There are few studies where dip 2 has been evaluated in bitches. Gilet al. (20) found that fetuses exhibited distress when the FHR was between 160 and 180 bpm for 60 s or more. Contrary to the findings of Gil et al. (20), who observed that both primiparous and multiparous dams could present fetuses with HR decelerations, we found that dip 2 developed in fetuses of 44 primiparous bitches and 23 multiparous bitches; thus, dip 2 presentation was more likely to occur in fetuses of primiparous bitches, and in 29 bitches of the total study population, dip 2 was not observed.

Bradycardia

In some studies (67, 68), the welfare of canine fetuses has been evaluated based on fetal movements and heartbeat, and severe fetal distress was considered with an FHR <180 bpm. According to Gil et al. (20), the day of parturition can be predicted using the FHR, which

could help provide a timely intervention and reduce animal losses. Studies carried out in humans by Hon and Hon et al. (69–71) revealed fetal heartbeat variations when administering exogenous oxytocin in the mother during delivery or when the mother exercised. In contrast to these previous studies, in the present study, no drug was administered to the bitches, nor were they subjected to any exercise or stress, so the results obtained could be closer to events in a normal whelping in bitches.

In this study, 79 newborn puppies presented bradycardia, and these decreases in heart rate were more evident in pups born to primiparous dams. This is likely because the most intense uterine contractions were observed in primiparous dams, which makes the presentation of dip 2 decelerations more likely.

Cyanosis

The number of cyanotic puppies was higher in primiparous dams, possibly due to the complications and lack of experience reported in animals at the first parity. Fetal asphyxia, hypoxia, and cyanotic mucous membranes are indicators of low vitality scores in several domestic species (72, 73). Fetal asphyxia due to constant uterine contractions and umbilical cord blood vessel occlusion increases the whelping duration (74).

Hypothermia and adynamia

Primiparous bitches, having little or no experience with parturition or maternal behavior, tend to be less skillful in the maternal care of their pups. These newborns are altricial and require the help of the dam to move and thermoregulate (1, 75) as they are unable to do so on their own; this aligns with what was found in this study, where 69 newborn puppies from primiparous dams exhibited adynamia and hypothermia compared to 47 newborn puppies from multiparous dams. In this sense, maternal experience influences their care of the offspring.

Sex of the puppies

The relationship between higher birth weight and male sex is observed in different mammal species. This is attributed to sexual dimorphism, as males tend to be larger than females, as reported in newborn piglets (76). Moreover, as previously discussed, the body weight of the bitches also influences the birth weight of the newborns. Therefore, for dams with high body weights, it is important to consider the adverse effects on the mother and on the puppy's growth and survival.

Conclusion

Electronic fetal and uterine monitoring is a tool that should be implemented in bitches in all veterinary clinics, hospitals, and dog breeding sites to ensure the well-being of pregnant bitches and newborns, as well as to decrease the high rates of perinatal mortality in this species. It is a practical, noninvasive technique that is easy to use and accessible in most cases.

Weight can affect the vitality of newborns and the uterine dynamics of bitches, as weight groups differed in the frequency, intensity, and duration of myometrial contractions. The greater the weight of the bitches was, the more uterine dynamics changed, with the most intense and frequent contractions occurring in the heaviest primiparous dams. The expulsion interval between puppies was highest in the lightest primiparous dams and lowest in the heaviest multiparous dams. The duration of the expulsion phase, as well as the number of stillbirths, was greater in the heavier primiparous females. Similarly, the heaviest pups were born to the heaviest primiparous dams. The highest number of stillbirths (16) was observed in primiparous females of G_4, with a total of 72 stillborn pups (23.61%).

Newborn male puppies were significantly heavier than newborn females, and birth weight also differed significantly according to dam weight group. However, no significant differences in the expulsion interval were found between female and male puppies. Thus, these findings suggest that the sex of the newborn does not influence its survival.

Future directions

Veterinarians in the field of obstetrics of domestic canines and felines have several objectives: to increase the proportion of fetuses born alive, to minimize mothers' morbidity and mortality, and to increase newborn survival during the first week of life. Electronic fetal and uterine monitoring is a tool in veterinary medicine that could facilitate perinatal care, thereby improving the survival of pups and the welfare of the animals, saving valuable time when making decisions of vital importance, and helping reduce production costs due to losses or deaths. The evaluation and correct interpretation of decelerations of the fetal heartbeat can indicate whether a bitch will require a cesarean section in a timely manner, and thereby reduce mortality rates. Although this tool has many advantages, some authors (77) caution that it is not helpful in preventing cerebral palsy and other neurodevelopmental disorders. Thus, several techniques that, when combined, provide the most complete fetal and maternal evaluation possible should be used (4). For example, MFE using cardiotocography, thermography (78, 79), evaluation of newborn vitality (APGAR) (6, 80–82), gasometry (83, 84), and evaluation of the morphology of the umbilical cord can be performed.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by Before carrying out the study, an informed consent was provided to the animals' owners evaluated, so they authorized carrying out the procedures. All work was performed under Mexico's Official Norm NOM-062-ZOO-1999

guidelines on the technical specifications for animal production, care, and ethical use in applied ethological studies (32). This project was approved by Ph.D. Program in the Biological and Health Science Academic Committee with number CBS.114.19. All the females evaluated in this study were treated gently, avoiding to the maximum the stress that manipulation could generate, and the fact that using an electronic fetal and uterine monitor greatly facilitated this aspect because it is a non-painful or invasive technique. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

KL-G: Investigation, Methodology, Writing – original draft. JM-B: Project administration, Writing – review & editing. UB-E: Formal analysis, Methodology, Writing – review & editing. DV-G: Project administration, Resources, Writing – review & editing. AO-H: Methodology, Project administration, Writing – review & editing. IH-Á: Supervision, Writing – review & editing. PM-M: Methodology, Supervision, Writing – review & editing. AD-O: Investigation, Supervision, Writing – review & editing. DM-R: Conceptualization, Investigation, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CAPÍTULO 4.

Assessment of Vitality, Blood Profile, and Degree of Meconium Staining on the Skin in Newborn Dogs According to Its Birth Weight

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Article

Assessment of Vitality, Blood Profile, and Degree of Meconium Staining on the Skin in Newborn Dogs According to Its Birth Weight

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Simple Summary: Preventing neonatal mortality is a critical aspect of canine perinatology. Among the leading causes of mortality, a low vitality score at birth, hypothermia, hypoxia, and hypoglycemia can be mentioned. This study aimed to assess vitality, blood values, and the degree of meconium staining on newborns' skin concerning their birth weight. It is concluded that larger newborns tend to present greater problems in surviving.

Abstract: Neonatal mortality in dogs reaches up to 40%. Due to the high rates, promptly detecting the causes and preventing newborns from dying are extremely important. Vitality evaluation, blood parameters, and the degree of meconium staining on the skin are valuable resources in canine perinatology. In this study, 435 puppies from 85 bitches close to parturition were recruited and divided into four quartiles according to the puppy's birth weight: Q_1 (127–200 g) n = 110 puppies, Q_2 (201–269 g) n = 108 puppies, Q_3 (270–388 g) n = 108 puppies, and Q_4 (389–464 g) n = 109 puppies. This experimental article aimed to report the effect of birth weight on the blood profile variables, the vitality of newborn puppies, and the meconium staining degree, integrating these three aspects. It was concluded that the weight of newborns was correlated with the degree of meconium staining, presenting more cases of severe meconium staining in the puppies of the highest birth weight group. The weight of the newborns was correlated with a higher number of stillbirths and alterations in the blood variables, showing the most severe cases of metabolic acidosis, hypoxia, and hypoglycemia in the puppies of the Q_4 quartile. On the contrary, no statistically significant correlations were found between the weight of newborns and vitality. Nevertheless, the analysis of the results showed that the most vigorous puppies were found at Q_1; however, at minute 60 after birth (AB), all the puppies in the four quartiles standardized their vitality scores.

Keywords: whelping; animal perinatology; puppy welfare; newborn puppy; vitality; meconium staining; blood biomarkers

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1. Introduction

During whelping, dogs' mortality rates can reach up to 40% [1]. For this reason, these high rates worry dog owners and breeders [1,2] since one in ten puppies can die before

60 days of age [3]. In addition, newborn puppies are highly immature, making them extremely vulnerable [4,5]. Perinatal death could occur before parturition when the fetus is forming in the uterus, during expulsion, immediately after birth, or during the first weeks of life [2,6,7], especially on the first seven days [8,9], but during birth is when the majority of stillbirths occur [10].

In dogs, similarly to humans, pigs, and cattle [11], puppies with less birth weight are more likely to die. For example, in piglets, this risk is a dozen times more likely than in animals with normal weight at birth [11].

One factor observed to affect newborns' adaptation to extrauterine life negatively is asphyxia [12,13], including both their vitality and viability. This, in turn, can delay the newborn reaching the dam's teat [8,14,15]. There have been some reports in dogs that have presented a certain level of transitory asphyxiation during parturition. In a certain way, this can become normal during parturition, generating in puppies transitory acidosis and hypercapnia [16–18]. The gas exchange could be altered if these conditions continue [19], decreasing respiration rates and generating metabolic acidosis in newborns [20]. Fortunately, blood gases can be evaluated through the umbilical cord in other species, such as humans. In this way, we can obtain valuable information regarding the neonatal acid–base status [21,22]. Therefore, gasometry is currently an important tool for assessing the health status of newborns [8,23]. In small species, the assessment of umbilical blood parameters is limited. However, it is a technique that has been applied to newborn piglets to evaluate concentrations of pH, partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), glucose, lactate, hematocrit, sodium, potassium, and ionized calcium [24].

Newborn mortality risk also includes the proportion of liveborn (LB) vs. stillbirth (SB) puppies and their viability [25,26], with hypoxia being a determinant factor that can alter a newborn's blood profile [27–29].

On the other hand, meconium staining of the skin at birth and aspiration of meconium reflect dystocia processes with severe intrauterine hypoxia [30]. Newborns exposed to meconium aspiration develop Meconium Aspiration Syndrome(MAS) [31]. MAS increases neonatal mortality due to hypoxemia, acidosis, respiratory distress [31–33], and pulmonary edema due to the proinflammatory mediators contained in meconium [34]. MAS has been reported to occur in diverse species; for example, in puppies, the reported mortality from MAS can reach 1–3% [35]. Various articles explain the pathophysiology of MAS; however, Swarman et al. [36], Martínez-Burnes et al. [31], and Mota-Rojas et al. [33] describe the association between MAS, airway obstruction, and fetal hypoxia, this being one of the most critical factors that can cause a loss of vitality in newborns.

Neonatal vitality refers to the capacity of newborns to respond to parturition stress. Vitality is evaluated by the Apgar scoring system adapted for human and animal newborns [2,37]. According to Randall [38], low viability scores in piglets are related to low birth weights and hypercapnia and, as stated by Zaleski and Hacker [39] and De Roth and Downie [40], are also positively associated with pH and negatively with PCO_2.

Although studies in dogs assessing the effect of the dam's weight on the puppy's birth weight, litter size, vitality, survival [1,41,42], and the occurrence of asphyxia and newborn hematological values have been evaluated [27], the effect of the birth weight on the blood profile, meconium staining degree, and vitality has not been comprehensively studied. Therefore, this study aimed to evaluate the effect of birth weight on blood profile variables, the vitality of newborn puppies, and the meconium staining degree, integrating the three aspects. We hypothesized that bigger newborns would have lower vitality scores, significant blood profile alterations, and more cases of meconium staining than smaller puppies.

2. Materials and Methods

2.1. Facilities

This study was developed in the facilities of 10 veterinary hospitals in the municipality of Campeche, Campeche State, Mexico, where there is a tropical climate with a temperature

between 36 and 40 °C. To carry out this study, the tutors of pregnant bitches were asked for their collaboration. The bitches were given medical attention and monitoring from day 25 of gestation until 48 after the puppy's birth. All births took place in the clinics. Once the probable date of parturition was calculated, some bitches stayed sheltered in the clinics, or the guardians took them to the clinics when they began to notice changes in the bitches' behavior. The tutors did not participate during the whelping; only veterinary staff attended and monitored it.

2.2. Study Population

Four hundred thirty-five puppies from eighty-five parturient bitches were recruited and divided into four groups classified in quartiles, following Mugnier et al. [43] and Tesi et al.'s [44] method. The first quartile (Q_1) represents the lowest 25% of registered values, the second quartile (Q_2) represents 25–50%, the third quartile (Q_3) represents 50–75%, and the fourth quartile represents 75–100% (Q_4). Animals in group Q_1 were considered low-weight, while those belonging to Q_4 were considered high-weight puppies. This classification is based on the great variety of dog breeds, ranging from Chihuahuas weighing 500 g as adults to mastiffs weighing 100 kg [45]. Within the breeds included in this study, we can mention Chihuahua, Yorkshire Terrier, Poodle, Scottish Terrier, Cocker Spaniel, Standard Schnauzer, German Shepherd, Labrador, Golden Retriever, Great Dane, and Belgian Shepherd. Quartiles were calculated at the puppy level with this formula: Qa = Li ((aN/4 + Fi-1)/Fi) Ai, where Li is the lower limit of the class where the quartile is located, N is the sum of the absolute frequencies, Fi-1 is the accumulated frequency of the previous class, and Ai is the amplitude of the class, that is, the number of values contained in the interval. The groups were Q_1 (127–200 g) n = 110 puppies, Q_2 (201–269 g) n = 108 puppies, Q_3 (270–388 g) n = 108 puppies, and Q_4 (389–464 g) n = 109 puppies.

The inclusion criteria were the same used in previous studies by Reyes-Sotelo et al. [27,28] and Lezama-García et al., 2022. Mota-Rojas et al.'s [29,46] classification of type I and type II stillbirths in piglets was used to define which could be considered for the study and which could not. According to Mota-Rojas et al. [30,46], stillbirths can be classified into two types: type I, also known as prepartum or antepartum deaths, includes fetuses that die before the end of gestation, usually of infectious causes and have a rather characteristic hemorrhagic and edematous appearance with a grayish-brown discoloration; type II stillbirths, which are also referred to as intrapartum deaths, can die during parturition typically from intrauterine asphyxia and rarely from infectious diseases, and they have the same appearance of their normal littermates, but they do not breathe. Type I stillbirths were excluded from the study, and only type II stillbirths were included, classified by necropsy.

Puppies were weighed using a digital scale from Salter Weight Tronix Ltd., West Bromwich, UK, immediately after the dam stopped licking and cleaning their amniotic fluids and placental membranes.

2.3. Blood Sampling

Blood samples were taken by a veterinarian when the bitch finished removing the chorioallantoic membranes. When necessary, an assistant held the puppy in a supine position and exposed the abdominal region. When the puppies allowed it, they were only placed in lateral decubitus on an adult diaper to perform the sampling. Venous blood from the umbilical cord (0.3 mL) was obtained with a tuberculin syringe and a lithium heparin-impregnated needle. All samples (150 µL) were processed using a GEM Premier® critical blood variable analyzer (Instrumentation Laboratory Diagnostics, Lexington, KY, USA/Milano, Italy). The metabolites analyzed were glucose (mg/dL), lactate (mg/dL), blood gases pCO_2 (mmHg), pO_2 (mmHg) pH, HCO_3 − (mmol/L), EB (mEq/L), Ca++ (mmol/L), and hematocrit (Htc %). All profiles were tested for each LB and type II SB pup.

2.4. Meconium Staining Degree on Skin

To assess the degree of meconium staining in the skin of the puppies, they were divided according to the methodology of Mota-Rojas et al. [30] into absent, mild, moderate, and severe (Figure 1). It is important to emphasize that the process of resuscitation of the newborns by the mother was not interrupted since the puppies were taken just after the bitch finished removing the chorioallantoic membranes and as soon as possible to prevent the bitch from licking the meconium stain; once the stain was impregnated in the white towel, the puppy was returned to the dam so that she could continue with the licking and maternal care.

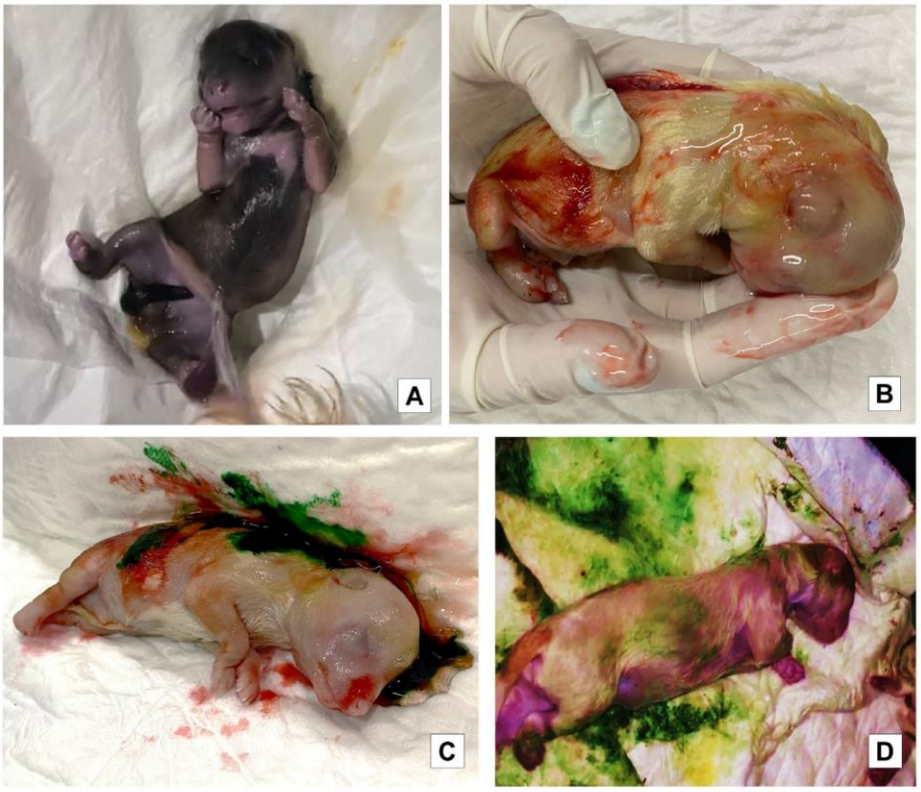

Figure 1. Different degrees of meconium staining on the skin in liveborn (LB) puppies. (**A**). Liveborn (LB) with amniotic fluid and no meconium staining. (**B**). Liveborn (LB) with mild meconium staining. (**C**). Liveborn (LB) with moderate meconium staining. (**D**). Liveborn (LB) with severe meconium staining.

The puppies born free of meconium staining on the skin were considered absent of staining, a mild degree was considered when the body was covered less than 30% of its surface, a moderate degree was considered by 30 to 60%, and a severe degree was considered when its body was covered over 60%. A white human bed diaper was used to identify the degree of meconium staining in the dark-colored animals, pressing it on the newborn body to more clearly observe the impregnated color (Figures 2 and 3). It is worth mentioning that the puppies were removed from the mother as soon as possible at birth to prevent the bitch from licking the meconium stain; once the stain was impregnated in the white towel, the puppy was returned to the dam so that she could continue with the licking and maternal care.

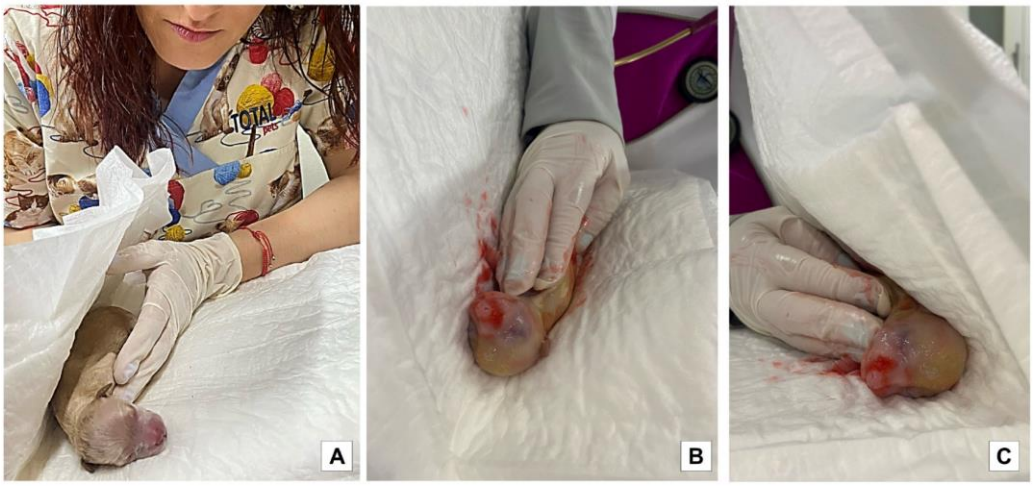

Figure 2. Methodology used to evaluate the degree of meconium staining in puppies. (**A**): The pup was gently picked up before the bitch began to lick it to prevent her from removing meconium staining if it was present. (**B**,**C**): The puppy was placed in a white diaper and surrounded with it to impregnate the meconium staining.

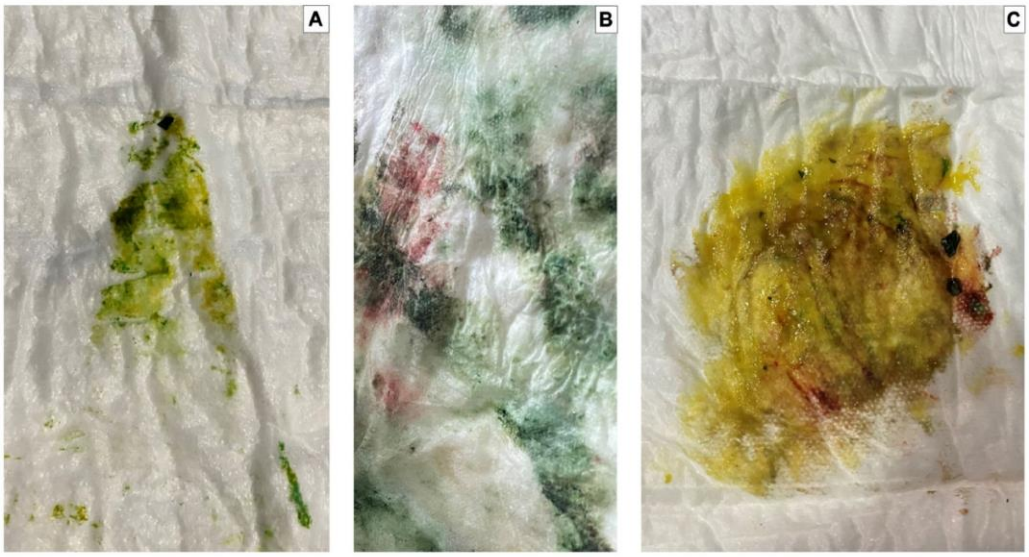

Figure 3. Different degrees of meconium staining. Different degrees of meconium staining were observed using the methodology described in the previous figure. (**A**): Mild. (**B**): Moderate. (**C**): Severe.

2.5. Vitality Score

For the evaluation of vitality in the newborns, the Veronesi [1] scale was used with Randall's [38] adaptation of the Apgar score for human newborns and modified by Mota-Rojas et al. [47]. The variables measured in the first minute after birth were respiratory effort (no crying/< 6 respiratory rates (rr), mild crying/6 to 15 rr, and crying/>15 rr); motility (flaccid, some flexions, and active motion); heart rate (beats per min): <180, between 180 and 220, and >220; mucus color (cyanotic, pale, and pink). Also, the meconium staining on the skin was classified as severe, moderate, mild, or absent based on the methodology used before in pigs by Mota-Rojas et al. [30,47]. The score for vitality was 0 (the least favorable)

to 2 (the most favorable), and a global score ranging from 1 to 10 was obtained for each newborn puppy (Figure 4). A vitality score of 6 or less was considered as failure.

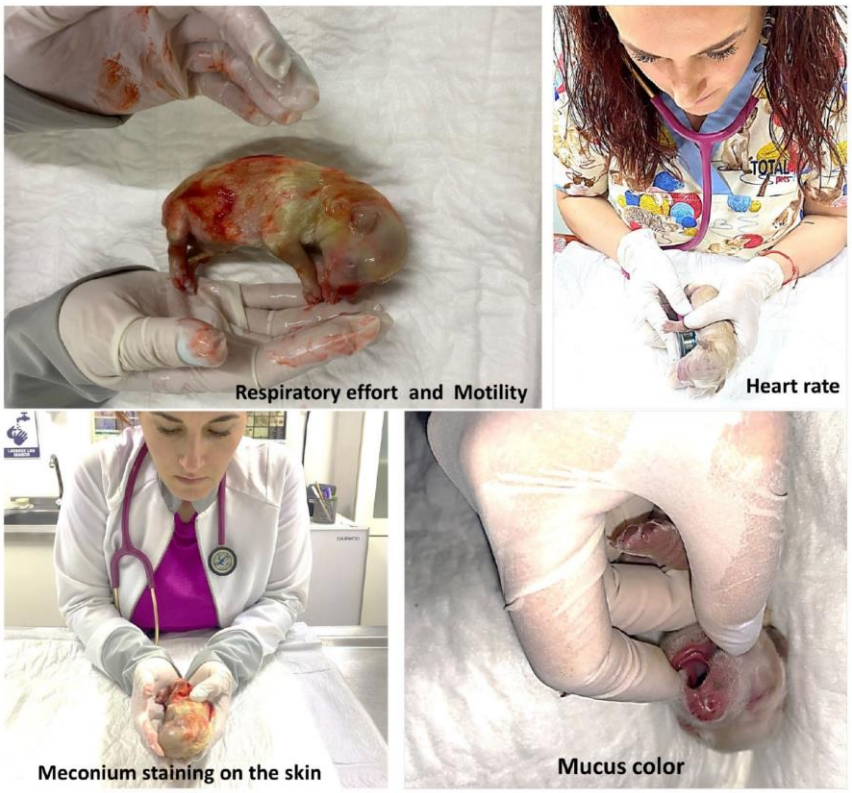

Figure 4. Evaluation of vitality score in first minute after birth.

2.6. Statistical Analysis

Statistical analyses were performed in R version 4.2.2 (R Core Team, Vienna, Austria) using the packages "moments", "ggpubr", "stats", "emmeans", and "multcompView". The significance level was set at $p < 0.05$.

The effect of puppy weight on blood profile variables was analyzed using separate one-way ANOVA with the four puppy weight groups (quartiles) as categorical predictors. The normality of blood-profile-dependent variables was assessed through a visual inspection of histograms, Q-Q plots, and the skewness and kurtosis of data. Post hoc pairwise comparisons between quartiles were carried out using Tukey HSD tests.

The effect of puppy weight on newborn puppy vitality scores at different time points (1 min AB, 5 min AB, 60 min AB) was evaluated using a linear mixed model with puppy vitality score set as the response variable. Puppy weight groups (quartiles) and timepoint were fitted as fixed effects, and puppy ID was set as a random effect to account for the nonindependence of puppies across time points. The normality and homoscedasticity of model residuals were assessed with a visual inspection of Q-Q plots and residuals vs. predicted values plots. Post hoc pairwise comparisons between groups and time points were conducted using Tukey HSD tests.

Differences in the proportion of stillbirths grouped according to birth weight (quartiles) and the presence of meconium stain on their skin were assessed using a chi-square test.

2.7. Ethical Statement

The Ph.D. Program in Biological and Health Science Academic Committee approved the present study (CBS.114.19). The care and use of animals was performed according

to guidelines for the ethical use of animals in applied ethology studies [48]. The owners provided informed consent.

3. Results

A one-way ANOVA was used to determine significant differences between puppy weight quartiles in pH ($F(3) = 102.70$, $p < 0.001$), pCO_2 ($F(3) = 30.97$, $p < 0.001$), pO_2 ($F(3) = 54.53$, $p < 0.001$), glucose ($F(3) = 4.581$, $p = 0.004$), Ca^{++} ($F(3) = 67.36$, $p < 0.001$), lactate ($F(3) = 48.27$, $p < 0.001$), hematocrit ($F(3) = 51.98$, $p < 0.001$), and HCO_3 ($F(3) = 40.28$, $p < 0.001$). The post hoc Tukey HSD pairwise comparisons between quartiles for the blood profile variables of puppies born alive are shown in Table 1.

Table 1. Mean and standard error of blood profile values from puppies born alive and classified according to their birth weight.

Blood Trait	Q_1 n = 110	Q_2 n = 108	Q_3 n = 108	Q_4 n = 109
pH	7.49 ± 0.0126 ^{a}	7.24 ± 0.0136 ^{b}	7.25 ± 0.0116 ^{b}	7.24 ± 0.0125 ^{b}
pCO_2 (mmHg)	46.9 ± 0.879 ^{a}	56.5 ± 1.41 ^{b}	58.8 ± 1.46 ^{b}	64.0 ± 1.42 ^{c}
pO_2 (mmHg)	18.0 ± 0.325 ^{a}	14.4 ± 0.323 ^{b}	14.0 ± 0.302 ^{b}	12.5 ± 0.306 ^{c}
Glucose (mg/dL)	93.7 ± 1.83 ^{a}	92.7 ± 2.44 ^{a}	93.4 ± 2.76 ^{a}	81.9 ± 2.99 ^{b}
Ca^{++} (mmol/L)	1.59 ± 0.0196 ^{a}	1.90 ± 0.0206 ^{a}	1.90 ± 0.0200 ^{a}	1.94 ± 0.0208 ^{b}
Lactate (mmol/L)	4.74 ± 0.226 ^{a}	6.86 ± 0.198 ^{a,b}	7.54 ± 0.216 ^{b}	8.29 ± 0.252 ^{c}
Hematocrit (%)	45.3 ± 0.408 ^{a}	48.7 ± 0.348 ^{b}	50.0 ± 0.404 ^{b}	51.8 ± 0.316 ^{c}
HCO_3^- (mmol/L)	21.7 ± 0.357 ^{a}	19.0 ± 0.322 ^{b}	17.8 ± 0.305 ^{b,c}	17.0 ± 0.321 ^{c}

(One-way ANOVA.) Q_1, 127–200 g; Q_2, 201 g–269 g; Q_3, 270 g–388 g; Q_4, 389 g–464 g. ^{a,b,c} Different superscripts between columns indicate statistically significant differences between quartiles with $p < 0.05$.

Table 1 shows how some blood values in the puppies born alive presented marked variations between Q_1 and Q_4, such as PCO_2, with the highest significant difference between these quartiles. If we compare the blood values obtained in Q_1 with Q_4, the biggest changes were reflected in Q_4: PO_2 decreased by 5.5 mmHg, glucose decreased by 11.8 mg/dL, hematocrit increased by 6.5%, and HCO_3 decreased by 4.7 mmol/L. Lactate showed significant changes between Q_1 and Q_4, doubling its value in the quartile of puppies with the highest weight. All these findings can be assumed as indicators of stress to which larger puppies were subjected, which can be evidenced by hypercapnia, hypoxia, hypoglycemia, and marked metabolic acidosis, as well as polycythemia, hypercalcemia, and marked hyperlactatemia.

A statistically significant difference between puppy weight quartiles in the blood profile variables pCO_2 ($F(3) = 10.74$, $p < 0.001$), pO_2 ($F(3) = 12.04$, $p < 0.001$), lactate ($F(3) = 11.88$, $p < 0.001$), and HCO_3 ($F(3) = 7.316$, $p < 0.001$) were found. Unlike these results, pH ($F(3) = 2.217$, $p = 0.0943$), glucose ($F(3) = 0.177$, $p = 0.912$), Ca^{++} ($F(3) = 2.073$, $p = 0.112$), and hematocrit ($F(3) = 0.672$, $p = 0.572$) did not show a significant difference between puppy weight quartiles. The post hoc Tukey HSD pairwise comparisons between quartiles for the blood profile variables of the stillbirth puppies are shown in Table 2.

Table 2. Mean and standard error of blood profile values in stillbirth puppies classified according to their birth weight.

Blood Trait	Q_1 n = 100	Q_2 n = 108	Q_3 n = 108	Q_4 n = 109
pH	6.82 ± 0.0479 ^{a}	6.80 ± 0.0449 ^{a}	6.73 ± 0.0325 ^{a}	6.82 ± 0.0176 ^{a}
pCO_2 (mmHg)	82.5 ± 2.37 ^{a}	83.2 ± 1.34 ^{ab}	91.7 ± 1.48 ^{c}	95.1 ± 1.36 ^{b,c}
pO_2 (mmHg)	9.24 ± 0.531 ^{a}	8.2 ± 0.932 ^{a}	5.71 ± 0.486 ^{b}	4.34 ± 0.386 ^{b}
Glucose (mg/dL)	41.0 ± 6.06 ^{a}	40.0 ± 4.34 ^{a}	37.7 ± 2.10 ^{a}	37.7 ± 2.26 ^{a}
Ca^{++} (mmol/L)	2.12 ± 0.0630 ^{a}	2.12 ± 0.0650 ^{a}	2.26 ± 0.0474 ^{a}	2.17 ± 0.0216 ^{a}
Lactate (mmol/L)	12.3 ± 0.682 ^{a}	12.1 ± 0.480 ^{a}	14.3 ± 0.340 ^{b}	14.7 ± 0.226 ^{b}
Hematocrit (%)	59.9 ± 1.01 ^{a}	60.5 ± 0.423 ^{a}	59.3 ± 0.725 ^{a}	59.1 ± 0.585 ^{a}
HCO_3^- (mmol/L)	12.8 ± 0.685 ^{a}	12.8 ± 0.685 ^{a}	13.2 ± 0.571 ^{a,b}	10.6 ± 0.343 ^{b}

(One-way ANOVA.) Q_1, 127–200 g; Q_2, 201 g–269 g; Q_3, 270 g–388 g; Q_4, 389 g–464 g. ^{a,b,c} Different superscripts between columns indicate statistically significant differences between quartiles with $p < 0.05$.

Table 2 reports the blood profile alterations between the four quartiles in the SB puppies, and as shown in Table 1 for LB, many parameter alterations were observed. However, in this case, all the blood profile indicators in SB showed acid–base imbalance, manifested with a pH below 7 that denotes severe metabolic acidosis. In the same way, there was hypercapnia in the four quartiles, which was more marked in Q_4. Also, there were marked decreases in glucose, with values ranging from 37.7 mg/dL (Q_4) to 41 mg/dL (Q_1), and a notable increase in Ca^{++} in all quartiles regardless of weight. In addition, lactate showed extremely high values—practically tripled if we compare them with the lactate values for LBs (LB 8.29 mmol/L and SB 14.7 mmol/L). Elevated hematocrit again denoted splenic contraction because of the release of catecholamines, and HCO_3 was found in values decreased to half of those presented in this parameter in LBs due to the marked metabolic acidosis that the newborns experienced until death.

A linear mixed model (LMM) revealed that newborn puppies' vitality score was significantly affected by the interaction between puppy weight group (quartile) and timepoint ($F(6,722) = 5.652$, $p < 0.001$). The post hoc Tukey HSD pairwise comparisons between quartiles and time after birth (AB) are reported in Table 3.

Table 3. Estimated marginal mean and standard error of vitality score of newborn puppies evaluated at different times and classified according to their birth weight.

Time	Q_1 n = 110	Q_2 n = 108	Q_3 n = 108	Q_4 n = 109
Minute 1 AB	$7.28 \pm 0.173^{a,1}$	$6.80 \pm 0.180^{a,1}$	$6.62 \pm 0.187^{a,b,1}$	$5.77 \pm 0.207^{b,1}$
Minute 5 AB	$7.64 \pm 0.173^{a,1,2}$	$7.38 \pm 0.180^{a,2}$	$7.28 \pm 0.187^{a,2}$	$6.81 \pm 0.207^{a,2}$
Minute 60 AB	$7.95 \pm 0.173^{a,2}$	$7.73 \pm 0.180^{a,2}$	$7.68 \pm 0.187^{a,2}$	$7.48 \pm 0.207^{a,3}$

(Linear mixed model.) Q_1, 127–200 g; Q_2, 201 g–269 g; Q_3, 270 g–388 g; Q_4, 389 g–464 g. AB: After birth. ^{a,b} Different superscripts between columns indicate statistically significant differences between quartiles with $p < 0.05$. ^{1,2,3} Different numbers among rows indicate statistically significant differences between times of evaluation.

In Table 3, we can see that Q_1 puppies were the most vigorous, and Q_4 had the lowest scores in terms of vitality. However, after 60 min AB, the vitality began to become uniform, showing an increase in the vitality score to almost double in Q_4 (from 5.77 min 1 AB to 7.48 min 60 AB), with statistically significant differences ceasing to exist between the four quartiles 60 min AB.

The results of the two-way ANOVAs for blood profile variables showed that the interaction between birth weight quartiles and puppy vitality score groups significantly affected the levels of pCO_2 ($F(6) = 3.389$, $p = 0.003$), pO_2 ($F(6) = 3.539$, $p = 0.002$), Ca^{++} ($F(6) = 2.738$, $p = 0.013$), lactate ($F(6) = 3.091$, $p = 0.006$), hematocrit ($F(6) = 6.628$, $p < 0.001$), and HCO_3 ($F(6) = 6.628$, $p < 0.001$) in the newborn puppies. Furthermore, pH and glucose levels differed significantly between puppy weight quartiles (pH: $F(3) = 184.034$, $p < 0.001$; glucose: $F(3) = 8.686$, $p < 0.001$) and across puppies classified according to vitality score (pH: ($F(2) = 140.863$, $p < 0.001$; $F(2) = 159.742$, $p < 0.001$). The post hoc Tukey HSD pairwise comparisons between puppy weight quartiles and vitality score groups are reported in Table 4.

A statistically significant effect on vitality score for all blood values on puppies classified according to weight was observed (Table 4). Glucose and pH had no interactions, while pCO_2, pO_2, Ca^{++}, lactate, hematocrit, and HCO_3 had interactions and were significantly affected. Also, in all quartiles, the values that were most affected in puppies with failed vitality were pCO_2 (increased), pO_2 (decreased), lactate (doubled), glucose (halved), and HCO_3 (almost halved). The results demonstrate how the puppies that presented the lowest vitality scale (failed) fought to survive, thereby causing extreme metabolic acidosis because of the activation of all the compensatory factors the newborn uses to survive.

Table 4. Mean and standard error of blood profile values in newborn puppies born alive classified according to their vitality score (failed, medium, and high) and birth weight.

Blood Trait	V 0–5 (Failed) *n* = 102	V 6–7 (Medium) *n* = 105	V 8–10 (High) *n* = 158
	Q_1		
pH	$7.29 \pm 0.0207^{a,1}$	$7.50 \pm 0.0197^{b,1}$	$7.55 \pm 0.0108^{b,1}$
pCO_2 (mmHg)	$62.3 \pm 2.30^{a,1}$	$42.5 \pm 1.07^{b,1}$	$43.8 \pm 0.362^{b,1}$
pO_2 (mmHg)	$13.7 \pm 0.578^{a,1}$	$18.7 \pm 0.697^{b,1}$	$19.0 \pm 0.306^{b,1}$
Glucose (mg/dL)	$62.8 \pm 4.73^{a,1}$	$99.4 \pm 2.55^{b,1}$	$101.0 \pm 0.943^{b,1}$
Ca^{++} (mmol/L)	$1.92 \pm 0.0519^{a,1}$	$1.56 \pm 0.0259^{b,1}$	$1.51 \pm 0.0108^{b,1}$
Lactate (mmol/L)	$8.09 \pm 0.639^{a,1}$	$4.25 \pm 0.325^{b,1}$	$3.91 \pm 0.163^{b,1}$
Hematocrit (%)	$52.2 \pm 0.858^{a,1}$	$43.6 \pm 0.555^{b,1}$	$43.8 \pm 0.273^{b,1}$
HCO_3^- (mmol/L)	$15.1 \pm 0.665^{a,1}$	$22.2 \pm 0.328^{b,1}$	$23.6 \pm 0.189^{b,1}$
	Q_2		
pH	$7.09 \pm 0.0214^{a,2}$	$7.27 \pm 0.0159^{b,2}$	$7.31 \pm 0.0196^{b,2}$
pCO_2 (mmHg)	$75.3 \pm 2.83^{a,2}$	$53.9 \pm 1.84^{b,2}$	$48.6 \pm 0.447^{b,1,2}$
pO_2 (mmHg)	$11.0 \pm 0.502^{a,2}$	$14.4 \pm 0.443^{b,2}$	$16.4 \pm 0.370^{c,2}$
Glucose (mg/dL)	$65.3 \pm 4.67^{a,1}$	$95.1 \pm 3.21^{b,1}$	$106.0 \pm 2.59^{b,1}$
Ca^{++} (mmol/L)	$2.08 \pm 0.0500^{a,1}$	$1.87 \pm 0.0294^{b,2}$	$1.82 \pm 0.0231^{b,2}$
Lactate (mmol/L)	$9.08 \pm 0.416^{a,1,2}$	$6.96 \pm 0.249^{b,2}$	$5.50 \pm 0.145^{c,2}$
Hematocrit (%)	$51.5 \pm 0.871^{a,1}$	$47.9 \pm 0.519^{b,2}$	$47.8 \pm 0.380^{b,2}$
HCO_3^- (mmol/L)	$14.6 \pm 0.608^{a,1}$	$19.8 \pm 0.385^{b,2}$	$20.6 \pm 0.213^{b,2}$
	Q_3		
pH	$7.14 \pm 0.0183^{a,1,2}$	$7.26 \pm 0.0164^{b,2}$	$7.32 \pm 0.0122^{b,2}$
pCO_2 (mmHg)	$71.0 \pm 2.70^{a,1}$	$57.1 \pm 2.65^{b,2}$	$52.1 \pm 1.28^{b,2}$
pO_2 (mmHg)	$11.3 \pm 0.472^{a,1,2}$	$14.2 \pm 0.429^{b,2}$	$15.6 \pm 0.371^{b,2}$
Glucose (mg/dL)	$71.3 \pm 5.24^{a,1}$	$92.7 \pm 4.69^{b,1}$	$108.0 \pm 2.44^{c,1}$
Ca^{++} (mmol/L)	$2.05 \pm 0.0427^{a,1,2}$	$1.89 \pm 0.0336^{b,2}$	$1.81 \pm 0.0171^{b,2}$
Lactate (mmol/L)	$9.75 \pm 0.226^{a,2}$	$7.16 \pm 0.377^{b,2}$	$6.36 \pm 0.212^{b,2}$
Hematocrit (%)	$52.2 \pm 0.976^{a,1}$	$48.9 \pm 0.538^{b,2}$	$49.2 \pm 0.498^{b,2,3}$
HCO_3^- (mmol/L)	$14.4 \pm 0.382^{a,1}$	$18.6 \pm 0.505^{b,2,3}$	$19.5 \pm 0.204^{b,2}$
	Q_4		
pH	$7.17 \pm 0.0150^{a,2}$	$7.28 \pm 0.0147^{b,2}$	$7.33 \pm 0.0169^{b,2}$
pCO_2 (mmHg)	$71.4 \pm 1.49^{a,2}$	$61.8 \pm 3.13^{b,2}$	$51.9 \pm 0.782^{b,2}$
pO_2 (mmHg)	$11.2 \pm 0.395^{a,2}$	$14.4 \pm 0.507^{b,2}$	$13.3 \pm 0.471^{a,b,3}$
Glucose (mg/dL)	$62.6 \pm 1.82^{a,1}$	$89.8 \pm 6.21^{b,1}$	$111.0 \pm 1.96^{c,1}$
Ca^{++} (mmol/L)	$2.06 \pm 0.0178^{a,2}$	$1.90 \pm 0.0448^{b,2}$	$1.76 \pm 0.0193^{b,2}$
Lactate (mmol/L)	$9.68 \pm 0.228^{a,2}$	$8.26 \pm 0.428^{a,2}$	$5.75 \pm 0.226^{b,2}$
Hematocrit (%)	$53.1 \pm 0.369^{a,1}$	$50.1 \pm 0.546^{b,2}$	$50.6 \pm 0.593^{a,b,3}$
HCO_3^- (mmol/L)	$15.4 \pm 0.349^{a,1}$	$17.2 \pm 0.690^{a,3}$	$19.7 \pm 0.230^{b,2}$

(Two-way ANOVA.) Q_1, 127–200 g; Q_2, 201 g–269 g; Q_3, 270 g–388 g; Q_4, 389 g–464 g. V: Vitality. ^{a,b,c} Different superscripts between columns indicate statistically significant differences between vitality score and quartiles with $p < 0.05$. ^{1,2,3} Different numbers among rows indicate statistically significant differences between blood values on different quartiles.

All blood profile variables maintained a significant interaction between birth weight and the variation in the degree of meconium staining (pH: $F(9) = 4.538$, $p < 0.001$; pCO_2: $F(9) = 4.349$, $p < 0.001$; pO_2: $F(9) = 3.287$, $p < 0.001$; glucose: $F(9) = 5.318$, $p < 0.001$; Ca^{++}: $F(9) = 4.792$, $p < 0.001$; lactate: $F(9) = 3.751$, $p < 0.001$; hematocrit: $F(9) = 5.599$, $p < 0.001$; and HCO_3: $F(9) = 6.013$, $p < 0.001$). The post hoc Tukey HSD pairwise comparisons are shown in Table 5.

Table 5. Effect of the meconium staining degree on blood profile values of newborn puppies classified according to birth weight.

Blood Trait	Absent	Mild	Moderate	Severe
		Q_1		
pH	7.54 ± 0.0098 ^{a,1}	7.47 ± 0.0323 ^{a,1}	7.11 ± 0.0919 ^{b,1}	7.21 ± 0.0476 ^{b,1}
pCO_2 (mmHg)	43.5 ± 0.378 ^{a,1}	45.4 ± 2.45 ^{a,1}	69.8 ± 2.45 ^{b,1}	67.9 ± 3.79 ^{b,1}
pO_2 (mmHg)	18.9 ± 0.314 ^{a,1}	18.5 ± 0.666 ^{a,1}	11.8 ± 0.869 ^{b,1}	12.2 ± 0.482 ^{b,1}
Glucose (mg/dL)	101.00 ± 0.877 ^{a,1}	95.4 ± 4.70 ^{a,1}	55.0 ± 5.59 ^{b,1}	51.0 ± 3.76 ^{b,1}
Ca^{++} (mmol/L)	1.51 ± 0.0102 ^{a,1}	1.60 ± 0.0485 ^{a,1}	2.04 ± 0.0371 ^{b,1}	1.98 ± 0.0659 ^{b,1}
Lactate (mmol/L)	3.87 ± 0.145 ^{a,1}	4.96 ± 0.604 ^{a,1}	8.95 ± 0.736 ^{b,1,2}	10.1 ± 0.749 ^{b,1}
Hematocrit (%)	43.8 ± 0.259 ^{a,1}	44.9 ± 1.49 ^{a,1}	55.0 ± 1.21 ^{b,1}	54.0 ± 1.15 ^{b,1}
HCO_3^- (mmol/L)	23.3 ± 0.189 ^{a,1}	21.9 ± 0.468 ^{a,1}	14.1 ± 0.435 ^{b,1}	13.5 ± 0.402 ^{b,1}
		Q_2		
pH	7.29 ± 0.0154 ^{a,2}	7.26 ± 0.0224 ^{a,1,2}	7.14 ± 0.0280 ^{a,1}	6.90 ± 0.0380 ^{b,2}
pCO_2 (mmHg)	49.6 ± 0.774 ^{a,1,2}	60.5 ± 3.56 ^{a,b,1,2}	68.2 ± 4.42 ^{b,1}	82.6 ± 1.31 ^{c,2}
pO_2 (mmHg)	15.5 ± 0.371 ^{a,2}	13.8 ± 0.824 ^{a,2}	12.5 ± 0.484 ^{a,b,1}	9.04 ± 0.637 ^{b,1,2}
Glucose (mg/dL)	103.0 ± 1.99 ^{a,1}	87.6 ± 5.28 ^{a,b,1}	76.0 ± 6.78 ^{b,1}	44.3 ± 3.75 ^{c,1}
Ca^{++} (mmol/L)	1.82 ± 0.0176 ^{a,2}	1.95 ± 0.0453 ^{a,b,2}	1.99 ± 0.0560 ^{b,1}	2.15 ± 0.0595 ^{b,1}
Lactate (mmol/L)	6.02 ± 0.184 ^{a,2}	7.74 ± 0.373 ^{a,1,2}	8.18 ± 0.596 ^{a,,1}	11.0 ± 0.470 ^{b,1}
Hematocrit (%)	48.2 ± 0.435 ^{a,2}	49.0 ± 1.20 ^{a,1,2}	52.3 ± 1.14 ^{a,b,1}	54.2 ± 1.44 ^{b,1}
HCO_3^- (mmol/L)	20.6 ± 0.247 ^{a,2}	19.2 ± 0.469 ^{a,1,2}	14.8 ± 0.562 ^{b,1}	13.1 ± 0.380 ^{b,1}
		Q_3		
pH	7.28 ± 0.0185 ^{a,2}	7.14 ± 0.0614 ^{a,b,2,3}	7.05 ± 0.0704 ^{b,1}	7.00 ± 0.0409 ^{b,2}
pCO_2 (mmHg)	53.9 ± 1.58 ^{a,2}	64.9 ± 5.03 ^{a,b,2}	77.2 ± 4.34 ^{b,1}	77.7 ± 2.92 ^{b,1,2}
pO_2 (mmHg)	14.9 ± 0.405 ^{a,2,3}	12.8 ± 0.976 ^{a,b,2}	9.75 ± 1.01 ^{b,1}	9.42 ± 0.709 ^{b,1,2}
Glucose (mg/dL)	105.0 ± 2.79 ^{a,1}	74.9 ± 7.15 ^{b,1,2}	63.4 ± 8.55 ^{b,1}	58.7 ± 4.92 ^{b,1}
Ca^{++} (mmol/L)	1.83 ± 0.0219 ^{a,2}	1.98 ± 0.0630 ^{a,b,2}	2.10 ± 0.0688 ^{b,1}	2.12 ± 0.0378 ^{b,1}
Lactate (mmol/L)	6.51 ± 0.258 ^{a,2}	9.46 ± 0.899 ^{b,2,3}	10.8 ± 0.832 ^{b,1,2}	11.4 ± 0.399 ^{b,1}
Hematocrit (%)	49.7 ± 0.445 ^{a,2}	51.2 ± 1.60 ^{a,b,2,3}	53.6 ± 1.89 ^{a,b,1}	54.2 ± 0.941 ^{b,1}
HCO_3^- (mmol/L)	19.5 ± 0.207 ^{a,2}	17.8 ± 0.712 ^{a,2,3}	14.2 ± 0.526 ^{b,1}	13.2 ± 0.273 ^{b,1}
		Q_4		
pH	7.29 ± 0.0275 ^{a,2}	7.00 ± 0.0670 ^{b,3}	7.08 ± 0.0445 ^{b,1}	7.02 ± 0.0283 ^{b,2}
pCO_2 (mmHg)	55.3 ± 2.13 ^{a,2}	83.5 ± 3.65 ^{b,3}	76.2 ± 3.05 ^{b,1}	83.1 ± 2.43 ^{b,2}
pO_2 (mmHg)	12.7 ± 0.614 ^{a,3}	8.12 ± 1.13 ^{b,3}	10.0 ± 0.854 ^{a,b,1}	8.10 ± 0.731 ^{b,2}
Glucose (mg/dL)	103.0 ± 4.41 ^{a,1}	49.8 ± 4.14 ^{b,2}	61.2 ± 4.79 ^{b,1}	51.9 ± 3.05 ^{b,1}
Ca^{++} (mmol/L)	1.78 ± 0.0218 ^{a,2}	2.08 ± 0.0288 ^{b,2}	2.07 ± 0.0320 ^{b,1}	2.13 ± 0.0212 ^{b,1}
Lactate (mmol/L)	6.75 ± 0.443 ^{a,2}	11.9 ± 0.880 ^{b,3}	11.1 ± 0.630 ^{b,2}	12.0 ± 0.436 ^{b,1}
Hematocrit (%)	51.3 ± 0.689 ^{a,2}	56.7 ± 1.50 ^{b,3}	54.8 ± 0.970 ^{a,b,1}	55.0 ± 0.558 ^{b,1}
HCO_3^- (mmol/L)	19.1 ± 0.422 ^{a,2}	15.6 ± 0.970 ^{b,3}	12.8 ± 0.565 ^{c,1}	13.2 ± 0.514 ^{b,c,1}

(Two-way ANOVA.) Q_1, 127–200 g; Q_2, 201 g–269 g; Q_3, 270 g–388 g; Q_4, 389 g–464 g. ^{a,b,c} Different superscripts between columns indicate statistically significant differences between the degree of meconium staining and quartiles with $p < 0.05$. ^{1,2,3} Different numbers among rows indicate statistically significant differences between blood values on different quartiles.

Table 5 shows that puppies with a higher degree of meconium staining presented low pH values. pCO_2 and Ca^{++} increased in severe meconium staining, and pO_2 decreased. These findings suggest that as the degree of meconium staining increased, more significant blood profile alterations were observed regardless of weight or quartile. Ca^{++}, pCO2, hematocrit, and lactate values increased directly in severe meconium staining. On the contrary, in severe meconium staining, the pH, pO_2, glucose, and HCO_3 decreases were inversely proportional.

A chi-square test was performed to determine whether the proportion of stillbirths differed between puppies classified according to birth weight groups and the presence of meconium staining. The proportion of stillbirth puppies significantly differed between

puppy weight quartiles and degrees of meconium staining ($\chi^2 = 89.475$, df = 15, $p < 0.001$). The number and percentage of liveborn (LB) and stillbirths (SBs) can be observed in Table 6.

Table 6. Number and percentage of liveborn (LB) and stillbirth (SB) puppies according to their weight and the presence of meconium staining on the skin: absent, mild, moderate, and severe, and significant statistical differences.

Staining Degree	Q_1 *n* = 110		Q_2 *n* = 108		Q_3 *n* = 108		Q_4 *n* = 109	
	LB (%)	SB (%)	LB (%)	SB (%)	LB (%)	SB (%)	LB (%)	SB (%)
Absent	77 (70) ^{a,1}	0 ^{a,1}	58 (53) ^{a,1}	3 (2.7) ^{a,1}	51 (47.2) ^{a,1}	2 (1.8) ^{a,1}	26 (23.8) ^{a,1}	2 (1.8) ^{a,1}
Mild	12 (10.9) ^{a,1,2}	0 ^{a,1,2}	14 (12.9) ^{a,1}	0 ^{a,1}	11 (10.1) ^{a,1}	3 (2.7) ^{a,1}	6 (5.5) ^{a,1}	6 (5.5) ^{a,1}
Moderate	6 (5.4) ^{a,2}	3 (2.7) ^{a,2}	12 (11.1) ^{a,1}	2 (1.8) ^{a,1}	10 (9.2) ^{a,1}	5 (4.6) ^{a,1}	18 (16.5) ^{a,1}	10 (9.1) ^{a,1}
Severe	10 (9) ^{a,1,2}	2 (1.8) ^{a,1,2}	12 (11.1) ^{a,1}	7 (6.4) ^{a,1}	18 (16.6) ^{a,1}	8 (7.4) ^{a,1}	23 (21.1) ^{a,1}	18 (16.5) ^{a,1}

(X^2 test). Q_1, 127–200 g; Q_2, 201 g–269 g; Q_3, 270 g–388 g; Q_4, 389 g–464 g. LB: liveborn; SB: stillbirth. ^{a} Different superscript between columns indicates statistically significant differences between quartiles and meconium staining degree with $p < 0.05$. ^{1,2} Different numbers among rows indicate statistically significant differences between the degree of meconium staining in liveborn and stillbirth puppies.

Table 6 shows that regardless of weight or quartile, the degree of meconium staining significantly affected the number of SBs, which was greater in severe meconium staining and lower or even null in absent meconium staining degree.

The correlation coefficients for the blood profile values, the degree of meconium staining, and the vitality score are reported in Table 7, Table 8, and Table 9, respectively.

Table 7. Significant correlations between puppies' weight and blood profile values.

Variables	Correlation Coefficient (*rho*)	*p*-Value
	Q_1	
pH	−0.125	0.193
pCO_2 (mmHg)	0.273	0.004 *
pO_2 (mmHg)	−0.137	0.154
Glucose (mg/dL)	−0.165	0.085
Ca^{++} (mmol/L)	0.197	0.039
Lactate (mmol/L)	0.138	0.151
Hematocrit (%)	0.119	0.217
HCO_3^- (mmol/L)	−0.109	0.257
	Q_2	
pH	0.178	0.065
pCO_2 (mmHg)	−0.205	0.034 *
PO_2 (mmHg)	0.168	0.082
Glucose (mg/dL)	0.324	<0.001 *
Ca^{++} (mmol/L)	−0.038	0.695
Lactate (mmol/L)	−0.214	0.026 *
Hematocrit (%)	−0.266	0.005 *
HCO_3^- (mmol/L)	0.224	0.019 *
	Q_3	
pH	0.167	0.084
pCO_2 (mmHg)	−0.006	0.954
pO_2 (mmHg)	0.012	0.900
Glucose (mg/dL)	0.304	0.001 *
Ca^{++} (mmol/L)	−0.213	0.027 *
Lactate (mmol/L)	0.063	0.516
Hematocrit (%)	0.017	0.861
HCO_3^- (mmol/L)	0.039	0.686
	Q_4	
pH	−0.587	<0.001 *
pCO_2 (mmHg)	0.621	<0.001 *
PO_2 (mmHg)	−0.512	<0.001 *
Glucose (mg/dL)	−0.579	<0.001 *
Ca^{++} (mmol/L)	0.412	<0.001 *
Lactate (mmol/L)	0.603	<0.001 *
Hematocrit (%)	0.532	<0.001 *
HCO_3^- (mmol/L)	−0.541	<0.001 *

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their blood profile values. * Indicates significant statistical differences.

Table 8. Significant correlations between puppies' weight and meconium staining degree. The meconium staining degree has been recoded to numerical values. Higher the stain degree = higher number. Absent = 1, mild = 2, moderate = 3, and severe = 4.

Variables	Correlation Coefficient (*r*)	*p*-Value
All puppies (quartiles)	0.3319875	$p < 0.001$
Q_1	0.111	0.248
Q_2	−0.200	0.038 *
Q_3	0.023	0.811
Q_4	0.214	0.025 *

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their meconium staining degree. * Indicates significant statistical differences.

Table 9. Significant correlations between puppies' weight and its vitality.

Variables	Correlation Coefficient (*r*)	*p*-Value
	Q_1	
Vitality Score min 1	0.0644	0.514
Vitality Score min 5	0.116	0.238
Vitality Score min 60	0.0325	0.742
	Q_2	
Vitality Score min 1	0.0587	0.568
Vitality Score min 5	0.148	0.148
Vitality Score min 60	0.174	0.0882
	Q_3	
Vitality Score min 1	−0.0323	0.762
Vitality Score min 5	0.0430	0.687
Vitality Score min 60	0.143	0.179
	Q_4	
Vitality Score min 1	0.0912	0.443
Vitality Score min 5	0.0822	0.489
Vitality Score min 60	0.0887	0.456

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their vitality.

Table 7 shows that in Q_1, weight significantly affected pCO2, with a positive correlation of 0.273 ($p = 0.004$). In Q_2, there was a significant effect of weight on pCO_2, lactate, glucose, hematocrit, and HCO_3. Q_3 had a positive correlation with glucose ($p < 0.001$) and calcium ($p = 0.027$). In the case of Q_4, there were significantly high positive and negative correlations, for all the variables with statistically significant levels of $p < 0.001$, so it can be concluded that having more weight causes high positive correlations with all blood variables. We have marked with an asterisk the values in which there were statistically significant correlations.

Table 8 reports that birth weight was correlated with the degree of meconium staining in Q_2, with $r^2 = -0.200$ and $p = 0.038$, and in Q_4, with $r^2 = 0.214$ and $p = 0.025$, so it can be stated that birth weight was correlated with meconium staining in Q_2 and Q_4. We have marked with an asterisk the values in which there were statistically significant correlations.

Table 9 shows that no statistically significant correlations were found in any of the groups or at any time due to the effect of birth weight and vitality.

4. Discussion

The results show that birth weight influences the thermoregulation, hemodynamic changes, and degree of meconium staining in newborn dogs, as reported in previous studies performed by the authors [4,49].

4.1. Blood Profile Values

The most notable blood profile changes were associations between Q_4 puppies (heavier newborns) with hypoxia, hypoglycemia, hypercalcemia, hyperlactatemia, and marked metabolic acidosis.

The plasma levels of HCO_3^- observed in this study were 21.7 mmol/L for Q_1 LB, 12.8 mmol/L for Q_1 SBs, 17 mmol/L for Q_4 LB, and 10.6 mmol/L for Q_4 SB. These values could demonstrate severe pulmonary hypoxia and elevated lactic acid production because when its production is greatly increased, the organism activates the use of HCO_3 as a buffer. However, because of the increased consumption of HCO_3^-, newborns experience mixed (respiratory and metabolic) acidosis and metabolic deficiencies due to these anaerobic and respiratory processes that ultimately lead to hypoxia [50].

Variations in the balance of glucose levels during the neonatal stage depend on endogenous production and glucose metabolism, resulting in hyper- or hypoglycemia. According to Vannucchi et al. [51], hypoglycemia is the leading cause of neonatal mortality because a rapid depletion of energy resources can cause acute hypothermia. In other words, the rapid reduction in fetal circulation at delivery caused by uterine contractions and the pressure they can exert on the fetal umbilical cord is associated with rapid liver glycogen depletion and decreased glucose homeostasis [52]. In our study, within these adverse circumstances, we can mention that for Q_4, the whelping usually took longer because they are breeds that give birth to litters that are larger in number and size, which can prolong the expulsion interval. The significant differences in blood glucose concentration in the quartiles (62.8 mg/dL in Q_1; 65.3 mg/dL in Q_2; 71.3 mg/dL in Q_3, and 62.6 mg/dL in Q_4) could be associated with neonatal stress and the consequent release of catecholamine and promotion of liver glycogenolysis [53,54].

The reduction in the oxygen supply observed in the present study was also reported by Mota-Rojas et al. [46,47] in piglets, in which umbilical cord hypoxia leads to increased pCO_2 due to respiratory acidosis, acting as a risk for prenatal mortality. Similarly, in another study by Massip [16] in fetal sheep near term, the animals showed a compensatory mechanism to respiratory acidosis by increasing pCO_2. The contrary is observed during metabolic acidosis, leading to alveolar hyperventilation secondary to increased H^+ ions in the plasma and a decrease in pCO_2.

These mentioned findings coincide with what was found in our study, where once again, the quartile of the heaviest puppies (Q_4) developed severe hypercapnia, ranging from 64 mmHg in LB to 95.1 mmHg in SB, and this could be explained by the expectation that during birth, there may be variations in cardiorespiratory parameters. Tachycardia, hypercapnia, and respiratory acidosis may arise immediately after birth. According to Alonso-Spilsbury et al. [55] and Vannucchi et al. [51], physiological hypoxia triggers an increase in respiratory rate and its consequent apnea. The important variations in pH in all quartiles, especially in Q_4 (LB 7.24; SB 6.82), show that bicarbonate and pCO_2 indicate neonatal acidosis and possible fetal hypoxic stress [56].

4.2. Vitality Score

Puppies of Q_4 had the lowest vitality rating of all the quartiles at minute 1 AB. This could be associated with some factors, such as the expulsion interval, since in smaller puppies, it is faster than in larger ones. Therefore, unlike larger puppies, which take longer to be expelled, the smaller ones do not consume energy reserves as much. Hence, the energy consumption in larger puppies to try to compensate for the stress of hypoxia is more significant in the expulsion phase. These biggest newborns have an increased risk of intrauterine asphyxia, affecting their survival chances [26].

Birth weight can also predispose to asphyxia. For example, in humans, low-birth-weight individuals have poor vitality scores [14]. Similarly, Okere et al. [57] found that piglet viability score was highly correlated with their weight (r = 0.66). In contrast to these studies, we found that the biggest puppies (Q_4) had low vitality scores at the first minute AB, but their score improved at minute 60 AB. Our results are similar to the ones reported by Trujillo et al. [58], who concluded that heavy piglets had scores ≤ 5 and showed asphyxia signs. A possible explanation could be that heavier piglets had greater difficulty passing through the birth canal, affecting the health and vitality of the newborns [4,59].

Additionally, Veronesi et al. [60] mention that small-sized puppies may have the highest levels of distress but higher chances of survival when compared to large-sized animals.

Vitality also influences the capability of a newborn to stand and ingest colostrum, limiting the absorption of nutrients and energy obtention [61], parameters that are important in the postpartum period because the disposition of energy resources, such as brown adipose tissue and glucose, differs between low- and high-birth-weight animals. Moreover, blood oxygenation, particularly oxygen saturation (SpO2), has shown a negative correlation with birth weight, meaning that heavier newborns have lower SpO2, and might influence the time to receive oxygen [62].

Surprisingly, as the minutes passed, it was possible to observe that the time factor favored Q_4 because the consumption of colostrum probably matched these puppies, and because at minute 60 AB, almost always, all newborns have already consumed colostrum, in addition to the fact that larger puppies will have more suction force and, therefore, consume more colostrum. This could be sustained with a study by Mila et al. [42], who mentioned that glucose in the colostrum is essential to newborn puppies because only 1.3% of the body fat is available. On the contrary, the colostrum of small-breed bitches (less than 10 kg) provides 10% more energy than the colostrum of large-breed females [3].

In the same way that Herpin et al. [53] and Trujillo-Ortega et al. [58,63] reported, newborn puppies that failed the vitality scale in our study showed a blood rise in pCO_2 and lactic acid, with a low pO_2 in plasma, finding that would confirm that these newborns suffered early tissue hypoxia.

Correlations between birth weight and vitality did not show a statistically significant difference, which can be explained by the fact that, even though the puppies in Q_4 showed failed vitality scores (5.77) at minute 1 AB, they managed to reach high vitality scores (7.48) at minute 60 AB, practically matching the vitality scores of the puppies of the other quartiles (Q_1, Q_2, and Q_3).

4.3. Meconium Staining Degree

Studies performed on piglets suggest that low-weight newborns have a higher frequency of intrapartum mortality [38,64,65]. In contrast, human babies with higher birth weights appear to have a higher predisposition to hypoxia and meconium aspiration [66,67]. This association could be related to parturition problems due to the size and weight of the newborn rather than problems with the umbilical cord. This also could explain why heavier intrapartum piglets showed more severe meconium staining in a study by Mota-Rojas et al. [30].

The present results show that 16.5% of stillbirths had severe meconium staining of the skin in Q_4 puppies. Most stillbirths had mild, moderate, and severe meconium staining (6, 10, and 18 puppies, respectively), suggesting that careful observation is required to assess intrapartum anoxia based on epidermal staining alone correctly. In other words, the highest percentages of stillbirths occurred in Q_4, with 33.02% (36 SB puppies from 109 in total), of which 5.5% were mild meconium staining, 9.1% were moderate, and 16.5% were severe.

The present study found an association between low blood pH values and severe meconium staining, regardless of birthweight, coinciding with previous studies where meconium staining demonstrated the ability to lead to fetal hypoxia and, consequently, acidosis [68]. The presence of meconium can obstruct the airways, leading to acute hypoxemia, hypercapnia, and metabolic acidosis due to tissular anaerobic metabolism that causes a progressive decrease in pulmonary blood flow, exacerbating hypoxia and acidosis [69]. A similar result was reported in humans, in whom 81.82% of newborns that presented thick meconium-stained fluid and low Apgar scores (around 6.45) had blood pH below 7.5 [70]. Therefore, observing meconium-stained puppies could indicate physiological alterations that might not always be evaluated during whelping but could be inferred to adopt a proper perinatal control of stained puppies.

One of the main limitations of this study was that the monitoring of the animals was carried out in 10 different clinics or hospitals to have a larger number of samples. For this

reason, factors such as standardizing the temperatures where the births took place were not possible, but to standardize the areas where the dams gave birth, foam mats and adult bed cover diapers were used. However, parturitions were carried out in Campeche, Mexico, where there is a tropical climate, so situations such as hypothermia due to environmental factors were not observed. As it is a species where the breeds have a great diversity of weights and sizes, the authors decided to classify the animals according to their weight and not their breed. Therefore, another limitation could be that some breeds could have more intense maternal care than others or have longer parturitions due to the size of the litter or newborns.

5. Conclusions

In the present study, we could observe that the weight of newborns was correlated with the degree of meconium staining, presenting more cases of severe meconium staining in the puppies of the highest-birth-weight group. In addition, the weight of the newborns was correlated with the alterations in the blood variables, showing the most severe cases of metabolic acidosis, hypoxia, hypoglycemia, and higher stillbirths in the puppies of the Q_4 quartile. On the contrary, no statistically significant correlations were found between the weight of newborns and vitality. However, it could be observed throughout the analysis of the results that the most vigorous puppies were found in Q_1. However, at minute 60 AB, all the puppies in the four quartiles standardized their vitality scores. According to this, our hypothesis that bigger newborns would have lower scores in vitality, more blood profile alterations, and more cases of meconium staining than smaller puppies was confirmed.

One of the main perspectives that we were able to elucidate with this study, and according to Groppetti et al. [71] and Forsberg [72], is that the use of both fetal and uterine monitoring before, during, and after parturition could be a valuable tool to help predict cases of hypoxia, asphyxia, dystocia, and uterine inertia, which could lead to prolonged deliveries with complications and, therefore, an increase in the mortality rate as well as a decrease in the vitality of newborns [73–76].

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CAPÍTULO 5.

Relation between the Dam's Weight on Superficial Temperature of Her Puppies at Different Stages of the Post-Partum

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Article

Relation between the Dam's Weight on Superficial Temperature of Her Puppies at Different Stages of the Post-Partum

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Simple Summary: Newborn puppies have great difficulty achieving a stable temperature, and it has been seen that this is influenced by the weight of the mother. In the present study, the temperature of the puppies was evaluated in eight different areas of the body and at seven different times after birth. It is concluded that the weight of the mothers significantly influences the thermoregulatory capacity of the puppies and that the areas where the lowest puppies' temperatures were recorded were the thoracic and pelvic limbs, and the highest were the abdominal, thoracic, nasal, and upper left palpebral, especially when newborns dry and at 24 h after birth.

Abstract: The thermal stability of newborns is an essential parameter that can be recorded to evaluate neonatal care. Knowing the thermal windows to evaluate and maintain a constant temperature helps significantly reduce neonatal mortality. This study aimed to assess the superficial temperature alterations in the distinct thermal windows of puppies with mothers of diverse weights and their repercussions. We evaluated the superficial temperature using infrared thermography at eight thermal windows and seven different times: when wet due to the fetal fluid immediately after birth until 24 h of life in newborn puppies from bitches divided into four bodyweight groups. The results revealed a positive correlation between the dam's weight and the ability to achieve thermostability in the newborn puppies in all the evaluated thermal windows. The time effect showed the lowest temperatures when the puppies were still wet, a gradual increase, and the highest temperature at 24 h after birth. The thermal windows with the highest temperatures were abdominal, thoracic, nasal, and upper left palpebral, and those with the lowest were the thoracic limb brachial biceps, thoracic limb elbow, metacarpal, and femoral pelvic limb. A significant increase in the temperatures in the thermal windows of the abdominal, thoracic, and upper left palpebral immediately after ingesting colostrum was observed. The weight of the dams is an important factor that positively intervenes in the thermoregulatory capacity of the puppies, especially when newborns are dry and have been passed 24 h after birth.

Keywords: thermographic image; whelping; dogs; animal perinatology; puppy welfare; newborn puppy

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1. Introduction

Birth is a great challenge for the newborn since it must adapt to extrauterine life and survive the neonatal period. Low vitality is a recurrent issue in veterinary perinatology, and several factors can culminate in newborn death [1]. Various studies on neonate mammals demonstrate that maternal behavior directly impacts newborn survival [2–4]. Moreover, mortality rates between birth and weaning can be linked to maternal and newborn behavior [1,5–8]. Other than this, neonatal viability is related to fetal maturity, the environmental circumstances, and maternal care [9]. In dogs (*Canis familiaris*), mortality in newborn puppies varies from 5 to 35% [10]. Among several maternal, pregnancy, and newborn-related factors, hypothermia is considered to be a condition that could adversely affect newborn survival [11]. One of the main parameters to be controlled during birth is thermal stability that is a fundamental aspect of neonatal care, and maintaining a constant temperature helps significantly reduce neonatal mortality [12]. In other words, thermoregulation plays an essential role in the survival of all altricial species and poikilothermic organisms, especially in neonates [13–17]. Individuals that make the transition from the warm environment of the uterus to an extrauterine environment cause a significant decrease in their corporal temperature at birth [18,19], considering that 35–37 °C is the normal rectal temperature in newborn puppies of less than 1 week [20,21]. This thermal change impacts the newborn pup's ability to thermoregulate since mechanisms such as the shivering reflex and vasoconstriction are still underdeveloped [22,23].

Various methods measure temperature in animals, but most can be invasive, altering the final value [24,25]. This can be due to the stress of being manipulated [26,27]. For this reason, in recent years, it has been seen that a non-invasive technique to evaluate the temperature changes, both on farms and in companion animals, is infrared thermography (IRT) [28]. IRT can also record the exact measurements of the body surface temperature of any organism from a distance of 30 cm or more [29]. This technique detects changes in the blood flow of the microvasculature in response to pathophysiological or environmental events such as heat or cold stress [30].

Studies have been carried out on sheep [5,31,32] and in pigs [33,34] where it has been seen that the survival of the newborn can be influenced by elements such as the dam weight and body condition, parity, breed, age, newborn's weight at birth, and the litter size, as well as by the maternal and offspring behavior [2,35]. In dogs, studies have evaluated the effect of the dam's weight on the puppy's weight at birth, the litter size, vitality, and the puppy's survival [10,36–38]. Additionally, the effect of the dam's weight on the presentation of asphyxia and newborn hematological values were evaluated [39]. In some species, newborns from larger females have reported less thermoregulatory problems, and a higher temperature in central zones of the body [12–17,25,26], so similar results can be expected in dogs. However, there is still no information on thermal windows in puppies (e.g., ocular or auricular regions), known as a body region of the neonate where superficial temperatures can be assessed [40]. The use of these regions could help understand the process of thermoregulation in newborn dogs, and thus help reduce the high mortality rates in this species. In addition, the effect of the dam weight and the pups on their thermoregulatory capacity during the first 24 h after birth is also unknown.

For this reason, this study aims to evaluate the alterations in the different thermal windows of puppies with mothers of different body weights and their repercussions on the presentation of hypothermia in newborns. The present authors have the following hypothesis: (1) newborns from bitches with a high body weight will have fewer thermoregulation problems, and those from low body weight bitches will have more thermoregulation complications; in the same way, the body weight of puppies can affect thermoregulation due to the large body surface that they present; (2) thermal windows with more elevated temperatures will be the ones closest to the brain and vital organs (palpebral, thoracic, abdominal, and nasal); and (3) the thermal windows with the lowest temperatures will be the ones furthest from the vital structures (thoracic limbs and pelvic limbs).

2. Materials and Methods

2.1. Facilities

A network of 10 participating veterinary clinics was formed to monitor canine and puppy births. The study region is located in southeastern Mexico, specifically in the Yucatan peninsula, bordering to the north and northeast with Yucatan, to the east with Quintana Roo, to the south with Guatemala and Belize, to the west with the Gulf of Mexico, and to the southwest with Tabasco. The owners of pregnant bitches were offered medical attention for prenatal control from day 25 of pregnancy to the first 48 h after parturition.

2.2. Study Population

Seventy-two pregnant young multiparous bitches (2–4 births) were recruited. However, 12 bitches required an emergency C-section during parturition and were excluded. In total, 290 puppies from 60 parturient bitches were included in the present study. Within the breeds included in this study we found Chihuahua, German Shepherd, Labrador, Golden Retriever, Great Dane, Standard Schnauzer, Cocker Spaniel, Poodle, Scottish Terrier, and Belgian Shepherd. A total of 60 bitches were divided into 4 groups of 15 bitches each, according to their body weight as follows: G_1 (4–8 kg) $n = 47$ puppies, G_2 (8.1–16 kg) $n = 68$ puppies, G_3 (16.1 to 32 kg) $n = 79$ puppies, and G_4 (32.1 to 39.6 kg) $n = 96$ puppies. The obstetric condition of the bitches, as well as the gestation from the beginning to the term (from day 28 to day 30 after mating), was monitored as reported later in the prenatal procedure section.

The body weight of the bitches was obtained using a digital scale from Salter Weight Tronix Ltd., West Bromwich, UK, immediately at the first stage of whelping, when the contractions started. The inclusion criteria were: (a) clinically healthy dogs with valid vaccination/deworming records; (b) no clinical records of reproductive problems; and (c) bitches with ultrasonographic and radiographic studies to sustain a natural birth. The exclusion criteria were: (a) bitches with previous cases of dystocia or pyometra; (b) primiparous dams; (c) malformed fetuses; (d) the administration of birth inducers or accelerators; (e) behavioral problems (aggressive females); (f) bitches with a body condition over 8 (obese) as per the WSAVA scale [41]; (g) any large breeds and brachycephalic bitches due to their reported high incidence of dystocia; and (h) those that required an emergency C-section [42]. The bodyweight ranges were based on the Federation Cynologique Internationale (FCI) [43].

2.3. Clinical History

The clinical history of the sampled animals was carried out by collecting data such as their age, type of diet, parity number, bodyweight, breed, history of preventive medicine, and description of where they lived. These data were collected with diagnostic and monitoring methodologies controlled by the veterinary software SmartZooft® LAN, 14 K version, developed by SQUENDA®, Mexico City, Mexico.

2.4. Prenatal Procedures

The pregnancy diagnosis was carried out from days 28 to 30 post-mating in the bitches, using a Mindray® model DP-30VetPower ultrasonography equipment (Shenzhen, China) with Doppler and Pulsed Doppler (PW) advanced technologies, equipped with a 3.5 MHz convex transducer. The gestation was corroborated by visualizing the gestational sacs and the presence of a heartbeat in the embryos. The following ultrasonographic assessment was carried out between days 40 and 43 of gestation to determine the viability of the fetuses and their growth and health status. Subsequently, X-ray studies were carried out between days 48 and 50 to determine the number of fetuses and their head sizes and to make measurements to predict if there could be a dystocic whelping, which could even end in cesarean section due to some cephalopelvic disproportion [44]. From day 60 post-mating, the bitches and fetuses were monitored using a Sonolife® (Chihuahua, Mexico) brand antepartum monitor, model Smart Monitor Color, with a multi-crystal pulsed Doppler transducer to evaluate the health status of both the mother and the fetuses, uterine activity,

number, duration, interval and frequency of the contractions, and the fetal heart rate, following a methodology previously reported in piglets by other authors [45]. In cases where fetal heart rate decelerations type 2 (DIP 2) arose (a drop in the fetal heart rate that begins after the onset of a uterine contraction and returns to the baseline only after the uterine contraction has ended, caused by uteroplacental insufficiency), an emergency cesarean section was performed, and these bitches were excluded from the group, that is why none of the puppies used for this study presented meconium-stained amniotic fluid. In the same way, the monitoring of the vital signs of the dams was carried out using a veterinary monitor DESEGO® (Mexico City, Mexico) model M8i SVGA to evaluate the electrocardiographic tracings, respiratory rate, oxygen saturation, temperature, and blood pressure from the probable date of whelping. However, the bitches were only hospitalized in cases where delivery care took place in hospital/clinic facilities and not when delivery took place at the bitches' homes.

2.5. Puppies

Once parturition started, 290 puppies were evaluated when expelled and the bitch began to lick them to separate them from the membranes. The temperature of the room where the bitches gave birth was not controlled because the study was carried out in different clinics, hospitals, or even in the homes of the animals. However, in the city where the study was carried out, the climate is tropical, and the temperatures oscillate between 36 and 40 °C. In all cases, when parturition started, the air conditioners and fans were turned off. The temperature measurements in the puppies were recorded in 7 different stages: (1) wet puppy (wet) with amniotic fluid, once the dam released the puppy of the membranes and temporarily stopped licking it; (2) dry puppy (dry), which was dried by rubbing for 1 min with rag towels and immediately afterwards, the puppy was returned to the mammary gland area of the bitch, and until the puppy made contact with the teat on its own; (3) colostrum pup (colostrum), immediately after it ingested colostrum and separated from its mother's teat; (4) at 30 min of birth (30 min AB); (5) at the first hour of birth (1 h AB); (6) at 4 h after birth (4 h AB); and (7) at 24 h after birth (24 h AB). It is worth mentioning that all the temperatures were obtained without manipulating the puppies except to dry and weight them and to evaluate their vitality.

Finally, the weight of the puppies at birth was obtained using a digital scale from Salter Weight Tronix Ltd., West Bromwich, UK, after drying them. The puppies' weight was obtained only once when the bitch stopped licking the placental membranes. All the puppies of each litter were evaluated. For their identification we used a quick-drying indelible ink marker. It is worth mentioning that the puppies were only fed milk from the dam and were not supplemented with additional milk formulas, and the puppy was returned to the area of the mammary gland so that it could start suckling itself.

2.6. Infrared Thermography

A total of 16,240 thermographic data were evaluated with their minimum, average, and maximum values. These data resulted from 290 puppies, from which 3 thermograms were taken: one from the facial area, another from the left lateral, and another from the right lateral regions. The facial thermogram included the thoracic limbs to not only record the nasal (N) and upper left palpebral (UPL) temperature but also to have a frontal image of the thoracic limb metacarpals (TLM) region. Likewise, the thermogram taken from the right lateral region recorded the thoracic limb elbow (TLE) and femoral pelvic limb (FPL) The temperature records of the thorax and abdomen were obtained from the left lateral image. In each puppy, 8 thermal windows were identified at 7 different times, which are explained in detail in Figure 1; the thoracic limb brachial biceps (TLBB) and thoracic limb metacarpals (TLM) windows were obtained from the area where the armpit begins to half the width of the thoracic limb to the joint formed by the metacarpals, covering the area from the medial to lateral end, respectively. The thoracic limb elbow (TLE) and femoral pelvic limb (FPL) windows were delimited from the elbow area covering the vertex formed

by the humerus-radio-ulnar joint to the space bounded by the edge of the pelvic limb in the biceps femoris region, respectively. The thoracic (T) window was delimited by the axillary area, the anatomical position of the last rib, and from the region of the spinal vertebrae to the ventral part of the abdominal region (A). This window was delimited by two millimeters after the last rib to the inguinal area, and from the region of the spinal vertebrae to the ventral part of the abdomen. The nasal (N) and upper left palpebral (ULP) windows were delimited by the edges of the nasal mucosa and at the edge of the left upper eyelid, respectively.

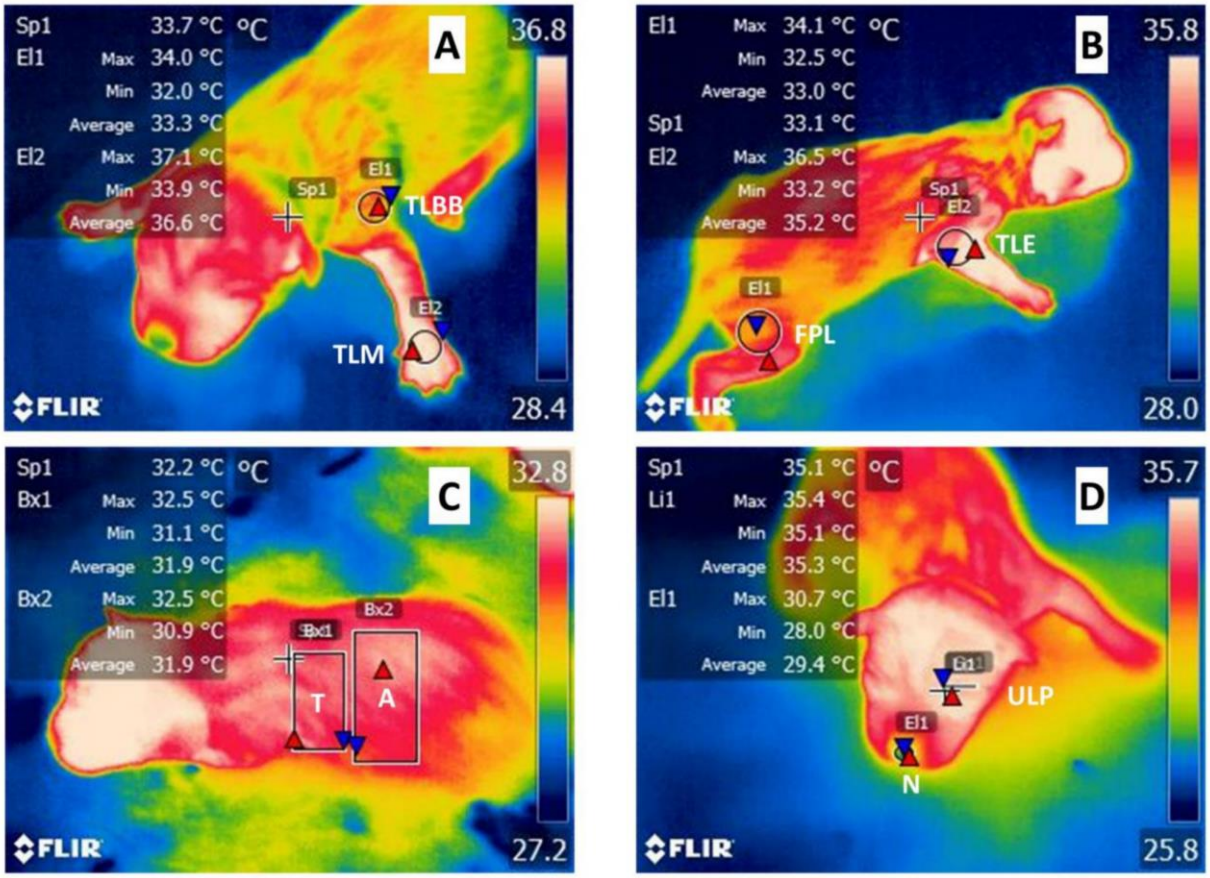

Figure 1. Thermal windows in newborn puppies. (**A**) Thoracic limb brachial biceps (TLBB) and thoracic limb metacarpals (TLM) windows were obtained by placing circular figures from the area where the armpit begins to half the width of the thoracic limb and in the joint formed by the metacarpals covering the area from medial to lateral end, respectively. (**B**) Thoracic limb elbow (TLE) and femoral pelvic limb (FPL) windows were obtained by placing circular figures in the elbow area covering the vertex formed by the humerus-radio-ulnar joint and in the space bounded by the edge of the pelvic limb in the biceps femoris region, respectively. (**C**) Thoracic (T) window was made with rectangular figures delimited by the axillary area, the area of the last rib, and from the region of the spinal vertebrae and to the ventral part of the abdominal region (A) window was delimited by two millimeters after the last rib, to the inguinal area, and from the region of the spinal vertebrae and to the ventral part of the abdomen. (**D**) Nasal (N) and upper left palpebral (ULP) windows were made with circular figures delimited by the edges of the nasal mucosa and at the area of the edge of the left upper eyelid, respectively. Sp1: Spot 1; El1: Ellipse 1; El2: Ellipse 2; Bx1: Box 1; Bx2: Box 2; Li1: Line 1; red triangles maximum temperature of that zone; blue triangles minimum temperature of that zone.

Thermographic images were obtained with an infrared camera FLIR® model Thermal TM E80, FLIR Systems, Wilsonville, OR, USA, with the following specifications: IR resolution 320 × 240 pixels, thermal sensitivity < 0.045 °C, accuracy ± 2 °C or 2% of reading in the ambient temperature of 10 °C to 35 °C and image frequency of 60 Hz. All the images were collected with an emissivity of 0.95 at a uniform distance of 30 cm. Thermographic images were taken to evaluate 8 different zones: (1) thoracic limb brachial biceps (TLBB); (2) thoracic limb elbow (TLE); (3) thoracic limb metacarpals (TLM); (4) femoral pelvic limb (FPL); (5) thoracic (T); (6) abdominal (A); (7) nasal (N); and (8) upper left palpebral (ULP). The thermographic images were saved in JPEG format to analyze later using specialized s FLIR Tools software® 6.x (FLIR Systems, Wilsonville, OR, USA). The maximum, minimum, and mean temperature of each thermal window was obtained in each of the 7 different stages.

It is important to mention that to avoid the type of floor where the puppy is expelled, which could influence the gain or loss of heat, a thermosetting mat based on foam rubber (ethyl vinyl acetate) with a surface area of 1 m^2, 1 cm deep, with a weight of 0.032 kg, and a matte finish was used in all cases.

2.7. Statistical Analysis

Descriptive statistics were obtained for all the variables examined following the procedure outlined in the two-factor Analysis of Variance (ANOVA StatSoft Inc. 0.8, Tulsa, Oakland, CA, USA) to compare the effects of the dam's bodyweight groups (4 categories: G_1, G_2, G_3, and G_4) and time (7 categories: wet, dry, colostrum, 30 min, 1 h, 4 h, and 24 h) by eight different zones (TLBB, TLM, TLE, FPL, T, A, N, and UPL). Additionally, a three-factor ANOVA was used to compare the effects of the dam's body weight (4 categories: G_1, G_2, G_3, and G_4), time (7 categories: wet, dry, colostrum, 30 min, 1 h, 4 h, and 24 h) and zones (8 categories: TLBB, TLM, TLE, FPL, T, A, N, and ULP). The Tukey test ($p < 0.05$) showed a contrast of means. Spearman's rank test was used to establish the correlation between the variables and the relationship between the temperatures and the dam's weights.

2.8. Ethical Statement

All the owners of the study animals were asked to sign their informed consent to carry out the procedures. All work was performed under the guidelines and lineaments of Mexico's Official Norm NOM-062-ZOO-1999 on the technical specifications for animal production, care, and ethical use in applied ethological studies [46]. The Ph.D. Program in the Biological and Health Science Academic Committee approved this project with approval number CBS.114.19. The animals included in the present study were treated gently and were not touched or stressed, since infrared thermography is a non-invasive technique. The only time the puppies were taken was when they were finished drying; they were weighed and their vitality was assessed, and this procedure did not take more than 2 min.

3. Results

3.1. Infrared Thermography

The standard error (SE) of the mean of the eight thermal windows, including the different puppies' time-point, was analyzed between the four groups of bitches. As seen in Figure 2, there are statistically significant differences ($p < 0.0001$) between the four groups (G_1, G_2, G_3, and G_4), but in general and in all thermal windows, groups G_2 and G_3 were similar.

In the same way, the evidence indicates that there were significant differences between the temperature means of the thermal windows and the different puppies' evaluation times (wet, dry, colostrum, 30 min AB, 1 h AB, 4 h AB, and 24 h AB), as can be seen in Figure 3.

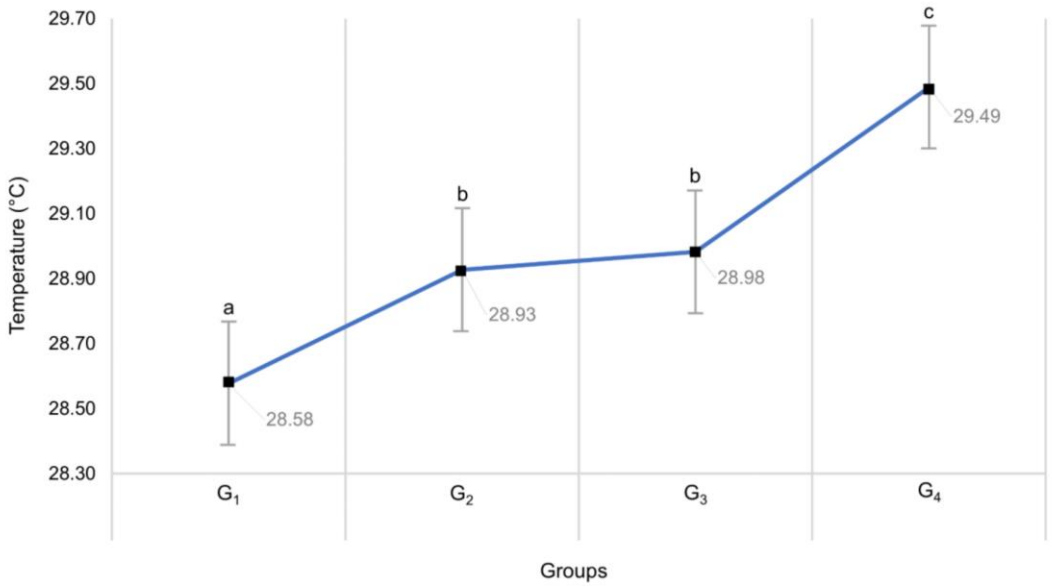

Figure 2. Mean ± standard error of the temperatures in the 4 groups of bitches. Different letters [(a,b,c)] indicate significant differences in temperatures between the 4 groups of bitches (G_1, 4–8 Kg; G_2, 8.1–16 Kg; G_3, 16.1–32 Kg; G_4, 32.1–39.6 Kg) and in the 7 measurement times.

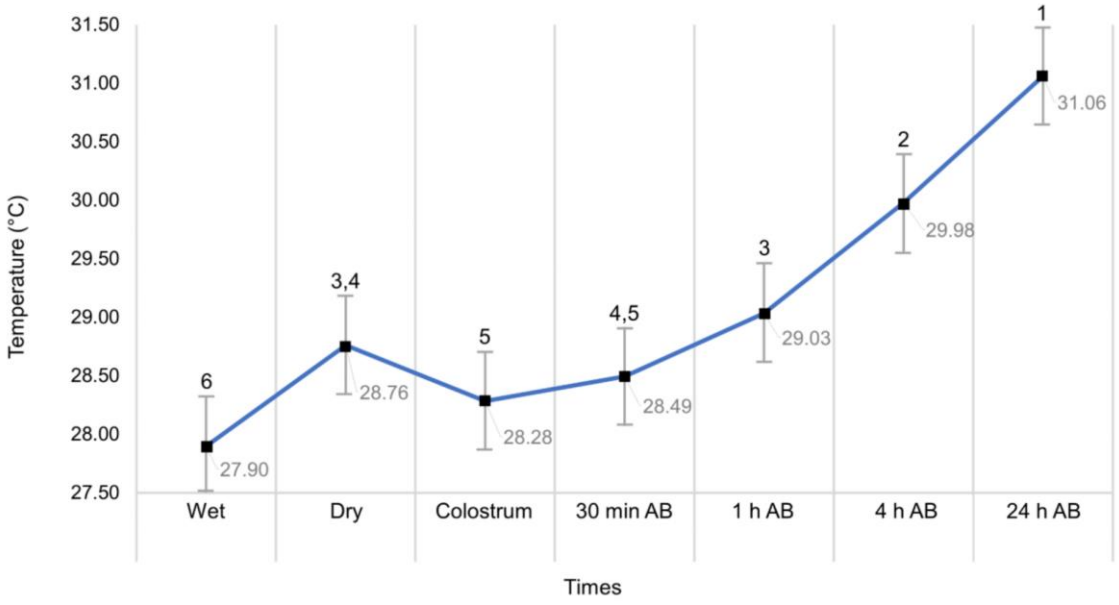

Figure 3. Neonatal average temperature values throughout the experimental period regardless of the surface region or dam's weight. Means ± standard errors. Different numbers [(1,2,3,4,5,6)] indicate significant differences between times in the same dam's weight group. AB: after birth.

There was no interaction between the groups and times ($p > 0.05$), except on the thoracic limb elbow (TLE) thermal window, in which there was an interaction between the group and time ($p < 0.0001$), as it can be observed in Figure 4.

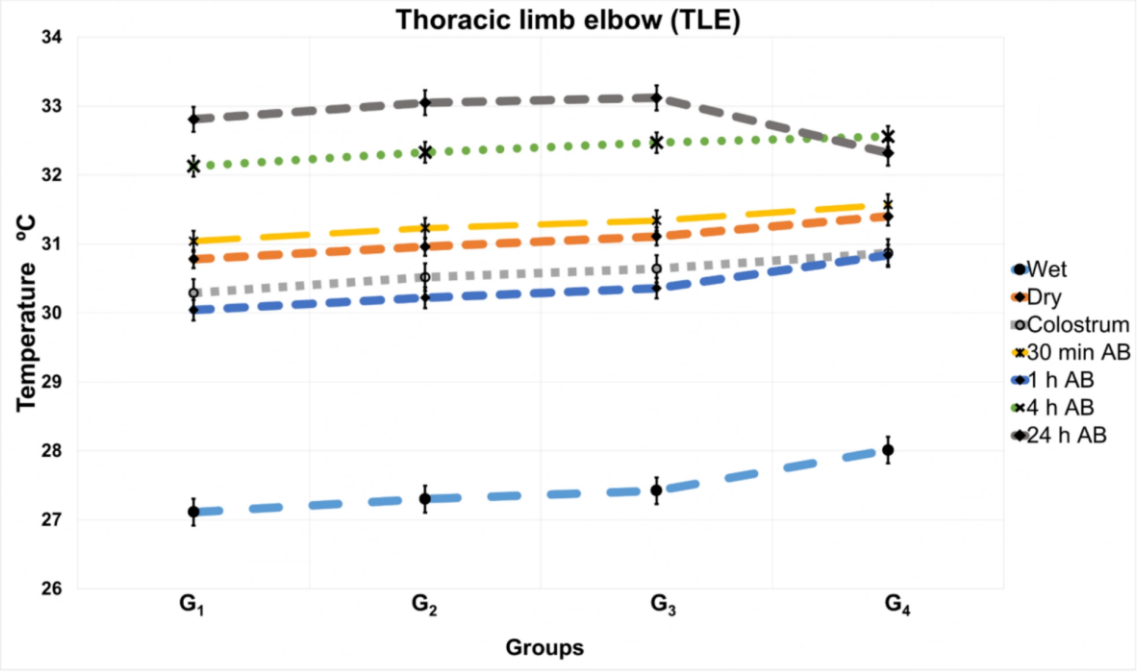

Figure 4. Interaction between group and time in thoracic limb elbow (TLE) thermal window at different times in newborn puppies from bitches classified into 4 groups according to their weight. G_1, dams between 4 and 8 Kg; G_2, dams between 8.1 and 16 Kg; G_3, dams between 16.1 and 32 Kg; G_4, dams between 32.1 and 39.6 Kg, AB: after birth.

On the three-way ANOVA results (the group, thermal window, and time), there were differences between the groups ($p < 0.0001$), between the thermal windows ($p < 0.0001$), and differences between the times ($p < 0.0001$). There was an interaction between the group and thermal window ($p < 0.0001$), there was no interaction between the group and time ($p > 0.05$), there was an interaction between the thermal window and time ($p < 0.0001$), and there was no interaction between the three factors (the group, thermal window, and time) ($p > 0.005$).

3.2. Effect of Maternal Weight and Litter Size on Neonatal Superficial Temperature Changes

The highest temperatures were recorded in puppies born to bitches from the G_4 group, and in all the thermal windows evaluated, the lowest temperatures were in G1 bitches. Regarding G_2 and G_3, similarities were observed between them (Tables 1–8). The average litter size for G_1 was 3.13 puppies, for G_2 was 4.4 puppies, for G_3 was 5.2 puppies, and finally for G_4 was 6.4 puppies. The average of the puppies' weight for each group was G_1 195.12 g, G_2 233.31 g, G_3 332 g, and G_4 396.65 g. It is also important to mention that the mean gestational age by groups was G_1 58 days, G_2 61 days, G_3 60 days, and G_4 67 days.

3.3. Effect of Colostrum Consumption on Thermoregulation

Once the pups ingest colostrum, the temperature decreased significantly ($p < 0.0001$) at windows TLBB, TLE, TLM, FPL, and N. For example, in the TLBB window (Table 1) and TLE (Table 2), the range of this decrease was 0.48 °C in all the groups (G_1, G_2, G_3, and G_4). For the TLM window (Table 3), the average of this decrease was 0.85 °C. However, in thermal window T, the temperature increased immediately after the ingestion of colostrum by an average of 0.21 °C in the four groups (Table 4). The same occurred in thermal window A, with an average of 0.14 °C above the temperature of dry puppies (Table 5). On the FPL window (Table 6), the decrease was 0.55 °C; for the N window (Table 7), the decrease range

was 0.29 °C. Similarly, the temperature in the ULP thermal window increased immediately after the pups ingested colostrum with an average of 1.61 °C above the dry stage (Table 8).

Table 1. Mean temperature (°C) of thoracic limb brachial biceps (TLBB) window at different times in newborn puppies from bitches classified into 4 groups according to its weight.

Time	G_1 $n = 47$	G_2 $n = 68$	G_3 $n = 79$	G_4 $n = 96$
Wet	$27.43 \pm 0.065^{c,5}$	$27.76 \pm 0.051^{b,6}$	$27.80 \pm 0.046^{b,6}$	$28.29 \pm 0.042^{a,6}$
Dry	$28.27 \pm 0.120^{b,3,4}$	$28.61 \pm 0.114^{b,3,4}$	$28.65 \pm 0.114^{b,3,4}$	$29.19 \pm 0.096^{a,3,4}$
Colostrum	$27.75 \pm 0.057^{c,4,5}$	$28.11 \pm 0.047^{b,5}$	$28.20 \pm 0.049^{b,5}$	$28.72 \pm 0.045^{a,5}$
30 min AB	$28.03 \pm 0.104^{c,4}$	$28.33 \pm 0.093^{b,c,4,5}$	$28.40 \pm 0.075^{b,4,5}$	$28.91 \pm 0.073^{a,4,5}$
1 h AB	$28.53 \pm 0.094^{c,3}$	$28.86 \pm 0.078^{b,3}$	$28.95 \pm 0.075^{b,3}$	$29.47 \pm 0.063^{a,3}$
4 h AB	$29.43 \pm 0.110^{c,2}$	$29.85 \pm 0.096^{b,2}$	$29.91 \pm 0.082^{b,2}$	$30.38 \pm 0.076^{a,2}$
24 h AB	$30.59 \pm 0.103^{c,1}$	$30.95 \pm 0.083^{b,1}$	$30.95 \pm 0.070^{b,1}$	$31.45 \pm 0.068^{a,1}$

(ANOVA, StatSoft Inc. 8.0, Tulsa, Oakland, CA, USA). *n*, number of puppies; weight of dams according to category: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; AB, after birth; least-squares mean ± standard error. *p* values for the different temperatures of the pups between groups and times in the regions evaluated were <0.0001 in all cases. ^{a,b,c} Different superscripts among columns indicate significant temperature differences between dam's weight groups at the same time. ^{1,2,3,4,5,6} Different numbers among rows indicate significant differences between times in the same dam's weight group.

Table 2. Mean temperature (°C) of thoracic limb elbow (TLE) window at different times in newborn puppies from bitches classified into 4 groups according to its weight.

Time	G_1 $n = 47$	G_2 $n = 68$	G_3 $n = 79$	G_4 $n = 96$
Wet	$27.11 \pm 0.057^{c,5}$	$27.30 \pm 0.051^{b,c,7}$	$27.42 \pm 0.047^{b,7}$	$28.01 \pm 0.039^{a,4}$
Dry	$30.78 \pm 0.074^{c,4}$	$30.96 \pm 0.058^{b,c,4}$	$31.11 \pm 0.058^{b,4}$	$31.40 \pm 0.060^{a,3}$
Colostrum	$30.29 \pm 0.067^{c,3}$	$30.52 \pm 0.048^{b,c,5}$	$30.64 \pm 0.049^{b,5}$	$30.87 \pm 0.078^{a,2}$
30 min AB	$31.04 \pm 0.059^{c,4}$	$31.23 \pm 0.046^{b,c,3}$	$31.34 \pm 0.044^{a,b,3}$	$31.57 \pm 0.086^{a,3}$
1 h AB	$30.04 \pm 0.071^{c,3}$	$30.22 \pm 0.061^{b,c,6}$	$30.36 \pm 0.060^{b,6}$	$30.84 \pm 0.055^{a,2}$
4 h AB	$32.13 \pm 0.068^{b,2}$	$32.33 \pm 0.057^{a,b,2}$	$32.47 \pm 0.059^{a,2}$	$32.56 \pm 0.078^{a,1}$
24 h AB	$32.81 \pm 0.073^{a,b,1}$	$33.05 \pm 0.066^{b,1}$	$33.12 \pm 0.059^{b,1}$	$32.62 \pm 0.094^{a,1}$

(ANOVA, StatSoft Inc. 8.0, Tulsa, Oakland, CA, USA). *n*, number of puppies; weight of dams according to category: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; AB, after birth; least-squares mean ± standard error. *p* values for the different temperatures of the pups between groups and times in the regions evaluated were <0.0001 in all cases. ^{a,b,c} Different superscripts among columns indicate significant temperature differences between the dam's weight groups at the same time. ^{1,2,3,4,5,6,7} Different numbers among rows indicate significant differences between times in the same dam's weight group.

Table 3. Mean temperature (°C) of thoracic limb metacarpals (TLM) window at different times in newborn puppies from bitches classified into 4 groups according to its weight.

Time	G_1 $n = 47$	G_2 $n = 68$	G_3 $n = 79$	G_4 $n = 96$
Wet	$26.98 \pm 0.073^{c,5}$	$27.14 \pm 0.061^{b,c,5}$	$27.30 \pm 0.054^{b,5}$	$27.75 \pm 0.056^{a,6}$
Dry	$29.84 \pm 0.073^{c,2,3}$	$30.02 \pm 0.062^{b,c,2,3}$	$30.19 \pm 0.062^{b,2}$	$30.46 \pm 0.063^{a,4}$
Colostrum	$28.96 \pm 0.043^{c,4}$	$29.16 \pm 0.033^{b,4}$	$29.25 \pm 0.036^{b,4}$	$29.71 \pm 0.031^{a,7}$
30 min AB	$29.43 \pm 0.10^{b,3,4}$	$29.63 \pm 0.078^{b,3}$	$29.73 \pm 0.081^{b,3}$	$30.14 \pm 0.072^{a,5}$
1 h AB	$30.24 \pm 0.063^{c,1,2}$	$30.38 \pm 0.058^{b,c,2}$	$30.49 \pm 0.053^{b,2}$	$30.95 \pm 0.047^{a,3}$
4 h AB	$30.08 \pm 0.33^{b,2}$	$30.37 \pm 0.24^{b,2}$	$30.46 \pm 0.20^{b,2}$	$31.19 \pm 0.052^{a,2}$
24 h AB	$30.83 \pm 0.10^{b,1}$	$30.89 \pm 0.091^{b,1}$	$31.04 \pm 0.083^{b,1}$	$31.54 \pm 0.073^{a,1}$

(ANOVA, StatSoft Inc. 8.0, Tulsa, Oakland, CA, USA). *n*, number of puppies; weight of dams according to category: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; AB, after birth; least-squares mean ± standard error. *p* values for the different temperatures of the pups between groups and times in the regions evaluated were <0.0001 in all cases. ^{a,b,c} Different superscripts among columns indicate significant temperature differences between the dam's weight groups at the same time. ^{1,2,3,4,5,6,7} Different numbers among rows indicate significant differences between times in the same dam's weight group.

Table 4. Mean temperature (°C) of thoracic (T) window at different times in newborn puppies from bitches classified into 4 groups according to its weight.

Time	G_1 $n = 47$	G_2 $n = 68$	G_3 $n = 79$	G_4 $n = 96$
Wet	$28.79 \pm 0.18^{a,6}$	$28.80 \pm 0.19^{a,6}$	$29.06 \pm 0.13^{a,5}$	$29.20 \pm 0.13^{a,5}$
Dry	$31.44 \pm 0.049^{c,5}$	$31.64 \pm 0.042^{b,5}$	$31.69 \pm 0.040^{b,4}$	$31.93 \pm 0.035^{a,4}$
Colostrum	$31.66 \pm 0.074^{b,4,5}$	$31.87 \pm 0.056^{b,4,5}$	$31.86 \pm 0.052^{b,4}$	$32.16 \pm 0.047^{a,4}$
30 min AB	$31.99 \pm 0.052^{c,3,4}$	$32.17 \pm 0.039^{b,3,4}$	$32.26 \pm 0.037^{b,3}$	$32.51 \pm 0.033^{a,2}$
1 h AB	$32.12 \pm 0.063^{c,2,3}$	$32.35 \pm 0.052^{b,2,3}$	$32.38 \pm 0.045^{b,2,3}$	$32.62 \pm 0.041^{a,2}$
4 h AB	$32.40 \pm 0.066^{c,2}$	$32.68 \pm 0.054^{b,2}$	$32.63 \pm 0.059^{b,c,2}$	$32.92 \pm 0.050^{a,2}$
24 h AB	$33.22 \pm 0.054^{c,1}$	$33.38 \pm 0.052^{b,c,1}$	$33.49 \pm 0.045^{b,1}$	$33.72 \pm 0.041^{a,1}$

(ANOVA, StatSoft Inc. 8.0, Tulsa, Oakland, CA, USA). *n*, number of puppies; weight of dams according to category: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; AB, after birth; least-squares mean ± standard error. *p* values for the different temperatures of the pups between groups and times in the regions evaluated were <0.0001 in all cases. ^{a,b,c} Different superscripts among columns indicate significant temperature differences between the dam's weight groups at the same time. ^{1,2,3,4,5,6} Different numbers among rows indicate significant differences between times in the same dam's weight group.

Table 5. Mean temperature (°C) of abdominal (A) window at different times in newborn puppies from bitches classified into 4 groups according to its weight.

Time	G_1 $n = 47$	G_2 $n = 68$	G_3 $n = 79$	G_4 $n = 96$
Wet	$29.47 \pm 0.12^{b,6}$	$29.77 \pm 0.10^{a,b,5}$	$29.76 \pm 0.098^{a,b,5}$	$29.95 \pm 0.085^{a,5}$
Dry	$32.23 \pm 0.046^{c,5}$	$32.49 \pm 0.037^{b,4}$	$32.55 \pm 0.037^{b,4}$	$32.75 \pm 0.032^{a,4}$
Colostrum	$32.46 \pm 0.075^{b,4,5}$	$32.66 \pm 0.062^{b,4}$	$32.59 \pm 0.060^{b,4}$	$32.90 \pm 0.054^{a,4}$
30 min AB	$32.75 \pm 0.073^{b,3,4}$	$32.99 \pm 0.061^{b,3}$	$32.96 \pm 0.060^{b,3}$	$33.22 \pm 0.055^{a,3}$
1 h AB	$32.84 \pm 0.086^{b,3}$	$33.09 \pm 0.072^{a,b,3}$	$33.08 \pm 0.063^{a,b,3}$	$33.30 \pm 0.061^{a,3}$
4 h AB	$33.20 \pm 0.083^{b,2}$	$33.46 \pm 0.068^{a,b,2}$	$33.38 \pm 0.070^{b,2}$	$33.63 \pm 0.063^{a,2}$
24 h AB	$33.74 \pm 0.061^{c,1}$	$33.92 \pm 0.060^{b,c,1}$	$34.02 \pm 0.053^{b,1}$	$34.23 \pm 0.047^{a,1}$

(ANOVA, StatSoft Inc. 8.0, Tulsa, Oakland, CA, USA). *n*, number of puppies; weight of dams according to category: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; AB, after birth; least-squares mean ± standard error. *p* values for the different temperatures of the pups between groups and times in the regions evaluated were <0.0001 in all cases. ^{a,b,c} Different superscripts among columns indicate significant temperature differences between the dam's weight groups at the same time. ^{1,2,3,4,5,6} Different numbers among rows indicate significant differences between times in the same dam's weight group.

Table 6. Mean temperature (°C) of femoral pelvic limb (FPL) window at different times in newborn puppies from bitches classified into 4 groups according to its weight.

Time	G_1 $n = 47$	G_2 $n = 68$	G_3 $n = 79$	G_4 $n = 96$
Wet	$27.54 \pm 0.071^{c,6}$	$27.90 \pm 0.057^{b,6}$	$27.97 \pm 0.051^{b,5}$	$28.29 \pm 0.046^{a,5}$
Dry	$28.49 \pm 0.12^{b,3,4}$	$28.83 \pm 0.11^{b,3,4}$	$28.84 \pm 0.11^{b,3}$	$29.24 \pm 0.095^{a,3}$
Colostrum	$27.86 \pm 0.065^{c,5,6}$	$28.25 \pm 0.057^{b,5,6}$	$28.39 \pm 0.062^{b,4}$	$28.72 \pm 0.055^{a,4}$
30 min AB	$28.15 \pm 0.12^{b,4,5}$	$28.54 \pm 0.10^{b,4,5}$	$28.51 \pm 0.087^{b,4}$	$28.89 \pm 0.085^{a,4}$
1 h AB	$28.61 \pm 0.097^{c,3}$	$28.97 \pm 0.084^{b,3}$	$29.03 \pm 0.077^{b,3}$	$29.40 \pm 0.066^{a,3}$
4 h AB	$29.62 \pm 0.082^{c,2}$	$30.12 \pm 0.071^{b,2}$	$30.08 \pm 0.064^{b,2}$	$30.43 \pm 0.061^{a,2}$
24 h AB	$30.70 \pm 0.11^{c,1}$	$31.06 \pm 0.088^{b,c,1}$	$31.15 \pm 0.087^{b,1}$	$31.46 \pm 0.075^{a,1}$

(ANOVA, StatSoft Ic. 8.0, Tulsa, Oakland, CA, USA). *n*, number of puppies; weight of dams according to category: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; AB, after birth; least-squares mean ± standard error. *p* values for the different temperatures of the pups between groups and times in the regions evaluated were <0.0001 in all cases. ^{a,b,c} Different superscripts among columns indicate significant temperature differences between the dam's weight groups at the same time. ^{1,2,3,4,5,6} Different numbers among rows indicate significant differences between times in the same dam's weight group.

Table 7. Mean temperature (°C) of nasal (N) window at different times in newborn puppies from bitches classified into 4 groups according to its weight.

Time	G_1 $n = 47$	G_2 $n = 68$	G_3 $n = 79$	G_4 $n = 96$
Wet	$28.09 \pm 0.079^{c,3}$	$28.53 \pm 0.069^{b,3}$	$28.64 \pm 0.061^{b,3}$	$29.05 \pm 0.055^{a,3}$
Dry	$29.56 \pm 0.067^{c,1,2}$	$30.02 \pm 0.059^{b,1,2}$	$30.07 \pm 0.053^{b,1,2}$	$30.52 \pm 0.046^{a,1,2}$
Colostrum	$29.32 \pm 0.090^{c,1,2}$	$29.79 \pm 0.081^{b,2}$	$29.89 \pm 0.072^{b,2}$	$30.28 \pm 0.065^{a,2}$
30 min AB	$29.20 \pm 0.098^{c,2}$	$29.65 \pm 0.088^{b,2}$	$29.75 \pm 0.080^{b,2}$	$30.17 \pm 0.070^{a,2}$
1 h AB	$29.31 \pm 0.18^{b,1,2}$	$29.66 \pm 0.15^{b,2}$	$29.81 \pm 0.14^{a,b,2}$	$30.21 \pm 0.12^{a,2}$
4 h AB	$29.20 \pm 0.19^{c,2}$	$29.64 \pm 0.15^{b,c,2}$	$29.89 \pm 0.14^{a,b,2}$	$30.35 \pm 0.12^{a,2}$
24 h AB	$29.75 \pm 0.14^{c,1}$	$30.26 \pm 0.11^{b,1}$	$30.35 \pm 0.097^{a,b,1}$	$30.68 \pm 0.093^{a,1}$

(ANOVA, StatSoft Inc. 8.0, Tulsa, Oakland, CA, USA). *n*, number of puppies; weight of dams according to category: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; AB, after birth; least-squares mean ± standard error. *p* values for the different temperatures of the pups between groups and times in the regions evaluated were <0.0001 in all cases. [a,b,c] Different superscripts among columns indicate significant temperature differences between the dam's weight groups at the same time. [1,2,3] Different numbers among rows indicate significant differences between times in the same dam's weight group.

Table 8. Mean temperature (°C) of upper left palpebral (ULP) window at different times in newborn puppies from bitches classified into 4 groups according to its weight.

Time	G_1 $n = 47$	G_2 $n = 68$	G_3 $n = 79$	G_4 $n = 96$
Wet	$30.69 \pm 0.079^{b,5}$	$30.78 \pm 0.066^{b,5}$	$30.83 \pm 0.060^{b,5}$	$31.09 \pm 0.058^{a,5}$
Dry	$31.56 \pm 0.097^{b,4}$	$31.67 \pm 0.084^{a,b,4}$	$31.68 \pm 0.081^{a,b,4}$	$31.95 \pm 0.075^{a,4}$
Colostrum	$32.00 \pm 0.089^{b,3}$	$32.01 \pm 0.077^{b,3}$	$32.07 \pm 0.068^{b,3}$	$32.39 \pm 0.062^{a,3}$
30 min AB	$32.88 \pm 0.063^{b,2}$	$32.92 \pm 0.054^{b,2}$	$32.99 \pm 0.049^{b,2}$	$33.30 \pm 0.045^{a,2}$
1 h AB	$32.85 \pm 0.048^{c,2}$	$32.93 \pm 0.040^{b,c,2}$	$33.03 \pm 0.036^{b,2}$	$33.29 \pm 0.032^{a,2}$
4 h AB	$33.02 \pm 0.095^{b,2}$	$33.01 \pm 0.079^{b,2}$	$33.16 \pm 0.073^{b,2}$	$33.48 \pm 0.069^{a,2}$
24 h AB	$33.62 \pm 0.066^{b,1}$	$33.65 \pm 0.050^{b,1}$	$33.79 \pm 0.048^{b,1}$	$34.07 \pm 0.044^{a,1}$

(ANOVA, StatSoft Inc. 8.0, Tulsa, Oakland, CA, USA). *n*, number of puppies; weight of dams according to category: G_1, 4–8 kg; G_2, 8.1–16 kg; G_3, 16.1–32 kg; G_4, 32.1–39.6 kg; AB, after birth; least-squares mean ± standard error. *p* values for the different temperatures of the pups between groups and times in the regions evaluated were <0.0001 in all cases. [a,b,c] Different superscripts among columns indicate significant temperature differences between the dam's weight groups at the same time. [1,2,3,4,5] Different numbers among rows indicate significant differences between times in the same dam's weight group.

3.4. Changes in Temperature according to the Regions Evaluated

The thermal windows with the lowest temperatures recorded were the thoracic limb metacarpals (TLM) in wet puppies (26.98 ± 0.073) (Table 3) and the femoral pelvic limb (FPL) thermal window in wet puppies (27.54 ± 0.071) (Table 6). The thermal windows with the highest temperatures recorded were the abdominal (34.23 ± 0.047) at 24 h after birth (Table 5) and the upper left palpebral (ULP) (34.07 ± 0.044) at 24 h after birth (Table 8).

3.5. Thermal Response of the Newborn Due to the Effect of Time

The data suggest that it is more difficult for wet puppies to reach thermostability. The lowest temperatures were observed when the puppies were humid (26.98 ± 0.073) in the thermal window TLM (Table 3), and the highest were observed 24 h after birth (34.23 ± 0.047) in the thermal window A (Table 5).

The findings reflected in Table 1 indicate that the temperatures of the puppies in the TLBB thermal window at 7 different times (wet, dry, colostrum, 30 min AB, 1 h AB, 4 h AB, and 24 h AB) show statistically significant differences ($p < 0.0001$) between the groups of the neonates of the bitches from the G_1 and G_4 groups. Observing a 0.86 °C difference between the temperatures recorded in the puppies born to small-sized bitches (G_1) when they were still wet, with the puppies born from large-sized bitches (G_4) when they were also still wet, showing this same difference of 0.86 °C when compared to the G_1 group with G_4 at 24 h AB according to descriptive statistics. When comparing the temperatures of the

wet puppies of the G_1 group with those recorded in the G_4 group at 24 h AB, a difference of 4.02 °C was recorded, the broadest f differences between the temperatures evaluated in this thermal window. There was no interaction between the group and time ($p > 0.05$).

Regarding the temperature of the puppies at the different times, the evidence indicates that there is a statistically significant difference ($p < 0.0001$) in the temperature of the TLE region between the puppies born to bitches that are included in group G_1 and the puppies born to bitches in G_4. Statistical differences among the timepoints were observed in all four experimental groups. For puppies evaluated at 24 h AB, no significant differences were registered between G_1, G_2, and G_3, but with differences when compared to G_4, which indicates that the surface temperature of the puppies in the TLE region tends to stabilize and reaches the higher temperature values in puppies from heavy weight bitches.

Regarding the puppies born to bitches of a lower weight (G_1), there were statistically significant differences ($p < 0.0001$) in the TLE temperatures between the wet times and 24 h AB in a range of 4.6 to 5.7 °C. It is also essential to observe that at the TLBB window (Table 1) and this thermal window (Table 2), the range of the decrease in the temperature immediately after consuming colostrum (between 31.04 °C and 31.57 °C) was roughly 0.5 °C in all groups (G_1, G_2, G_3, and G_4). There is an interaction between the group and time ($p < 0.0001$), as it can be observed in Figure 4. That is to say that there is an interaction between the groups and times, specifically in G_4 where there is an overlap of the temperature values for time 4 h AB and 24 h AB and also for time 1 h AB and colostrum time.

Like the previous tables, this thermal window (TLM) (Table 3) repeats that the lowest temperatures were recorded in the puppies born to the G_1 bitches, and the highest temperatures in the puppies born to the G_4 bitches. It is worth mentioning that the lowest temperature of this study (26.98 ± 0.073) was recorded in this thermal window in the wet puppies of the G_1 group. Another important piece of data to point out in this table is that the decrease in the temperature once the pups ingested colostrum was statistically significantly different ($p < 0.0001$) between the dry and colostrum time, showing an average of 0.85 °C less than in the dry time in all groups. There was no interaction between the group and time ($p > 0.05$).

Table 4 shows, unlike the previous tables, the most crucial point to remark, that the T window temperature of the puppies increased immediately after the ingestion of colostrum by an average of 0.21 °C in the four groups. Furthermore, it is observed that at all times, the temperature difference between group G_1 and G_4 was 0.5 °C, except in wet and dry times, in which the difference between these groups was 0.41 °C and 0.49 °C, respectively, showing statistically significant differences ($p < 0.0001$). There was no interaction between the group and time ($p > 0.05$).

Notably, the A temperature of newborn puppies from the G_1 at dry was 0.52 °C lower than in G_4. At the same time, the average temperature of the puppies in G_1 at 24 h AB was 0.49 °C lower than in G_4. This effect shows that the dam's weight influenced the thermal response at the abdominal level of newborn puppies at all the time-points.

When comparing the different times per group, the puppies from G_1 had a significant statistical difference ($p < 0.0001$) between the A temperature registered at wet and dry (an average difference of 2.76 °C), at 4 h (+3.73 °C when compared to wet values), and at 24 h AB, with an average increase in the A temperature of 4.27 °C for the G_1. Similarly, in G_2, G_3, and G_4, the A temperature at 24 h AB increased by approximately 4.24 °C and was statistically significant when comparing the wet time in all the groups. An increase in the A temperature can also be observed between dry and colostrum times, with an average of +0.14 °C. It is also worth mentioning that the highest temperatures in all the studies were recorded on this thermal window at 24 h AB in the puppies born to bitches of group G_4. There was no interaction between the group and time ($p > 0.05$).

When comparing the average temperature of FPL in the four groups, significant statistical differences ($p < 0.0001$) can be observed between G_4 and the other three groups at all the measured times; the newborn puppies from G_4 had higher temperatures and a mean

difference of 0.78 °C when compared to G_1, particularly at 30 min AB (mean difference of 0.86 °C) and at 4 h AB (mean difference of 0.81 °C).

Within the groups, a statistically significant difference was found in all the groups at 4 h AB and 24 h AB ($p < 0.0001$). In G_1, from the wet time to 4 h AB, the puppies from low-weight dams had an increase in the FPL temperature average of 2.08 °C, while the temperature from wet to 24 h AB increased by an average of 3.16 °C. A similar case was observed in G_2, G_3, and G_4 at wet and 24 h AB (3.16, 3.18, and 3.17 °C, respectively). Additionally, wet to 4 h showed similar increasing temperatures as those registered in G_1, 2.22 °C for G_2, 2.11 °C for G_3, and 2.14 for G_4. There was no interaction between the group and time ($p > 0.05$).

The average temperatures of the N window show a significant statistical difference ($p < 0.0001$) between G_1 and G_4, differing from the first time of evaluation (wet) by 0.96 °C and at 24 h AB by 0.93 °C.

The differences between the temperatures of N taken at the seven times per group show that in all the groups, the temperatures at wet were significantly different ($p < 0.0001$) from the rest of the evaluated times, with a mean increase in the temperature from wet to dry in the four groups of 5.86 °C. There was no interaction between the group and time ($p > 0.05$).

The mean ULP temperatures of G_4 have statistically significant differences ($p < 0.0001$) from the other three groups, except for G_2 during the dry period. When comparing the average temperature of G_4 in all the measured times (32.79 °C) to the same average values in G_1, G_2, and G_3, in all the times, the IRT temperatures were higher in G_4, with a mean temperature difference of +0.42 °C, +0.37 °C, and +0.29 °C, respectively.

A statistically significant difference ($p < 0.0001$) was recorded within all the groups at wet and 24 h AB.

For G_1, the temperature increased from the first evaluation to 24 h AB at an average of 2.93 °C; for G_2, it increased by 2.87 °C, G_3 by 2.96 °C, and G_4 2.98 °C. Interestingly, when considering the difference between the times dry and colostrum, the newborn puppies ULP average temperatures in all the groups increased by 1.61 °C as an effect of the colostrum intake.

Subsequently, the correlations between the mother's weight and the temperatures of the pups at different times and in the different thermal windows were evaluated using Spearman's rank correlations. Tables 9–12 report the existing correlations between the weight of the dam and the temperature of the puppies, where the weights of the four groups (G_1, G_2, G_3, and G_4) and their respective temperatures by the time and thermal window indicate how the temperature tends to vary at different times and in different thermal windows. In almost all cases, there was a positive correlation with statistically significant differences ($p < 0.0001$) (Tables 9, 11 and 12). These results mean that the higher the weight of the bitches, the higher the temperatures recorded in the pups were. Only one case was observed where the correlation was negative (Table 10), in the thermal window TLE at 24 h AB. In this case, as the weight of the dams increased, the temperature of the puppies decreased in the TLE window. The r values in all tables were between 0.127 and 0.634, except in Table 10 of the TLE thermal window, where $r = -0.177$ ($p = 0.0026$) at 24 h AB. The highest correlations were observed in the Table 9 TLBB window (colostrum $r = 0.601$) and Table 11 TLM window (colostrum $r = 0.634$). There was no interaction between the group and time ($p > 0.05$).

Table 9. Significant correlations between the thoracic limb brachial biceps (TLBB) temperatures of newborn puppies and the weight of the dams at different times: wet (with amniotic fluid), dry (once the puppy was rubbered), colostrum (after colostrum intake and separated from mother's teat), 30 min AB, 1 h AB, 4 h AB, and 24 h AB.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.540	<0.0001
Dry	0.301	<0.0001
Colostrum	0.601	<0.0001
30 min AB	0.399	<0.0001
1 h AB	0.435	<0.0001
4 h AB	0.369	<0.0001
24 h AB	0.365	<0.0001

Spearman's rank correlation coefficients and their statistical significance between the dam's weight and temperature of puppies at different times. AB: after birth.

Table 10. Significant correlations between the weight of the dam and the superficial temperature at thoracic limb elbow (TLE) thermal window in newborn puppies at different times: wet (with amniotic fluid), dry (once the puppy was rubbered), colostrum (after colostrum intake and separated from mother's teat), 30 min AB, 1 h AB, 4 h AB, and 24 h AB.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.610	<0.0001
Dry	0.380	<0.0001
Colostrum	0.326	<0.0001
30 min AB	0.286	<0.0001
1 h AB	0.484	<0.0001
4 h AB	0.222	<0.001
24 h AB	−0.177	0.0026

Spearman's rank correlation coefficients and their statistical significance between the dam's weight and temperature of puppies at different times. AB: after birth.

Table 11. Significant correlations between the weight of the dam and the superficial temperature at thoracic limb metacarpals (TLM) window in newborn puppies at different times: wet (with amniotic fluid), dry (once the puppy was rubbered), colostrum (after colostrum intake and separated from mother's teat), 30 min AB, 1 h AB, 4 h AB, and 24 h AB.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.475	<0.0001
Dry	0.358	<0.0001
Colostrum	0.634	<0.0001
30 min AB	0.334	<0.0001
1 h AB	0.485	<0.0001
4 h AB	0.241	<0.0001
24 h AB	0.342	<0.0001

Spearman's rank correlation coefficients and their statistical significance between the dam's weight and temperature of puppies at different times. AB: after birth.

Table 12. Significant correlations in the T thermal window temperatures of newborn puppies and the weight of the dams at different times: wet (with amniotic fluid), dry (once the puppy was rubbered), colostrum (after colostrum intake and separated from mother's teat), 30 min AB, 1 h AB, 4 h AB, and 24 h AB.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.127	0.031
Dry	0.429	<0.0001
Colostrum	0.316	<0.0001
30 min AB	0.467	<0.0001
1 h AB	0.342	<0.0001
4 h AB	0.286	<0.0001
24 h AB	0.391	<0.0001

Spearman's rank correlation coefficients and their statistical significance between the dam's weight and temperature of puppies at different times. AB: after birth.

4. Discussion

The present study assessed the superficial temperature at eight anatomical regions or thermal windows and at different times in newborn puppies born from bitches with distinct weights. According to the results, there is an association between the surface temperature of the pups and the weight of the dams, having the highest temperature in all the thermal windows at all the evaluation times in G_4 (bitches with the highest weight range of 32.1–39.6 kg). Additionally, although the temperatures at the wet and dry times differed between the four groups, both had the lowest values in all groups. At 24 h AB, the temperature in all the thermal windows showed a correlation with, regardless of the weight of the dam, similar values, meaning that the thermoregulatory capacity of the puppies after 24 h may depend on the dam's weight. It is also important to mention that the rectal temperature of the puppies or the bitches was not recorded, to avoid an invasive approach to temperature evaluation.

This study's results show the effect of the dam's weight on the superficial temperature of canine neonates. The statistical analyzes carried out in this study revealed that the thermoregulation capacity of the puppies is closely linked to the weight of the bitches, since significant differences ($p < 0.0001$) between the temperatures recorded in all the groups of bitches were observed. Newborns from low-weight bitches had lower temperature values for all the thermal windows at every measured time than puppies from the heaviest bitches. This thermoregulatory dependence on the weight and time has also been reported in dog puppies that cannot stabilize their temperature rhythm for several days after birth, but their rectal temperatures become stable six weeks after birth [47]. It also has been reported that dogs of a small size tend to lose heat quickly because they have a bigger surface area to volume ratio [48]; therefore, they require a greater heat production to maintain their thermostability [49]. Additionally, it can be attributed to the wide variability of canine breeds [49]. For example, short-haired dogs from breeds like Miniature Pinscher, pit bull mix, or pointer hound mix presented a higher surface temperature of approximately 2 °C more than long-haired breeds such as Shih-tzu, miniature schnauzer, Labrador mix, or Pomeranian dogs [49]. Due to this, the environment's temperature where the puppies live could have a favorable or unfavorable effect on their welfare [50]. In addition, their weight impacted their health and vitality [51]. Veronesi [52] mention that the body size of a breed also influences the viability of newborn puppies, assessed with Apgar score systems, where small-sized puppies may have the highest levels of distress but higher chances of survival when compared to large-sized animals.

Birth weight is essential for the survival of neonates, and it is one of the most important factors that influences temperature loss since the body mass index (BMI) determines the capacity of thermoregulation in newborns like foxes [53]. Regarding the dam's body weight, this depends on the fetus's presence (representing 1 to 3% of the total weight of the bitch), and this bodyweight also influences the birth weight of newborns [54]. In addition, according to Mila et al. [37], the birth weight is influenced statistically significant by litter size, presenting a higher number of low-birth-weight puppies in large litters compared to small litters. According to the data obtained in this study, it was observed that the larger the litter size and the greater the weight of the puppies at birth, the more easily stabilized thermoregulation was in the puppies that came from larger litters and had heavier weights raised at birth.

Attempts have been made to establish the most appropriate thermal regions or windows that provide better information on the thermal changes in veterinary medicine [55]. Thus, thermal utility windows are lacrimal caruncle, eye, ear, thorax flanks, appendicular area, and face [13,40,56]. However, other thermal windows have also been suggested in pathological cases, such as the mammary gland and the ventral window for cases of mammary gland cancer and the neck for cases of hypothyroidism in cats [57,58].

The most notable thermoregulatory changes in newborn puppies were low temperatures in the distal regions, that is, in the thermal windows of the pelvic and thoracic limbs (TLM and FPL), and the highest temperatures in the thermal windows A, T, and ULP.

The peripheral and central circulation could explain these findings. The most important structures related to the metabolism and vital functions are in the thoracic, abdominal, and cranial regions [40]. The appendicular windows presented the lowest temperatures. This can be explained by the limited locomotion of altricial species and their inability to walk or stand up immediately after birth. However, newborn puppies can crawl and have active movement of the limbs, although these movements are minimal and may not promote significant thermal changes detected with IRT because neonates have a low blood pressure and, thus, the blood flow in the periphery is lower than the central circulatory system [16]. When considering the thermographic changes in the skin of newborn dogs, it is important to highlight the degree of neurological development responsible for the mechanism of thermal modulation. Neonates rely on non-shivering thermogenesis by the lipolysis of brown adipose tissue and the oxidation of fatty acids in the mitochondria through ATP synthesis [59]. It is reported that neonatal muscles (skeletal muscles) are immature at birth, so shivering thermogenesis is ineffective in producing heat [59].

In altricial species, thermostability is reached until day 18 of life [60], and autonomic thermoregulation is wholly developed at the end of the fourth week [61].

According to the results of the present study, regardless of the mother's weight, puppies showed a decrease in the temperature from dry to before the intake of colostrum in the anatomical regions of TLBB, TLM, TLE, FPL, and N, and this could be associated with the physiological temperature drop in neonates but also to the limited energy resources that can be compensated through a colostrum intake [62]. Although a control group of non-colostrated puppies was not considered as a comparison for all the experimental groups, the decrease in the temperature observed in the puppies depends on their limited energy reserves immediately after birth [63]. Neonates have minimal glycogen reserves to maintain stable glucose levels, and the immature liver is inefficient at generating energy to thermoregulate when the glycogen stores are emptied, as they are not able to produce heat by shivering, and the brown adipose tissue in newborns is not highly developed [10]. The further increase in the superficial temperature of all the thermal windows after a colostrum intake and at 30 min AB confirm the provided energy source and the impact on the temperature. This effect can be worsened by difficulties in the colostrum intake [63] and may decrease the core temperature and, consequently, the superficial temperature assessed by IRT.

Mila et al. [64] stated that colostrum is an energy source since it can provide 1300–1800 kcal/L and is also high in IgG [65]. However, the variability of the colostrum properties depends on the birth order and suckling time [64], factors not assessed in this study and ones which may be suggested for further research. In general, newborn puppies have a daily energy requirement of approximately 20–26 kcal/100 g of their body weight [61] and need an average colostrum intake of 12 mL per 100 g of their body weight to cover these requirements [65]. In addition, in calves [66], pigs [67], and dogs [68], colostrum contributes to the correct maturation and function of the digestive system and the absorption of nutrients [13]. The amount of glucose that colostrum provides to puppies is crucial because only 1.3% of the body fat content is available in newborns, and hypoglycemia and hypothermia are significant causes of neonatal mortality [64]. Interestingly, the colostrum of small breed bitches (less than 10 kg) provides 10% more energy than the colostrum from large breed females [65], and the ingestion of colostrum could provide a 10% weight recovery during the first 24 h after birth, ensuring the newborn puppy's survival [13]. This could be why puppy temperatures at 24 h AB reached a certain thermostability between the groups after the colostrum intake, regardless of the dam's weight, another factor that could intervene is the environmental temperature.

Contrarily to the temperature decrease described in most thermal windows, in T, A, and ULP, there was an increase in the temperature from dry to before the colostrum times. These regions' anatomical location and vascular irrigation could explain this effect. As reported by various studies, ocular temperature assessed with IRT has shown a positive correlation with the body core temperature compared to other anatomical regions [26,69,70].

The above could be due to the proximity of the ocular orbit to the brain and the ample blood supply provided by the supra and infraorbital arteries [40,71–73].

Throughout the seven evaluation times, the significant temperature differences found from wet to 24 h AB in the evaluated regions reflect the disruption of the thermal stability of the newborn puppies and the activation of the thermoregulatory mechanisms. In the intrauterine environment, a heat transfer by maternal circulation maintains the fetal temperature 0.3 to 0.5 °C higher than the mother's body temperature. However, the maternally dependent puppies experience a rapid temperature loss at birth due to an exposure to the cold extrauterine environment and heat loss by the evaporation from the wet dermal surface by amniotic fluid [59].

Hypothermia is suggested to occur immediately after birth as a protective mechanism to prevent hypoxic damage in the neonate and reduce the metabolic rate to improve the survival of the newborn in the first hours [10,61,74]. This effect could be observed in the newborn puppies of the present study at the first three evaluation times (wet, dry, and colostrum), where the lowest superficial temperatures were recorded. Neonate thermoregulation involves biochemical, anatomical, physiological, and endocrine mechanisms that trigger respiratory and vascular changes and activate the metabolism to produce energy [39,75]. As stated previously, a colostrum intake is essential for newborns to prevent the consequences of hypothermia and reflect the thermal changes in the superficial temperature assessed by IRT.

Another element that influences the temperature differences between the evaluation times is the significant neonatal heat evaporation through the wet dermal surface since animals are born wet [59]. This happens because the insulation and protection function that the coat provides is not efficient since the water (amniotic fluid in this case) has a high thermal conductivity, which generates more significant hypothermia. In addition, other contributing factors include decreased body fat, age, and lack of acclimatization to the environment [76–79]. In this way, it is possible to explain that body temperatures increased when passing from the wet to dry time, either due to the decrease in the heat loss through evaporation or the decrease in the convection favored by air currents. Another critical factor is the rubbing effect on puppies, which stimulates the dermal vascular microcirculation of the peripheral blood vessels (peripheral vasodilation), increasing microvascular hyperemia at the dermal level.

The thermostability observed at 24 h AB In the puppies from all groups could be attributed to the efficacy of the thermoregulatory response of newborns regardless of the initial weight of the bitch, as shown in the values of IRT in all the thermal windows, where the four groups, when compared to the temperature at wet, had a similar average increase.

Several factors can influence this response, such as the glycogen reserves, colostrum intake, increased digestion, the dam's presence, and the litter's members. As observed in the presented results, at 24 h AB, all the puppies reached thermostability after the drying of their coat, colostrum intake, and heat transfer of the mother's temperature by convection. These thermoregulatory behaviors are important during newborns' first days since puppies cannot maintain their body temperature when exposed to cold environments up to six days after birth [10]. Therefore, the results suggest that, during the first hours of the life of newborn puppies, their thermoregulation mechanisms are deficient by anatomical and metabolic factors that can be reduced by a colostrum intake, maintaining an adequate body temperature, and its mechanisms to conserve or dissipate heat on the evaluated thermal windows.

One of the main limitations of this study was that since births did not always occur in the same place, the environmental temperatures could not be controlled and standardized for all the cases. It should be clarified that the thermoneutral zone, known as the range of environmental conditions where an animal can regulate heat loss with a minimum of effort [17], should be considered uniformly in puppies to maintain their body temperature within normal limits. Another important point is the great diversity of the breeds and sizes in this species, so the most practical way to group the bitches might be by their weight

and not by their breed. Additionally, the pregestational bodyweight of the dams was not recorded in the present study, but this could have given information about the original body condition of the dam before gestation. In some studies, made in humans, positive correlations between maternal obesity and the incidence of high-risk complications at delivery have been described. In this sense, the pre-pregnancy body mass index (BMI) is a major determinant of pregnancy outcome [80]. In addition, it has been seen that a mother's obesity during pregnancy could predispose the development of obesity in a child between 3 and 5 years old [81]. Therefore, considering the BMI in bitches before and during gestation and its association with the thermal parameters in newborn puppies could be a field for future research.

5. Conclusions

In the present study, the IRT reading of the superficial temperature of newborn puppies showed that the weight of the dam seems to influence the neonatal thermostability, where puppies born from bitches of a higher weight presented the highest temperatures from birth to 24 h AB.

Regarding the body regions where temperatures were evaluated, the findings suggest that the thermal windows with the highest temperatures were A, T, N, and ULP, and those with the lowest temperatures were TLBB, TLE, TLM, and FPL. In TLE, there was an interaction between the groups ($p < 0.0001$) and between the groups and times, specifically in G4 where there was an overlap of the temperature values for the time 4 h AB and 24 h AB, as well as for the time 1 h AB and the colostrum time; the other thermal windows had not presented interactions.

One of the most interesting data are that, unlike other studies, this study observed a significant increase in the temperatures of the puppies in the thermal windows A, T, and ULP immediately after ingesting colostrum, which could be an area for future research. An important aspect to note is that the use of the thermal windows used in this work in newborn puppies had not been reported previously, so further studies using these thermal windows in this species could be considered. Similarly, the concern arises to know how much the weight of newborn puppies can influence their ability to achieve thermostability.

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Informed Consent Statement: All the owners of the study animals were asked to sign the informed consent to carry out the procedures.

Data Availability Statement: Data are contained within the article.

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CAPÍTULO 6.

Is the Weight of the Newborn Puppy Related to Its Thermal Balance?

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Is the Weight of the Newborn Puppy Related to Its Thermal Balance?

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Simple Summary: Newborns experience a significant thermal change at birth, leading their bodies to adjust and reduce their metabolism to survive. In this study, newborn puppies' weights and their temperatures in different body areas and at different measure times were evaluated to determine if there is a relationship between weight and their ability to reach thermostability. It was observed that there is a positive relationship between the weight of the puppies and their thermoregulatory capacity.

Abstract: Hypothermia, a factor associated with neonatal mortality, can occur immediately after birth as a protective mechanism to prevent hypoxic damage in neonates, or to reduce the metabolic rate to improve the chances of survival in the first hours of life. The heat interchange through the superficial temperature of animals can be evaluated with infrared thermography (IRT). However, to date, there is no information on thermal windows in puppies. This study aimed to evaluate, with the use of IRT, the microcirculatory alterations in 8 different thermal windows identified at 7 different times in 289 newborn puppies assigned to different groups. Three thermograms were taken from four zones of each puppy: the facial, frontal, right lateral, and left lateral regions. Newborn puppies were grouped in 4 quartiles according to their weight: Q_1 (126–226 g) $n = 73$, Q_2 (227–330 g) $n = 72$, Q_3 (331–387 g) $n = 74$, and Q_4 (388–452 g) $n = 70$. A total of 8 thermal windows were considered at 7 evaluation times from Wet at birth until 24 h after birth (AB). Two-way mixed ANOVA within and between subjects' design for each thermal window (eight models) was performed. Results revealed a positive correlation between the puppy's weight and its ability to achieve thermostability in all the evaluated thermal windows. Statistically significant differences ($p < 0.0001$) between the 4 quartiles (Q_1, Q_2, Q_3, and Q_4) were found. The lowest temperatures were recorded when the pups were still wet and the highest at 24 h AB. Thermal windows with the highest temperatures were abdominal (34.234 ± 0.056 °C), thoracic (33.705 ± 0.049 °C), nasal (30.671 ± 0.110 °C), and upper left palpebral (34.066 ± 0.052 °C), while the lowest were thoracic limb brachial biceps (27.534 ± 0.051 °C), thoracic limb elbow (27.141 ± 0.049 °C), thoracic limb metacarpal (27.024 ± 0.062 °C), and femoral pelvic limb

(27.654 ± 0.055 °C). Assessing the thermal response in newborn puppies can help identify drastic temperature reductions or deficient thermoregulatory compensation during the first hours of life, preventing the consequences of hypothermia.

Keywords: dogs; puppy welfare; animal perinatology; newborn puppy; thermoregulation; infrared thermography

1. Introduction

Endothermic animals regulate their body temperature through heat interchange between the amount produced by metabolism and the environment [1]. The control of heat exchange between the body surface and external environment plays a significant role in the regulation of body temperature during different physiological phases and/or activities throughout the life of homeotherms. Thermoregulatory adjustments can be induced not only by changes in environmental temperature but also by a variety of physiological situations including age, fasting and food intake, stress circumstances, and inflammation status, inducing changes in internal temperature which are followed by changes in body surface temperature. Under stressful conditions, animal's reaction is mainly centered on the activation of the sympathetic system and the hypothalamic–hypophysis–adrenal axis (HPA) through the release of effector hormones, namely, catecholamine and glucocorticoid, respectively [2]. One of the known results of the sympathetic system's activation is stress-induced hyperthermia, consisting in increased core body temperature with consequent changes in involucre temperature. Therefore, that allows obtaining distance images of a specific body region—represents a valuable tool to monitor the physiologic status, welfare, and stress responses of animals [3–5].

Thermoregulation plays an important role in newborns, especially in altricial species [6]. Through its extensive dermal vascularization, the body avoids losing temperature by producing changes in the blood flow of specific body regions [7], which are called biological thermal windows [8].

These changes can be indirectly quantified by evaluating tissue heat radiation through infrared thermography (IRT) [9]. Using a tool that can record these changes could help to identify states of hypothermia, a factor related to mortality and one of the most important causes of death in neonates [10,11].

Perinatal mortality in some species, such as pigs [12] and dogs [13,14], is one of the biggest concerns for producers and breeders since it can represent an average of one puppy out of ten live births dying before two months of age [15]. In addition, the immaturity of newborn puppies makes them highly vulnerable. Neonate thermoregulation involves biochemical, anatomical, physiological [16,17], and endocrine mechanisms to trigger respiratory and vascular changes and activate metabolism to produce energy [18]. It has been suggested that hypothermia occurs immediately after birth as a protective mechanism to prevent hypoxic damage in the neonate and reduce the metabolic rate to improve the survival of the newborn in the first hours [19–21]. Another important risk factor for neonatal mortality in diverse species, such as humans, pigs, and cattle, is low birth weight [22,23].

Similarly, in dogs, low birth weight neonates have a higher risk of death. More precisely, this risk is 12 times greater than in animals with normal birth weight [22]. In this species, studies have been carried out on the effect of the dam's weight on the puppy's weight at birth, litter size, vitality, and survival [13,24,25]. Likewise, the effect of the dam´s weight on the presentation of asphyxia and newborn hematological values have been evaluated [26]. According to Plavec et al. [27], puppies that experienced severe distress postpartum had significantly worse survival rates in the first week of their lives (p = 0.0113 and p = 0.0231, respectively). Attempts have been made to establish the most appropriate thermal regions or windows that provide better information on thermal

changes in veterinary medicine [28]. Thus, thermal utility windows in dogs are the lacrimal caruncle, eye, ear, thorax flanks, appendicular area, and face [29–31]. However, there still needs to be more information on thermal windows in puppies. This could aid in our understanding of the process of thermoregulation in newborn dogs and, thus, help reduce the high mortality rates in this species.

This study aims to evaluate the microcirculatory changes in the different thermal windows of puppies assigned to different groups according to weight. The repercussions of their presentation of hypothermia were also analyzed. Our hypothesis assumed that (1) bigger newborns will have fewer thermoregulation problems than those of small sizes; (2) the highest temperatures will be obtained in thermal windows closest to the brain and vital organs (palpebral, thoracic, abdominal, or nasal); and (3) the lowest temperatures will be the ones recorded in thermal windows furthest from vital structures (thoracic limbs and pelvic limbs).

2. Materials and Methods

2.1. Facilities

The study was carried out in 10 veterinary clinics in the central zone of the municipality of Campeche, Campeche state, Mexico, a zone with a tropical climate and a temperature range between 36 and 40 °C. The participation of owners of pregnant bitches was requested. Bitches were monitored and offered medical attention to carry out prenatal stage control from day 25 of pregnancy to 48 h of the puppies' life.

2.2. Study Population

In total, 289 puppies from 60 parturient bitches were recruited and divided into 4 groups using size-specific quartiles, following Mugnier et al. [32] and Tessi et al.'s [33] method. The first quartile (Q_1) represents the lowest 25% of registered values, the second quartile (Q_2) represents 25–50%, the third quartile (Q_3) represents 50–75%, and the fourth quartile represents 75–100% (Q_4). Animals in the group Q_1 were considered low-weight, while those belonging to Q_4, the highest 25% of registered values, were considered high-weight puppies. This classification is due to the great variety of sizes between the breeds of dogs—the greatest morphological variability within any land mammal species. In this sense, in dogs, we can find adult body weight ranges from 500 g in miniature breeds, such as Chihuahuas, to more than 100 kg in giant breeds, such as mastiffs [34]. Among the breeds included in this study were Chihuahua, German shepherd, Labrador, golden retriever, Great Dane, standard schnauzer, cocker spaniel, poodle, Scottish terrier, and Belgian shepherd. As a result of this considerable variation in body weights, birth weight should be analyzed according to breed size. Quartiles were calculated at the puppy level with this formula: Qa = Li ((aN/4 + Fi-1)/Fi) Ai, where Li is the lower limit of the class where the quartile is located, N is the sum of the absolute frequencies, Fi-1 is the accumulated frequency of the previous class, and Ai is the amplitude of the class, that is, the number of values contained in the interval. The groups were Q_1 (126–226 g) n = 73 puppies, Q_2 (227–330 g) n = 72 puppies, Q_3 (331–387 g) n = 74 puppies, and Q_4 (388–452 g) n = 70 puppies.

The weight of the puppies was obtained using a digital scale from Salter Weight Tronix Ltd., West Bromwich, UK, as soon as the dam stopped licking and cleaning the amnionic fluids and placental membranes from them.

2.3. Infrared Thermography

In the present study, in each puppy, 8 thermal windows were identified at 7 different times, according to the methodology used by Lezama-García et al. [35]. The assessment times were: Wet (after the mother stopped licking the amniotic fluids from the pup), Dry (after drying the puppies with a towel for one minute), Colostrum (immediately after colostrum intake and after the puppy's release of the teat), 30 min after birth (AB), 1 h AB, 4 h AB, and 24 h AB. The thermal windows are explained in detail in Figure 1. A total of 16,240 records of the puppies' minimum, maximum, and average surface temperature were

obtained. These data resulted from 289 puppies, from which 3 thermograms were taken from 4 zones: facial, frontal, right lateral, and left lateral regions. The facial images included the following regions: the upper left palpebral (ULP) and nasal (N). Frontal included thoracic limb metacarpal (TLM). Right lateral recorded femoral pelvic limb (FPL), thoracic limb biceps brachial (TLBB), and thoracic limb elbow (TLE), while on the left side, thoracic (T) and abdominal temperatures were assessed. Thermographic images were obtained with an infrared camera, FLIR® model Thermal TM E80, FLIR Systems, Wilsonville, OR, USA, with the following specifications: IR resolution 320 × 240 pixels, thermal sensitivity <0.045 °C, accuracy ±2 °C or 2% of reading in the ambient temperature of 10 °C to 35 °C, and image frequency of 60 Hz. All images were collected with an emissivity of 0.95 at a uniform distance of 30 cm. The thermographic images were saved in JPEG format to analyze them later using specialized FLIR Tools software® (FLIR Systems, Wilsonville, OR USA). The different evaluation times registered the minimum, average, and maximum temperatures for every image.

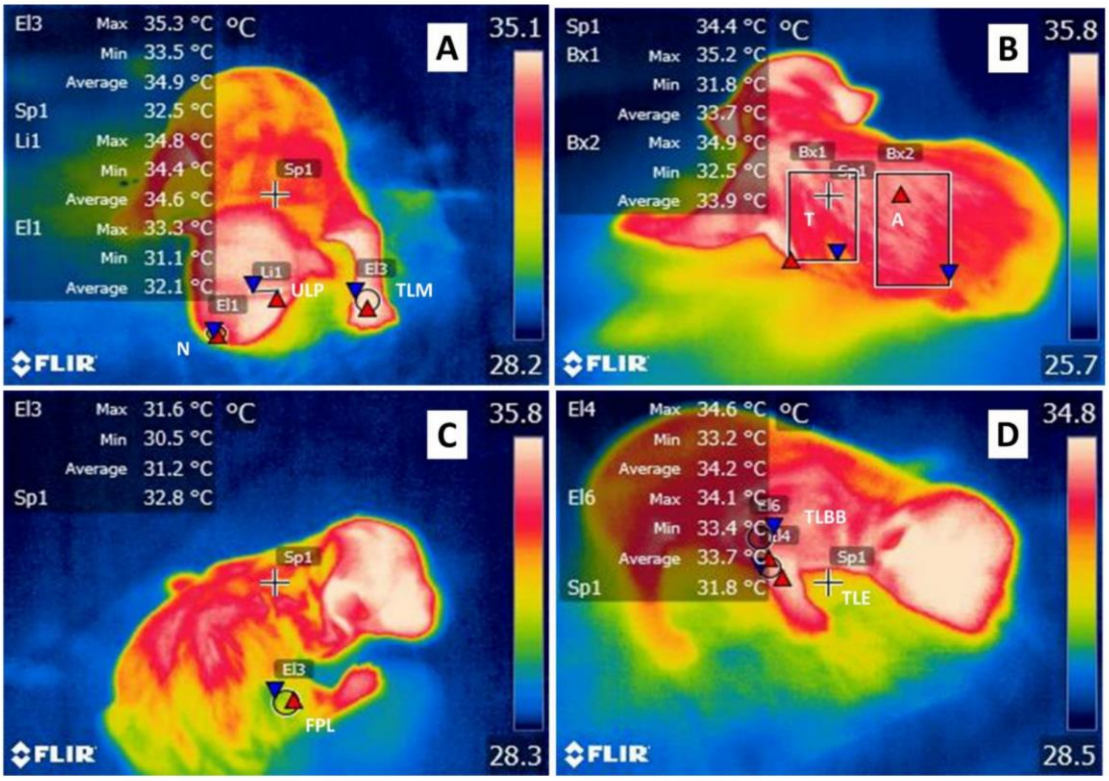

Figure 1. Thermal windows in newborn puppies. (**A**) Nasal (N) (El1), upper left palpebral (ULP) (Li1), and thoracic limb metacarpal (TLM) (El3) windows were made with circular figures delimited by the edges of the nasal mucosa, at the area of the edge of the left upper eyelid, and in the joint formed by the metacarpals covering the area from medial to lateral end, respectively. (**B**) Thoracic (T) window (Bx1) was made with rectangular figures delimited by the axillary area, the area of the last rib, and from the region of the spinal vertebrae to the ventral part of the abdomen. (**A**) Abdominal window (Bx2) was delimited by two millimeters after the last rib, to the inguinal area, and from the region of the spinal vertebrae to the ventral part of the abdomen. (**C**) Femoral pelvic limb (FPL) window (El3) was delimited by the space bounded by the edge of the pelvic limb in the biceps femoris region. (**D**) Thoracic limb brachial biceps (TLBB) (El6) and thoracic limb elbow (TLE) windows (El4) were obtained by placing circular figures from the area where the armpit begins to half the width of the thoracic limb and placing circular figures in the elbow area covering the vertex formed by the humerus-radio-ulnar joint, respectively.

2.4. Statistical Analysis

Two-way mixed ANOVA within and between subjects' design for each thermal window (eight models) was performed. This analysis has 4 independent categories (the groups according to weight of subjects) and 7 factors that were related or dependent (within subjects), also known as repeated measures.

Analysis components:

a. Within subjects' factor: 7 levels or times: (1) Wet; (2) Dry; (3) Colostrum; (4) 30 min; (5) 1 h; (6) 4 h; (7) 24 h.
b. Between subjects' factor: quartiles (4 levels): (1) Quartile 1 (Q_1): 126–226 g $n = 73$; (2) Quartile 2 (Q_2): 227–330 g $n = 72$; (3) Quartile 3 (Q_3): 331–387 g $n = 74$; (4) Quartile 4 (Q_4): 388–452 g $n = 70$.
c. Interaction between factors: groups.

2.5. Ethical Statement

All the animal owners were asked to grant informed consent to carry out the research. This project was approved by the Ph.D. Program in Biological and Health Science Academic Committee, with approval number CBS.114.19. The animals included in the present study were treated gently and were not touched or stressed, since infrared thermography is a non-invasive technique. All work was performed under lineaments of Mexico's Official Norm NOM-062-ZOO-1999 on technical specifications for animal production, care, and ethical use in applied ethological studies [36].

3. Results

The means and standard errors of the eight thermal windows were analyzed between the four quartiles. Statistically significant differences ($p < 0.0001$) between the 4 quartiles (Q_1, Q_2, Q_3, and Q_4) were found. However, in general and in all thermal windows, quartiles Q_2 and Q_3 were similar. In the same way, the evidence indicates significant differences between the temperature means of thermal windows and the different puppies' evaluation times (Wet, Dry, Colostrum, 30 min AB, 1 h AB, 4 h AB, and 24 h AB).

The highest temperatures were recorded in the Q4 group puppies and the lowest in the Q1 puppies in all evaluated thermal windows (Tables 1–8).

Table 1. Average of the temperature of upper left palpebral (ULP) window at different times in newborn puppies classified into 4 groups according to weight.

Time	Q_1 $n = 73$	Q_2 $n = 72$	Q_3 $n = 74$	Q_4 $n = 70$
Wet	$30.800 \pm 0.065^{b,5}$	$30.830 \pm 0.066^{b,5}$	$30.836 \pm 0.065^{a,b,5}$	$31.083 \pm 0.067^{a,5}$
Dry	$31.621 \pm 0.083^{b,4}$	$31.637 \pm 0.084^{b,4}$	$31.740 \pm 0.082^{a,b,4}$	$32.015 \pm 0.085^{a,4}$
Colostrum	$31.964 \pm 0.072^{b,3}$	$32.073 \pm 0.073^{b,3}$	$32.186 \pm 0.072^{a,b,3}$	$32.401 \pm 0.074^{a,3}$
30 min AB	$32.929 \pm 0.052^{b,2}$	$32.905 \pm 0.053^{a,b,2}$	$33.158 \pm 0.052^{a.2}$	$33.264 \pm 0.053^{a,2}$
1 h AB	$32.901 \pm 0.039^{c,2}$	$32.958 \pm 0.039^{b,c,2}$	$33.092 \pm 0.038^{b,2}$	$33.322 \pm 0.039^{a,2}$
4 h AB	$33.025 \pm 0.078^{b,2}$	$33.065 \pm 0.079^{a,b,2}$	$33.330 \pm 0.078^{a,2}$	$33.436 \pm 0.080^{a,2}$
24 h AB	$33.600 \pm 0.051^{b,1}$	$33.723 \pm 0.051^{b,1}$	$33.923 \pm 0.050^{a,1}$	$34.066 \pm 0.052^{a,1}$

(Two-way mixed ANOVA.) Notes: n, number of puppies; weight of puppies according to category (Q_1, 126–226 g; Q_2, 227–330 g; Q_3, 331–387 g; Q_4, 388–452 g); AB, after birth; least-squares mean ± standard error. ^{a,b,c}: different superscripts among columns indicate significant temperature differences between puppy's weight groups at the same time. ^{1,2,3,4,5}: different numbers among rows indicate significant differences between times in the same puppy's weight group.

Table 2. Average of the temperature of nasal (N) window at different times in newborn puppies classified into 4 groups according to its weight.

Time	Q_1 $n = 73$	Q_2 $n = 72$	Q_3 $n = 74$	Q_4 $n = 70$
Wet	28.187 ± 0.064 ^{c,4}	28.649 ± 0.064 ^{b,4}	28.711 ± 0.063 ^{b,3}	29.127 ± 0.065 ^{a,3}
Dry	29.757 ± 0.059 ^{c,1,2,3}	30.002 ± 0.060 ^{b,1,3}	30.331 ± 0.059 ^{a,1}	30.431 ± 0.060 ^{a,1,2}
Colostrum	29.445 ± 0.076 ^{c,2}	29.860 ± 0.077 ^{a,b,2,3}	30.036 ± 0.076 ^{a,2}	30.293 ± 0.078 ^{a,1,2}
30 min AB	29.385 ± 0.084 ^{b,2}	29.645 ± 0.085 ^{a,b,2}	29.924 ± 0.084 ^{a,2}	30.167 ± 0.086 ^{a,1,2}
1 h AB	29.494 ± 0.144 ^{b,1,2}	29.619 ± 0.145 ^{a,b,2}	29.994 ± 0.143 ^{a,1,2}	30.224 ± 0.148 ^{a,1}
4 h AB	29.366 ± 0.146 ^{b,2}	29.697 ± 0.147 ^{a,b,3}	30.080 ± 0.145 ^{a,1,2}	30.377 ± 0.149 ^{a,1}
24 h AB	29.977 ± 0.107 ^{b,c,1}	30.281 ± 0.108 ^{a,1,3}	30.453 ± 0.107 ^{a,1}	30.671 ± 0.110 ^{a,1}

(Two-way mixed ANOVA.) Notes: *n*, number of puppies; weight of puppies according to category (Q_1, 126–226 g; Q_2, 227–330 g; Q_3, 331–387 g; Q_4, 388–452 g); AB, after birth; least-squares mean ± standard error. ^{a,b,c}: different superscripts among columns indicate significant differences of temperature between puppy's weight groups at the same time. ^{1,2,3,4}: different numbers among rows indicate significant differences between times in the same puppy's weight group.

Table 3. Average of the temperature of thoracic limb metacarpal (TLM) window at different times in newborn puppies classified into 4 groups according to weight.

Time	Q_1 $n = 73$	Q_2 $n = 72$	Q_3 $n = 74$	Q_4 $n = 70$
Wet	27.024 ± 0.062 ^{c,6}	27.222 ± 0.062 ^{c,5}	27.482 ± 0.061 ^{b,6}	27.743 ± 0.063 ^{a,5}
Dry	29.858 ± 0.066 ^{c,3,5}	30.155 ± 0.066 ^{b,2,4}	30.281 ± 0.065 ^{a,b,2,5}	30.446 ± 0.067 ^{a,3}
Colostrum	29.028 ± 0.038 ^{c,4}	29.255 ± 0.038 ^{b,3}	29.344 ± 0.037 ^{b,4}	29.742 ± 0.038 ^{a,4}
30 min AB	29.582 ± 0.081 ^{b,3}	29.536 ± 0.082 ^{b,3}	29.886 ± 0.081 ^{a,3}	30.186 ± 0.083 ^{a,3}
1 h AB	30.294 ± 0.057 ^{b,c,2}	30.452 ± 0.057 ^{b,2}	30.644 ± 0.056 ^{b,2}	30.940 ± 0.058 ^{a,2}
4 h AB	30.239 ± 0.196 ^{b,1,2}	30.430 ± 0.197 ^{b,1,2}	30.664 ± 0.194 ^{a,b,1,2}	31.177 ± 0.200 ^{a,1,2}
24 h AB	30.778 ± 0.086 ^{b,1}	30.971 ± 0.086 ^{a,b,1}	31.271 ± 0.085 ^{a,1}	31.543 ± 0.088 ^{a,1}

(Two-way mixed ANOVA.) Notes: *n*, number of puppies; weight of puppies according to category (Q_1, 126–226 g; Q_2, 227–330 g; Q_3, 331–387 g; Q_4, 388–452 g); AB, after birth; least-squares mean ± standard error. ^{a,b,c}: different superscripts among columns indicate significant temperature differences between puppy's weight groups at the same time. ^{1,2,3,4,5,6}: different numbers among rows indicate significant differences between times in the same puppy's weight group.

Table 4. Average of the temperature of thoracic (T) window at different times in newborn puppies classified into 4 groups according to weight.

Time	Q_1 $n = 73$	Q_2 $n = 72$	Q_3 $n = 74$	Q_4 $n = 70$
Wet	28.884 ± 0.160 ^{a,6}	28.861 ± 0.161 ^{a,7}	29.089 ± 0.159 ^{a,5}	29.194 ± 0.163 ^{a,6}
Dry	31.525 ± 0.042 ^{c,5}	31.657 ± 0.042 ^{b,c,6}	31.806 ± 0.042 ^{a,b,4}	31.902 ± 0.043 ^{a,5}
Colostrum	31.718 ± 0.056 ^{c,4}	31.904 ± 0.057 ^{b,c,5}	31.982 ± 0.056 ^{a,b,4}	32.119 ± 0.057 ^{a,4}
30 min AB	32.067 ± 0.040 ^{c,3}	32.176 ± 0.040 ^{c,4}	32.358 ± 0.040 ^{b,3}	32.520 ± 0.041 ^{a,3}
1 h AB	32.218 ± 0.050 ^{c,3}	32.380 ± 0.050 ^{b,c,3}	32.453 ± 0.049 ^{a,b,3}	32.601 ± 0.051 ^{a,3}
4 h AB	32.504 ± 0.058 ^{c,2}	32.666 ± 0.059 ^{b,c,2}	32.734 ± 0.058 $^{a,b.2}$	32.910 ± 0.059 ^{a,2}
24 h AB	33.251 ± 0.048 ^{c,1}	33.451 ± 0.048 ^{b,1}	33.596 ± 0.048 ^{a,b,1}	33.705 ± 0.049 ^{a,1}

(Two-way mixed ANOVA.) Notes: *n*, number of puppies; weight of puppies according to category (Q_1, 126–226 g; Q_2, 227–330 g; Q_3, 331–387 g; Q_4, 388–452 g); AB, after birth; least-squares mean ± standard error. ^{a,b,c}: different superscripts among columns indicate significant temperature differences between puppy's weight groups at the same time. ^{1,2,3,4,5,6,7}: different numbers among rows indicate significant differences between times in the same puppy's weight group.

Table 5. Average of the temperature of abdominal (A) window at different times in newborn puppies classified into 4 groups according to weight.

Time	Q_1 $n = 73$	Q_2 $n = 72$	Q_3 $n = 74$	Q_4 $n = 70$
Wet	29.664 ± 0.100 ^{a,5}	29.739 ± 0.101 ^{a,5}	29.800 ± 0.099 ^{a,5}	29.939 ± 0.102 ^{a,5}
Dry	32.353 ± 0.039 ^{c,4}	32.494 ± 0.039 ^{b,c,4}	32.608 ± 0.038 ^{b,4}	32.764 ± 0.039 ^{a,4}
Colostrum	32.501 ± 0.063 ^{b,4}	32.696 ± 0.063 ^{a,b,4}	32.708 ± 0.062 ^{a,b,4}	32.862 ± 0.064 ^{a,4}
30 min AB	32.836 ± 0.062 ^{b,3}	33.007 ± 0.063 ^{a,b,3}	33.059 ± 0.062 ^{a,b,3}	33.191 ± 0.064 ^{a,3}
1 h AB	32.941 ± 0.069 ^{b,3}	33.094 ± 0.069 ^{a,b,3}	33.112 ± 0.068 ^{a,b,3}	33.337 ± 0.070 ^{a,3}
4 h AB	33.322 ± 0.071 ^{b,2}	33.432 ± 0.072 ^{a,b,2}	33.438 ± 0.071 ^{a,b,2}	33.642 ± 0.073 ^{a,2}
24 h AB	33.788 ± 0.055 ^{c,1}	33.995 ± 0.055 ^{b,1}	34.087 ± 0.055 ^{a,b,1}	34.234 ± 0.056 ^{a,1}

(Two-way mixed ANOVA.) Notes: *n*, number of puppies; weight of puppies according to category (Q_1, 126–226 g; Q_2, 227–330 g; Q_3, 331–387 g; Q_4, 388–452 g); AB, after birth; least-squares mean ± standard error. ^{a,b,c}: different superscripts among columns indicate significant temperature differences between puppy's weight groups at the same time. ^{1,2,3,4,5}: different numbers among rows indicate significant differences between times in the same puppy's weight group.

Table 6. The average temperature of the femoral pelvic limb (FPL) window at different times in newborn puppies classified into 4 groups according to weight.

Time	Q_1 $n = 73$	Q_2 $n = 72$	Q_3 $n = 74$	Q_4 $n = 70$
Wet	27.654 ± 0.055 ^{c,5}	27.979 ± 0.055 ^{b,5}	28.001 ± 0.055 ^{b,5}	28.348 ± 0.056 ^{a,5}
Dry	28.670 ± 0.111 ^{b,3}	28.826 ± 0.112 ^{a,b,3,4}	29.072 ± 0.111 ^{a,b,3,4}	29.116 ± 0.114 ^{a,3}
Colostrum	28.020 ± 0.062 ^{c,4}	28.280 ± 0.063 ^{b,4}	28.515 ± 0.062 ^{a,4}	28.714 ± 0.064 ^{a,4}
30 min AB	28.363 ± 0.097 ^{b,3,4}	28.535 ± 0.098 ^{a,b,4}	28.611 ± 0.097 ^{a,b,4}	28.846 ± 0.099 ^{a,3,4}
1 h AB	28.732 ± 0.081 ^{b,3}	29.072 ± 0.081 ^{a,3}	29.137 ± 0.080 ^{a,3}	29.366 ± 0.082 ^{a,3}
4 h AB	29.815 ± 0.070 ^{b,2}	30.068 ± 0.071 ^{a,b,2}	30.226 ± 0.070 ^{a,2}	30.434 ± 0.072 ^{a,2}
24 h AB	30.825 ± 0.089 ^{b,1}	31.163 ± 0.090 ^{a,1}	31.186 ± 0.088 ^{a,1}	31.483 ± 0.091 ^{a,1}

(Two-way mixed ANOVA.) Notes: *n*, number of puppies; weight of puppies according to category (Q_1, 126–226 g; Q_2, 227–330 g; Q_3, 331–387 g; Q_4, 388–452 g); AB, after birth; least-squares mean ± standard error. ^{a,b,c}: different superscripts among columns indicate significant temperature differences between puppy's weight groups at the same time. ^{1,2,3,4,5}: different numbers among rows indicate significant differences between times in the same puppy's weight group.

Table 7. Average of the temperature of thoracic limb biceps brachial (TLBB) window at different times in newborn puppies classified into 4 groups according to weight.

Time	Q_1 $n = 73$	Q_2 $n = 72$	Q_3 $n = 74$	Q_4 $n = 70$
Wet	27.534 ± 0.051 ^{c,6}	27.840 ± 0.051 ^{b,5}	27.883 ± 0.050 ^{b,5}	28.344 ± 0.052 ^{a,5}
Dry	28.439 ± 0.114 ^{b,3,4}	28.615 ± 0.115 ^{a,b,3}	28.948 ± 0.113 ^{a,3}	29.043 ± 0.116 ^{a,3}
Colostrum	27.884 ± 0.053 ^{d,5}	28.127 ± 0.053 ^{c,4}	28.440 ± 0.052 ^{b,4}	28.693 ± 0.054 ^{a,3,4}
30 min AB	28.206 ± 0.088 ^{c,4}	28.399 ± 0.088 ^{b,c,3,4}	28.543 ± 0.087 ^{a,b,4}	28.839 ± 0.089 ^{a,3,4}
1 h AB	28.630 ± 0.078 ^{c,3}	28.988 ± 0.079 ^{b,3}	29.111 ± 0.078 ^{b,3}	29.421 ± 0.080 ^{a,3}
4 h AB	29.567 ± 0.090 ^{c,2}	29.876 ± 0.091 ^{b,c,2}	30.149 ± 0.090 ^{a,b,2}	30.329 ± 0.092 ^{a,2}
24 h AB	30.712 ± 0.080 ^{c,1}	31.023 ± 0.081 ^{b,1}	31.024 ± 0.080 ^{b,1}	31.500 ± 0.082 ^{a,1}

(Two-way mixed ANOVA.) Notes: *n*, number of puppies; weight of puppies according to category (Q_1, 126–226 g; Q_2, 227–330 g; Q_3, 331–387 g; Q_4, 388–452 g); AB, after birth; least-squares mean ± standard error. ^{a,b,c}: different superscripts among columns indicate significant temperature differences between puppy's weight groups at the same time. ^{1,2,3,4,5,6}: different numbers among rows indicate significant differences between times in the same puppy's weight group.

Table 8. Average of the thoracic limb elbow (TLE) window temperature at different times in newborn puppies classified into 4 groups according to weight.

Time	Q_1 $n = 73$	Q_2 $n = 72$	Q_3 $n = 74$	Q_4 $n = 70$
Wet	27.141 ± 0.049 ^{d,7}	27.355 ± 0.049 ^{c,7}	27.675 ± 0.049 ^{b,5}	28.005 ± 0.050 ^{a,5}
Dry	30.798 ± 0.062 ^{b,6}	30.993 ± 0.062 ^{b,6}	31.341 ± 0.062 ^{a,4}	31.351 ± 0.063 ^{a,3}
Colostrum	30.365 ± 0.066 ^{b,5}	30.535 ± 0.067 ^{b,5}	30.787 ± 0.066 ^{a,3}	30.851 ± 0.068 ^{a,4}
30 min AB	31.095 ± 0.069 ^{c,4}	31.272 ± 0.069 ^{b,c,4}	31.450 ± 0.068 ^{a,b,4}	31.565 ± 0.070 ^{a,2,3}
1 h AB	30.093 ± 0.061 ^{c,3}	30.239 ± 0.062 ^{c,3}	30.540 ± 0.061 ^{b,3}	30.895 ± 0.062 ^{a,2,4}
4 h AB	32.149 ± 0.069 ^{b,2}	32.415 ± 0.070 ^{a,2}	32.615 ± 0.069 ^{a,2}	32.485 ± 0.071 ^{a,1}
24 h AB	32.848 ± 0.081 ^{a,b,1}	33.094 ± 0.082 ^{b,1}	33.012 ± 0.081 ^{b,1}	32.594 ± 0.083 ^{a,1}

(Two-way mixed ANOVA.) Notes: *n*, number of puppies; weight of puppies according to category (Q_1, 126–226 g; Q_2, 227–330 g; Q_3, 331–387 g; Q_4, 388–452 g); AB, after birth; least-squares mean ± standard error. ^{a,b,c,d}: different superscripts among columns indicate significant temperature differences between puppy's weight groups at the same time. ^{1,2,3,4,5,6,7}: different numbers among rows indicate significant differences between times in the same puppy's weight group.

Tables 1–8 show the estimated marginal means ± standard error. "Bonferroni corrections were used to adjust *p* values for multiple comparisons" to avoid a type-I statistical error, and the normal distribution of data was assessed using visual inspection of histogram and Q-Q plots.

Table 2 shows the average temperatures of the nasal window (N); a significant statistical difference ($p < 0.0001$) between group Q_1 and Q_4, with a difference of 2.48 °C between Wet time in Q_1 and 24 h AB on Q_4, can be observed.

Table 3 shows the range between different times on the TLM window in newborns with different weights classified into four quartiles. Moreover, like in previous tables, the average temperature in this thermal window shows that puppies with lower weights (Q_1) recorded low temperatures, and puppies with higher weights show the highest temperatures. It is important to note that the lowest temperature of all windows at Q_1 Wet time was recorded in this thermal window.

In Table 5, it can be observed that there is a significant difference ($p < 0.0001$) between the four groups at different times. However, there are similarities between groups Q_2 and Q_3, a pattern repeated in almost all thermal windows evaluated in this study. Similarly, it can be observed that the highest temperatures recorded in this study occurred in this thermal window at 24 h AB.

Table 6 shows statistically significant differences between the lowest and highest quartiles; however, at 24 h AB, the 4 quartiles practically remain without significant differences, except for Q_1.

Table 7 shows that when comparing the Q_1 group with Q_4 at 24 h AB, the difference between these groups was 4.78 °C, and when comparing the temperatures of wet puppies of the Q_1 group with temperatures recorded in the Q_4 group at 24 h AB, a difference of 3.96 °C was recorded, but similitudes were observed again between group Q_2 y Q_3.

Table 8 shows that statistically significant differences between Q_1 and Q_4 at different times were more evident than in the rest of the thermal windows, and these differences were observed between groups Q_2 and Q_3, unlike what was observed in the remainder of the tables.

Subsequently, the correlations between the puppy's weight and their temperatures at different times and in the different thermal windows were evaluated using Spearman's rank correlations. Tables 9–16 report the existing correlations between the puppies' weight and their temperature, where the weights of the four groups (Q_1, Q_2, Q_3, and Q_4) and their respective temperatures by time and thermal window indicate how the temperature tends to vary at different times and in different thermal windows. In almost all cases, there is a positive correlation with statistically significant differences ($p < 0.0001$) (Tables 9–15). These results mean that the higher the weight of the puppies, the higher temperatures recorded in them. Only one case was observed where the correlation was negative (Table 16) in

the thermal window TLE at 24 h AB. In this case, as the puppies' weight increased, their temperature decreased in the TLE window. The *r* values in all tables were between 0.146 and 0.651, except in Table 16 of the TLE thermal window, where $r = -0.024$ ($p = 0.679$) at 24 h AB. The highest correlations were observed in Table 15 TLBB window (Colostrum $r = 0.624$) and Table 11 TLM window (Colostrum $r = 0.632$).

Table 9. Significant correlations between puppies' weight and their superficial temperature in UPL thermal window.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.190	0.001
Dry	0.189	0.001
Colostrum	0.248	<0.001
30 min AB	0.379	<0.001
1 h AB	0.444	<0.001
4 h AB	0.292	<0.001
24 h AB	0.364	<0.001

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their temperature at different times. AB: after birth.

Table 10. Significant correlations between puppy's weight and their superficial temperature at N thermal window.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.542	<0.001
Dry	0.510	<0.001
Colostrum	0.423	<0.001
30 min AB	0.365	<0.001
1 h AB	0.226	<0.001
4 h AB	0.353	<0.001
24 h AB	0.331	<0.001

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their temperature at different times. AB: after birth.

Table 11. Significant correlations between puppies' weight and their superficial temperature at TLM thermal window.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.551	<0.001
Dry	0.372	<0.001
Colostrum	0.632	<0.001
30 min AB	0.315	<0.001
1 h AB	0.469	<0.001
4 h AB	0.452	<0.001
24 h AB	0.353	<0.001

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their temperature at different times. AB: after birth.

Table 12. Significant correlations between puppies' weight and their superficial temperature at T thermal window.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.182	0.002
Dry	0.409	<0.001
Colostrum	0.307	<0.001
30 min AB	0.449	<0.001
1 h AB	0.337	<0.001
4 h AB	0.328	<0.001
24 h AB	0.441	<0.001

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their temperature at different times. AB: after birth.

Table 13. Significant correlations between puppies' weight and their superficial temperature at A thermal window.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.146	0.013
Dry	0.419	<0.001
Colostrum	0.258	<0.001
30 min AB	0.252	<0.001
1 h AB	0.273	<0.001
4 h AB	0.205	<0.001
24 h AB	0.376	<0.001

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their temperature at different times. AB: after birth.

Table 14. Significant correlations between puppies' weight and their superficial temperature at FPL thermal window.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.429	<0.001
Dry	0.415	<0.001
Colostrum	0.494	<0.001
30 min AB	0.224	<0.001
1 h AB	0.338	<0.001
4 h AB	0.365	<0.001
24 h AB	0.292	<0.001

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their temperature at different times. AB: after birth.

Table 15. Significant correlations between puppies' weight and their superficial temperature at TLBB thermal window.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.520	<0.001
Dry	0.444	<0.001
Colostrum	0.624	<0.001
30 min AB	0.311	<0.001
1 h AB	0.397	<0.001
4 h AB	0.406	<0.001
24 h AB	0.360	<0.001

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their temperature at different times. AB: after birth.

Table 16. Significant correlations between puppies' weight and their superficial temperature at TLE thermal window.

Variables	Correlation Coefficient (*r*)	*p*-Value
Wet	0.651	<0.001
Dry	0.391	<0.001
Colostrum	0.429	<0.001
30 min AB	0.400	<0.001
1 h AB	0.525	<0.001
4 h AB	0.259	<0.001
24 h AB	−0.024	0.679

Spearman's rank correlation coefficients and their statistical significance between puppies' weight and their temperature at different times. AB: after birth.

4. Discussion

The present study assessed the superficial temperature in 289 newborn puppies born from 60 bitches with different weights at 8 anatomical regions or thermal windows. The

puppies were evaluated at different times and assigned to four groups using size-specific quartiles (Q_1, Q_2, Q_3, and Q_4). According to the results, there was an association between puppies' thermal response and their weight, with the highest temperature recorded in Q_4 in all thermal windows at all evaluation times. Additionally, although the temperatures at Wet and Dry times differed between the four groups (Q_1, Q_2, Q_3, and Q_4), both (Wet and Dry times) had the lowest values in all groups. Interestingly, at 24 h AB, the puppies' temperature in every thermal window was similar between the groups, showing a similar thermoregulatory capacity independent of their weight; however, it was still the lowest in group Q_1 and highest in Q_4. These results differed from those of another study carried out by this workgroup [35], where the groups were classified according to the dam's weight, and it was found that in the groups with greater dams' weight, the thermoregulation of the puppies stabilized at 24 h AB, showing positive correlations with higher weight of the mother, due to better thermoregulation of her pups.

The present results show the effect of puppies' weight on their superficial temperature. Newborns with low weight at birth had lower temperature values for all thermal windows at every measured time, compared with the heaviest puppies. According to Harri et al. [37], birth weight is important for the survival of neonates, and it is one of the most important factors that influence temperature loss, since the body mass index (BMI) determines the capacity of thermoregulation in newborns, as with foxes. However, the litter size also significantly influences ($p = 0.003$) the weight of the newborns. In this sense, it is common to have a higher number of low-birth-weight puppies in large litters compared to small litters [25]. This effect can be seen in piglets, where large litters can have smaller piglets with higher mortality rates, due to hypothermia or trauma generated by the mother when they are too weak to move [38].

This thermoregulatory dependence on weight and time has also been reported in dog puppies that cannot stabilize their temperature rhythm for several days after birth, but their rectal temperatures become stable at six weeks AB [39]. It is important to consider that the temperature of the environment in which puppies live could have a favorable or unfavorable effect on their welfare [40]. However, in this study, the temperature of the environment was not controlled, but similar conditions (34–40 °C) and foam mats were maintained in the place of parturition to avoid temperature loss by convection. Schrank et al. [41] reported that birth weight could impact newborns' vitality and the health. It also has been reported that dogs of small size tend to lose heat quickly because they have a greater surface-to-area volume ratios [42]; therefore, they require a greater heat production to maintain their thermostability [43]. This can also be attributed to the wide variability of canine breeds [39].

Significant differences have been observed throughout the times evaluated, from wet to 24 h AB, in the evaluated regions. When the fetuses are still in the uterus of the bitch, they maintain a temperature of 0.3 to 0.5 °C higher than the dam's body temperature. However, at birth, the puppies experience a significant thermal change and loss of temperature through evaporation occurs, because they are born wet, due to the amniotic fluid [44]. This happens because the insulation and protection function that the coat provides is not efficient, since the amniotic fluid has a high thermal conductivity which generates greater hypothermia. In addition to this, other contributing factors include decreased body fat, age, and lack of acclimatization to the environment [45,46]. Therefore, increased body temperatures when passing from Wet to Dry time may be explained either by the decrease in heat loss through evaporation, or the decrease in convection favored by air currents. Another critical factor is rubbing puppies, which stimulates dermal vascular microcirculation of peripheral blood vessels (peripheral vasodilation), increasing microvascular hyperemia at the dermal level.

Hypothermia was observed in newborn puppies of the present study at the first three evaluation times (Wet, Dry, and Colostrum), where the lowest superficial temperatures were recorded.

The thermostability observed at 24 h AB in puppies from all groups could be attributed to the efficacy of the thermoregulatory response of newborns regardless of their initial weight, as shown in the values of IRT in all thermal windows, where the four groups, when compared to the temperature at wet, had a similar average increase. Several factors can influence this response, such as glycogen reserves, colostrum intake, increased digestion, the presence of the dam, and the members of the litter. The thoracic windows demonstrate an important fact: as other studies carried out with other species showed, the temperature tends to rise after the consumption of colostrum; in this study, we can see that immediately after the consumption of colostrum, the temperature tends to rise in the four quartiles in the T window.

As observed in the results presented, at 24 h AB all puppies reached thermostability after their coat was dried and following colostrum intake and heat transfer of the mother's temperature by convection. According to Münnich and Küchenmeister [21], these thermoregulatory behaviors are important during newborns' first days since puppies cannot maintain their body temperature when exposed to cold environments up to six days after birth.

According to the results reported in this study, the most notable thermoregulatory changes in newborn puppies were low temperatures in the distal regions, that is, in the thermal windows of TLM and FPL, and high temperatures in thermal windows A, T, and ULP. This could be because the most important structures related to metabolism and vital functions are found in the thoracic, abdominal, and cranial regions [47,48]. The appendicular windows presented the lowest temperatures, which can be explained, in the case of newborn puppies, because they still do not present locomotion, as they are an altricial species, and their degree of neurological development, responsible for the mechanism of thermal modulation, is immature. Neonates rely on peripheral vasoconstriction and arteriovenous shunts at the skin level to reduce heat loss, but also use non-shivering thermogenesis by lipolysis of brown adipose tissue and the oxidation of fatty acids in the mitochondria through adenosine triphosphate (ATP) synthesis, because it is reported that neonatal skeletal muscles are immature at birth. It is precisely this point that provides the explanation for why thermogenesis by shivering in newborn puppies is not so effective [44]. The time the altricial species develop the ability to obtain thermostability also influences this. According to Pineda and Dooley [49], thermostability is not reached until day 18 of life, and autonomic thermoregulation is known to be wholly developed at the end of the fourth week [19].

Additional research that could be performed based on the present findings includes study of the influence of factors such as environmental temperature, size of litter, and quality of maternal care on the thermostability of newborn puppies during the first hours of life. It is important to mention that one of the main limitations of this study was that, since births did not always occur in the same place, the environmental temperatures could not be controlled and standardized for all cases. Likewise, applying IRT to study the association with the percentage of male puppies would be interesting, since it was found that the percentage of male puppies was positively correlated with lateral nursing, a position that provides puppies the easiest access to the milk. Looking at this data from an evolutionary point of view, the mothers spend more attention on male puppies because it is more convenient in terms of fitness [50] and could alter the peripheral thermal response of neonates.

5. Conclusions

Results suggest that, during the first hours of the life of newborn puppies, their thermoregulation mechanism is deficient in anatomical and metabolic factors that can be reduced by colostrum intake, maintaining an adequate body temperature, and their mechanisms to conserve or dissipate heat in the evaluated thermal windows.

These results also suggest a positive correlation between the puppy's weight and its ability to achieve thermostability, which is continuously observed in all the evaluated

thermal windows. The puppies born with lower weights (Q_1, 126–226 g) presented the lowest temperatures, while puppies with higher weights (Q_4, 388–452 g) presented the highest temperatures.

Likewise, the time effect of the temperature recording showed relevant changes in them; the lowest temperatures were obtained when the puppies were still wet and the highest at 24 h AB.

Regarding the body regions where temperatures were evaluated, the findings suggest that the thermal windows with the highest temperatures were A, T, N, and ULP, and those with the lowest temperatures were TLBB, TLE, TLM, and FPL. Assessing the thermal response in newborn puppies can help identify drastic temperature reductions or deficient thermoregulatory compensation during the first hours of life, preventing the consequences of hypothermia.

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Informed Consent Statement: All the owners of the study animals were asked to grant informed consent to carry out the procedures.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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9. DISCUSIÓN GENERAL

Fase 1. Dinámica uterina, perfil sanguíneo y monitoreo fetal electrónico en perras primíparas y multíparas clasificadas según su peso.

Los resultados obtenidos demuestran diferencias significativas entre perras primíparas y multíparas, las cuales no solo afectaron la salud sino también el desempeño de los recién nacidos.

1.1. Duración de la fase de expulsión

Aunque no se encontraron diferencias significativas entre madres primíparas y multíparas, la duración de la fase de expulsión estuvo influenciada por el peso de la madre, es decir, las perras más pesadas (G_4) tuvieron fases de expulsión más largas. Un estudio retrospectivo sobre distocia comparó diferentes razas según su rango de tamaño y peso: raza pequeña (<12,7 kg), mediana (12,7-20,5 kg) y grande (>20,5 kg) (Darvelid y Linde-Forsberg 1994), y contrariamente a los resultados del presente estudio, no se registraron diferencias entre el peso y el número de parto. Del mismo modo, Zonturlu y Kacar (2012) no encontraron diferencias significativas en cuanto a la duración de la fase de expulsión entre perras de Pastor Alemán (7,49 ± 2,44 h) y Labrador Retriever (7,38 ± 1 h). Sin embargo, el intervalo entre cachorros fue diferente, oscilando entre 20 y 415 minutos, y 5 y405 min, respectivamente, mientras que Baqueiro-Espinosa *et al.* (2022) encontraron que la duración más larga del parto (369,73 min) se observó en madres de diferentes razas y paridades que oscilaban entre 0 y 4.

Los hallazgos de este estudio respecto a las fases de expulsión más largas, éstas pueden asociarse con una disfunción en la contractilidad miometrial en animales con mayor peso, como se ha observado en modelos animales con sobrepeso. En un estudio realizado en ratas, las hembras con alto contenido de grasas y colesterol presentaron contracciones miometriales asincrónicas, lo que aumentó la duración del parto (Muir *et al.* 2023).

1.2. Intervalo de expulsión entre cachorros

Las perras primíparas y las del grupo G_1 tuvieron el intervalo de expulsión más largo entre cachorros (82,2 ± 4,86 min). La duración media de un intervalo normal oscila entre cinco minutos y dos horas (Romagnoli *et al.* 2004), mientras que los intervalos de 12 a 16 h entre el primer y el último feto se consideran distocia (Simões *et al.* 2016). Varios estudios han reportado hallazgos similares y el aumento en el intervalo entre crías se ha relacionado con un agotamiento fisiológico de la perra y contracciones miometriales ineficaces (Kuttan y Joseph 2017).

1.3. Cachorros nacidos muertos

El riesgo de muerte fetal se ha asociado con la paridad, como lo muestra el estudio de Münnich y Küchenmeister (2009), quienes concluyeron que las perras primíparas de más de seis años tenían la mayor frecuencia de muerte fetal (66,1%) y tenían partos retrasados (3,8%). Un resultado similar se obtuvo en el presente estudio, donde la proporción de cachorros nacidos muertos fue mayor en las perras primíparas del grupo G_4. Aparte de la mayor frecuencia de muertes fetales, se ha informado que las perras primíparas tienen un mayor riesgo de requerir cesáreas (p=0,004) y esto está directamente relacionado con la presencia de muertes fetales (Schrank *et al*. 2022b). Algunos autores atribuyen este efecto a la mayor duración del parto y a la falta de experiencia en perras primíparas (Schrank *et al.* 2022a). Sin embargo, otros informes indican que la paridad no está relacionada con los casos de muerte fetal (Oluwatoyin y Fayemi 2011; Van Egmond 2019), mientras que otros autores mencionan que sólo después de la cuarta paridad existe una tasa constante de muerte fetal (Gill 2001; Gill 2002).

En cuanto a la mayor proporción de muertes fetales en perras del grupo G_4, se ha documentado que el sobrepeso materno es un factor de riesgo de muerte fetal en mamíferos (Mitanchez y Chavatte-Palmer 2018). Esto se debe a una función placentaria deteriorada, lo que aumenta el riesgo de muerte fetal (Tajaddini *et al.* 2022).

1.4. Peso de nacimiento

La relación entre el mayor peso de las perras y el mayor peso al nacer de los cachorros observada en el presente estudio ha sido reconocida como un factor que podría afectar el desarrollo de los cachorros (Alberghina *et al.* 2021), hecho que también ha sido reportado en el ganado. En los corderos, el peso materno de las ovejas afectó significativamente su peso al nacer debido a la nutrición materna. En este sentido, la calidad y cantidad de nutrientes obtenidos durante la gestación influyen en el crecimiento fetal (Gardner *et al.* 2007). Otro estudio realizado en perras que evaluó la influencia de la raza y su peso promedio encontró que los cachorros de tamaño mediano (10-20 kg) tenían tasas de mortalidad perinatal más bajas que las razas grandes (> 20 kg) con probabilidades 0,99 veces menores (Baqueiro-Espinosa *et al.* 2022). En cuanto a la paridad, el mayor peso registrado fue el de los cachorros nacidos de perras multíparas, esto difiere de lo reportado por Tesi *et al*. (2020) en perros toy y de tamaño pequeño, en quienes la paridad no afectó el peso al nacer de los cachorros ni la mortalidad neonatal. Por el contrario, los corderos de las ovejas de primer parto tuvieron el peso más bajo (Elaref *et al*. 2020). Por lo que, en el caso de este estudio, podría deberse a un mayor rendimiento reproductivo en las perras multíparas.

1.5. Intensidad de las contracciones

En ratas (Muir *et al.* 2023), en bovinos (Doualla-Bell 1995) y en humanos (Chin *et al*. 2012), se ha observado que la obesidad está relacionada con la presentación de contracciones uterinas más intensas, y esto puede estar asociado con la regulación de la conexina-43 en los miocitos del miometrio. En el presente estudio, no se incluyeron perras obesas; sin embargo, las contracciones uterinas más intensas ocurrieron en perras primíparas de los grupos G_1 y G_2, siendo estas las pequeñas, pero también ocurrieron en perras multíparas de G_3 y G_4, siendo estas las más grandes. Por lo tanto, esto podría estar asociado, no sólo al peso de las madres, como en las primíparas y perras más livianas, sino también al peso de los recién nacidos al nacer y al tamaño de la camada, teniendo perras de mayor tamaño camadas más grandes (Snith, 2012; Okkens *et al.* 1993), y por tanto de menor espacio en el útero al estar totalmente ocupado por los fetos, así como fatiga uterina en partos muy prolongados (Kuttan y Joseph 2017).

1.6. Duración de las contracciones

En las primíparas la duración de las contracciones fue más prolongada que en las multíparas, pero en este caso su peso no afecta significativamente el tiempo de contracciones del miometrio. Factores como la respuesta de estrés desencadenada en el primer parto aumentan los niveles de epinefrina circulante, reduciendo la actividad contráctil del útero y aumentando su duración (Ulfsdottir *et al.* 2014). Asimismo, al comparar mujeres multíparas y nulíparas, las pacientes primíparas tuvieron fases de parto activo y pujo más prolongadas (Tilden *et al*. 2022), donde se requiere la oxitocina y su acción sobre los receptores uterinos de oxitocina para promover contracciones fuertes y efectivas (Buckley *et al*. 2023).

1.7. Número de contracciones

Algunas razas, como los Boxers, Border Collies, Labrador y Golden Retrievers, están predispuestas a presentar inercia uterina. Otros factores que predisponen a esta situación son la edad de la madre, camadas desproporcionadamente grandes o pequeñas, la obesidad y los desequilibrios hormonales o nutricionales (Egloff *et al*. 2020). A diferencia de estas razas predispuestas reportadas, en este estudio, las perras primíparas más pesadas (G_4), manifestaron más actividad uterina al tener más contracciones que las multíparas, y la mayoría de este grupo de perras eran Labrador y Golden Retriever.

1.8. Intervalo entre contracciones

El intervalo entre contracciones fue mayor para las perras multíparas más livianas, lo que coincide con que las perras primíparas tienen más contracciones, por lo que los intervalos entre ellas disminuyen. También hay que considerar que según Olsson *et al.* (2003), el inicio de la fase de expulsión se produce por el aumento de la concentración plasmática de vasopresina y probablemente en perras multíparas esta hormona podría disminuir a medida que avanza el momento del parto.

1.9. Perfil sanguíneo

En general, las perras primíparas más pesadas presentaron los desequilibrios sanguíneos más críticos. Cuanto mayor era el peso, mayor era la camada, más duraba el parto y mayor era la incidencia de inercia uterina y complicaciones del parto. Estas reacciones pueden estar asociadas con una mayor duración del parto que perjudica la actividad uterina y la consiguiente isquemia fisiológica, hipoxia y acidificación (Carlson *et al*. 2015) observadas como niveles elevados de lactato y pCO2, y una disminución de la pO2 reportada en perras del G_4. Las elevaciones en los niveles de glucosa registradas en perras de mayor peso son similares a las reportadas en perras y cachorros, donde la distocia fetal indujo un estado de hiperglucemia, junto con un aumento del cortisol, una hormona conocida por movilizar la glucosa a través de la glucogenólisis y la gluconeogénesis (Lúcio *et al.* 2021). Por lo tanto, las complicaciones del parto reportadas en perras G_4 son consistentes con el perfil bioquímico de los casos de distocia.

1.10. DIP 2

No existen muchos estudios donde se haya evaluado DIP 2 en perras. Un estudio de Gil *et al*. (2014) encontraron que los fetos estaban angustiados cuando los latidos por minuto estaban entre 160 y 180 lpm durante 60 segundos o más. Al contrario de lo encontrado por Gil *et al*. (2014), quienes observaron que tanto las perras primíparas como las multíparas podían presentar desaceleraciones, en el presente estudio se encontró que el DIP 2 se desarrolló en 44 perras primíparas, y en 23 multíparas, por lo que se pudo registrar que la presentación del DIP 2 era más probable que ocurriera en las perras primíparas y en 29 perras de la población total del estudio no se observó DIP 2.

1.11. Bradicardia

En algunos estudios (Zone y Wanke 2001) se ha observado que el bienestar de los fetos canideospuede evaluarse en función de los movimientos fetales y los latidos del corazón, y consideraron sufrimiento fetal grave cuando los fetos tenían <180 latidos por minuto. Según Gil *et al.* (2014), el día del parto se puede predecir mediante el uso de la FCF, lo que podría ayudar a proporcionar una intervención oportuna y reducir las pérdidas de animales. Existen otros estudios realizados en humanos por Hon (1958) y Hess y Hon (1960), donde evaluaron las variaciones de los latidos del corazón fetal al implementar oxitocina exógena en la madre durante el parto o al hacer ejercicio. A diferencia de dichos estudios, en el presente no se administró ningún fármaco a las perras ni se las sometió a ningún tipo de ejercicio o estrés, por lo que los resultados obtenidos podrían aproximarse más a lo que ocurre en un parto normal en las perras.

En este estudio, un total de 79 cachorros recién nacidos presentaron bradicardia y estas disminuciones en la frecuencia cardíaca fueron más evidentes en cachorros nacidos de perras primíparas. Esto se puede explicar por qué de igual manera en este estudio se ha observado que las contracciones uterinas más intensas se observaron en perras primíparas, lo que hace más probable la presentación de desaceleraciones DIP 2.

1.12. Cianosis

La observación de cachorros cianóticos fue mayor en perras primíparas, posiblemente debido a las complicaciones y falta de experiencia reportadas en animales del primer parto. La asfixia fetal, la hipoxia y las membranas mucosas cianóticas son indicadores de puntuaciones bajas de vitalidad en varias especies domésticas (Vannucchi *et al.* 2015; Mota-Rojas *et al.* 2018).

1.13. Hipotermia y adinamia

Se ha visto que las perras primíparas, al tener poca o ninguna experiencia en el desarrollo del parto y el comportamiento maternal, tienden a ser menos hábiles en el cuidado materno de sus crías. Estos recién nacidos son altriciales y requieren de la ayuda de la madre para moverse y termorregularse (Lezama-García *et al*. 2022c), no pueden hacerlo adecuadamente y esto coincide con lo encontrado en este estudio donde 69 cachorros recién nacidos de perras primíparas manifestaron adinamia e hipotermia. En comparación, 47 cachorros recién nacidos de perras multíparas lo manifestaron. En este sentido, la experiencia materna

influye en el cuidado de la descendencia. En la **Figura 17** se pueden observar las diferencias que presentan las especies altriciales y precociales en cuanto a su capacidad para termorregular.

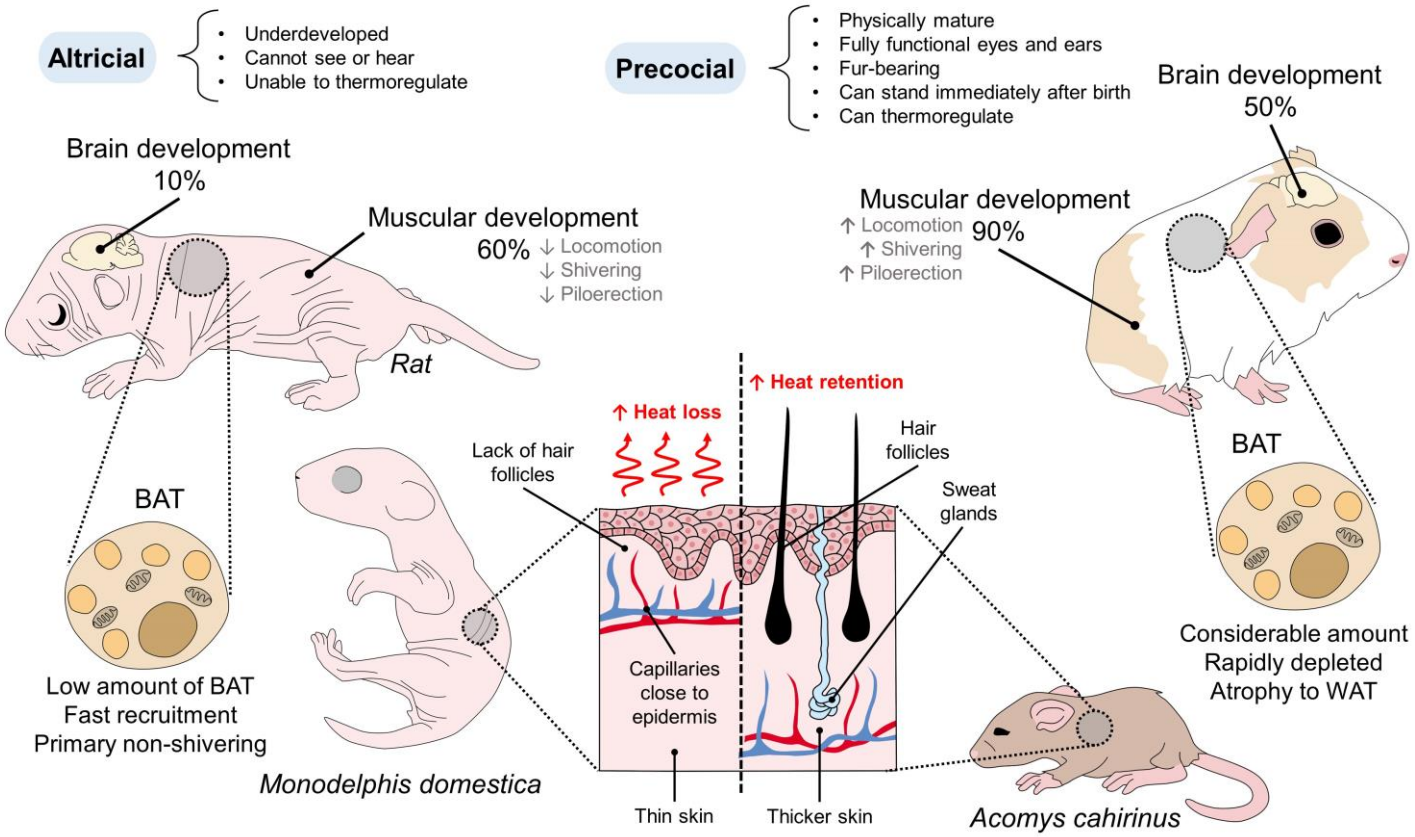

Figura 17. Diferencias morfoanatómicas en roedores recién nacidos altriciales y precociales (*Monodelphis domestica y Acomys cahirinus*), y su influencia en la termorregulación. En especies altriciales o subdesarrolladas y precociales o físicamente maduras, las características morfológicas promueven o dificultan la termorregulación. En los animales altriciales, la falta de pelaje, las bajas cantidades de Tejido Adiposo Pardo (BAT), la locomoción descoordinada y la piel fina con capilares cerca de la epidermis contribuyen a la pérdida de calor y la susceptibilidad a la hipotermia. Por el contrario, en las especies precociales, la presencia de pelaje, la locomoción coordinada y una cantidad considerable de BAT mantienen la retención de calor y una mejor termorregulación (Lezama-García *et al.* 2022c).

1.14. Sexo de los cachorros

La relación entre mayor peso al nacer y cachorros machos es una característica observada en diferentes especies de mamíferos. Se atribuye a un dimorfismo sexual en el que los machos tienden a ser más grandes que las hembras, como se ha informado en lechones recién nacidos (Stenhouse et al. 2022). Además, como se ha comentado anteriormente, el peso corporal de las perras también influye en el peso al nacer de los recién nacidos. Por ello, ante madres con pesos corporales elevados, es importante considerar los efectos adversos que esto puede provocar en la madre, y la importancia que tiene para el crecimiento y supervivencia del cachorro.

Un aspecto importante para considerar en futuras investigaciones podría ser el hecho de evaluar también el efecto de la raza en la presentación de determinadas conductas maternas y en el desempeño reproductivo y dinámica uterina de las perras, para de esta forma poder determinar qué razas pudieran ser reproducidas sin la presentación de factores que pudieran incrementar la mortalidad en esta especie.

Fase 2. Evaluación de la vitalidad, perfil sanguíneo y grado de tinción de meconio en la piel en cachorros recién nacidos clasificados de acuerdo con su peso al nacer

De acuerdo con los resultados se puede decir que el peso al nacer influye en la termorregulación, en los cambios hemodinámicos y en el grado de tinción de meconio en perros recién nacidos, como se informó en anteriores estudios realizados por los autores (Lezama-García *et al.* 2022b, 2022a).

2.1. Valores del perfil sanguíneo

Los cambios más notables en el perfil sanguíneo fueron la relación entre los cachorros Q_4 (recién nacidos más pesados) con la presentación de hipoxia, hipoglucemia, hipercalcemia, hiperlactatemia y marcada acidosis metabólica. Los niveles plasmáticos de HCO_3^- observados en este estudio fueron 21,7 mmol/L para Q_1 LB, 12,8 mmol/L para Q_1 SB, 17 mmol/L para Q_4 LB y 10,6 mmol/L para Q_4 SB. Estos valores podrían demostrar hipoxia pulmonar grave y elevada producción de ácido láctico, porque cuando su producción aumenta, el organismo activa el uso de HCO_3^- como un amortiguador. Sin embargo, debido al mayor consumo de HCO_3^-, los recién nacidos experimentan, tanto acidosis respiratoria, como acidosis metabólica debido a estos procesos respiratorios anaeróbicos que finalmente conducen a la hipoxia (Manning, 2001).

Las variaciones en el equilibrio de los niveles de glucosa durante la etapa neonatal dependen de la producción endógena y del metabolismo de la glucosa, lo que resulta en hiper o hipoglucemia. De acuerdo con Vannucchi *et al.* (2012), la hipoglucemia es la principal causa de mortalidad neonatal debido a que el rápido agotamiento de los recursos energéticos puede causar hipotermia aguda. En otras palabras, la rápida reducción de la circulación fetal en el momento del parto causada por las contracciones uterinas y la presión que pueden ejercer sobre el cordón umbilical fetal se asocia con un rápido agotamiento del glucógeno hepático y disminución de la homeostasis de la glucosa (Nowak y Poindron 2006). En el presente estudio, dentro de estas circunstancias adversas, se puede mencionar que para las perras del grupo Q_4 los partos suelen tardar más por ser razas que tienen camadas mayores en número y tamaño, lo que puede prolongar el intervalo de expulsión. Las diferencias significativas en la concentración de glucosa en sangre en los cuartiles (62,8 mg/dL en Q_1; 65,3 mg/dL en Q_2; 71,3 mg/dL en Q_3 y 62,6 mg/dL en Q_4) podrían estar asociados con el estrés neonatal y la consiguiente liberación de catecolaminas y promoción de la glucogenólisis hepática (Herpin *et al.* 1996; Tuchscherer *et al.* 2000).

En estudios previos realizados por Mota-Rojas *et al.* (2005b, 2006b) también se reportó la reducción en el suministro de oxígeno observada en lechones, en los que la hipoxia del cordón umbilical conduce a un aumento de la pCO2 por acidosis respiratoria, pudiendo con ello provocar mortalidad prenatal. De manera similar, en otro estudio realizado por Massip (1980) en fetos de ovejas cerca de término, los animales mostraron una compensación del mecanismo de acidosis respiratoria al aumentar la pCO2. Lo contrario se observa durante la acidosis metabólica, que conduce a hiperventilación alveolar secundaria al aumento de iones H^+ en el plasma y una disminución de la pCO2. Estos hallazgos coinciden con lo encontrado en el presente estudio, donde el cuartil de los cachorros más pesados (Q_4) desarrolló hipercapnia severa, la cual oscilaba entre 64 mmHg en LB y 95,1 mmHg en SB. La razón por la que esto sucede es porque durante el parto pueden existir variaciones en los parámetros cardiorrespiratorios (taquicardia) y la hipercapnia y la acidosis respiratoria pueden surgir inmediatamente después del nacimiento. De acuerdo con Alonso-Spilsbury *et al.* (2005) y Vannucchi *et al*. (2012), la hipoxia fisiológica desencadena un aumento de la frecuencia respiratoria y su consecuente apnea. Las variaciones importantes del pH en todos los cuartiles, especialmente en Q_4 (LB 7,24; SB 6,82), muestran que el bicarbonato y la pCO2 indican acidosis neonatal y posible estrés hipóxico fetal (Zamora-Moran, 2017).

2.2. Puntuación de vitalidad

Los cachorros de Q_4 tuvieron el índice de vitalidad más bajo de todos los cuartiles en el minuto 1 después del nacimiento (AB). Esto podría estar asociado a algunos factores, tales como el intervalo de expulsión, ya que en los partos en donde los cachorros son más pequeños, el parto es más rápido que en los más grandes. Por tanto, a diferencia de los cachorros más grandes, que tardan más para ser expulsados, los más pequeños no consumen tanto las reservas de energía. Por lo tanto, el consumo de energía en

cachorros más grandes para tratar de compensar el estrés de la hipoxia es más significativo en la fase de expulsión. Estos recién nacidos más grandes tienen un mayor riesgo de asfixia intrauterina, lo que afecta sus posibilidades de supervivencia (Lucia *et al.* 2002).

El peso al nacer también puede predisponer a la asfixia. Por ejemplo, en loshumanos, los individuos con bajo peso al nacer tienen puntuaciones bajas de vitalidad (Cavaliere 2016). De manera similar, Okere *et al*. (1997), encontraron que la puntuación de viabilidad de los lechones estuvo moderadamente correlacionada con su peso (r=0,66). En contraste con dichos autores, en el presente estudio se observó que los cachorros más grandes (Q_4) tenían puntuaciones de vitalidad bajas en el primer minuto AB, pero su puntuación mejoró en el minuto 60 AB. Del mismo modo, nuestros resultados son similares a los reportados por Trujillo *et al.* (2011), quienes concluyeron que los lechones pesados tenían puntuaciones ≤ 5 y mostraron signos de asfixia. Una posible explicación podría ser que los lechones más pesados tuvieran mayor dificultad para lograr pasar por el canal del parto, afectando la salud y vitalidad de los recién nacidos (Schrank *et al.* 2019). Además, Veronesi *et al*. (2022) mencionan que los cachorros de tamaño pequeño pueden presentar diversos niveles de angustia, pero mayores posibilidades de supervivencia en comparación con animales de gran tamaño.

La vitalidad también influye en la capacidad del recién nacido para ponerse de pie e ingerir calostro, limitando la absorción de nutrientes y la obtención de energía (Veronesi *et al.* 2022), parámetros que son importantes en el período posparto porque la disposición de los recursos energéticos, tales como el BAT y la glucosa, difiere entre animales de acuerdo con su peso al nacer. Además, la saturación de oxígeno (SpO_2), ha mostrado una correlación negativa con el peso al nacer, lo que significa que los recién nacidos más pesados tienen una SpO_2 más baja (Røsvik *et al.* 2009). Sorprendentemente, con el paso de los minutos se pudo observar que el factor tiempo favoreció al cuartil Q_4 porque el consumo de calostro probablemente coincidió con el de estos cachorros y al minuto 60 después del nacimiento, casi siempre, todos los recién nacidos ya han consumido calostro, además de que los cachorros más grandes tendrán más fuerza de succión y, por tanto, consumirán más calostro. Esto podría sostenerse con un estudio de Mila *et al*. (2015), quienes mencionaron que la glucosa que aporta el calostro es esencial para los cachorros recién nacidos porque sólo el 1,3% de la grasa corporal está disponible. Por el contrario, el calostro de las perras de razas pequeñas (menos de 10 kg) proporciona un 10% más de energía que el calostro de las hembras de razas grandes (Chastant-Maillard *et al.* 2017). Del mismo modo que Herpin *et al.* (1996) y Trujillo-Ortega *et al.* (2011) reportaron, los cachorros recién nacidos que no pasaron la escala de vitalidad en el presente estudio mostraron un aumento en la pCO_2 y ácido láctico en sangre y una pO_2 baja, hallazgo que confirmaría que estos recién nacidos sufrieron hipoxia tisular temprana.

Las correlaciones entre el peso al nacer y la vitalidad no mostraron una correlación estadísticamente significativa, lo cual puede explicarse por el hecho de que, aunque los cachorros Q_4 mostraron puntuaciones de vitalidad reprobatorias (5.77) en el minuto 1 después del nacimiento, lograron alcanzar puntuaciones de vitalidad altas (7.48) al minuto 60 después del nacimiento, prácticamente igualando las puntuaciones de vitalidad de los cachorros de los otros tres cuartiles (Q_1, Q_2 y Q_3). Esto de nuevo pudiera ser explicado con la posibilidad de que tuvieron estos cachorros de haber consumido calostro y con ello recuperar energía y mejorar su escala de vitalidad y de este modo su supervivencia.

2.3. Grado de tinción de meconio

Estudios realizados en lechones sugieren que los recién nacidos con bajo peso tienden a presentar con mayor frecuencia mortalidad intraparto (Randall 1971; Stanton y Carroll 1974; Marchant *et al.* 2000). Por el contrario, los bebés humanos con mayor peso al nacer parecen tener una mayor predisposición a la hipoxia y a la aspiración de meconio (Wiswell y Bent 1993; Cleary y Wiswell 1998). Esto podría estar relacionado con problemas en el parto debido al tamaño y peso del recién nacido. Del mismo modo, los lechones más pesados mostraron una tinción de meconio más intensa en un estudio realizado por Mota-Rojas *et al*. (2006a).

Los resultados actuales muestran que el 16,5% de los mortinatos tenían una tinción grave de meconio en la piel en cachorros del cuartil Q_4. La mayoría de los mortinatos tuvieron tinción de meconio leve, moderada y grave (6, 10 y 18 cachorros, respectivamente), lo que sugiere que se requiere una observación cuidadosa

para evaluar correctamente la anoxia intraparto basándose únicamente en la tinción epidérmica. En otras palabras, los porcentajes más altos de mortinatos (SB) se produjeron en Q_4, con un 33,02% (36 cachorros SB de 109 en total), de los cuales el 5,5% fueron tinciones de meconio leves, el 9,1% fueron moderadas y el 16,5% fueron severo.

El presente estudio encontró una asociación entre los valores bajos de pH sanguíneo y la presentación de tinción de meconio grave, independientemente del peso al nacer, coincidiendo con estudios previos donde la tinción con meconio como indicador demostró la capacidad de identificar hipoxia fetal y, en consecuencia, acidosis (Mitchell y Chandraharan 2018). La presencia de meconio puede obstruir las vías respiratorias, provocando hipoxemia aguda, hipercapnia y acidosis metabólica debido al metabolismo anaeróbico tisular que provoca una disminución progresiva del flujo sanguíneo pulmonar, exacerbando la hipoxia y la acidosis (Olicker *et al.* 2021). Un resultado similar se reportó en humanos, en quienes el 81,82% de los recién nacidos que presentaron líquido espeso teñido de meconio y puntuaciones bajas de Apgar (alrededor de 6,45) tenían un pH sanguíneo por debajo 7,5 (Kima *et al.* 2023). Por lo tanto, observar cachorros teñidos de meconio podría indicar alteraciones fisiológicas que no siempre se evalúan durante el parto, pero que podrían inferirse y adoptar un adecuado control perinatal de los cachorros manchados.

Fase 3. Relación entre el peso de la madre y la temperatura superficial de sus cachorros en diferentes etapas del posparto

El presente estudio evaluó la temperatura superficial en ocho regiones anatómicas o ventanas térmicas y en diferentes momentos en cachorros recién nacidos de perras con pesos distintos. Según los resultados, existe una asociación entre la temperatura superficial de las crías y el peso de las madres, teniendo la temperatura más alta en todas las ventanas térmicas en todos los tiempos de evaluación en el grupo G_4, es decir, en el de las perras con mayor peso (32,1 a 39,6 kg). Además, aunque las temperaturas en los tiempos húmedo y seco difirieron entre los cuatro grupos, ambos tuvieron los valores más bajos en todos los grupos. A las 24 h AB, la temperatura en todas las ventanas térmicas mostró una estabilización con valores similares, independientemente del peso de la madre, por lo que la capacidad termorreguladora de los cachorros después de 24 h puede verse influenciada por dicho peso.

Los resultados de este estudio muestran el efecto del peso de la perra sobre la temperatura superficial de los neonatos canídeos Los análisis estadísticos realizados en este estudio revelaron que la capacidad de termorregulación de los cachorros está estrechamente ligada al peso de las hembras. Los recién nacidos de perras de bajo peso tuvieron valores de temperatura más bajos para todas las ventanas térmicas en cada tiempo medido, a diferencia de los cachorros de las perras más pesadas. Esta dependencia termorreguladora del peso y del tiempo también se ha descrito en cachorros que no pueden estabilizar su ritmo de temperatura durante varios días después del nacimiento, pero su temperatura rectal se estabiliza seis semanas después del nacimiento (Piccione *et al.* 2009). También se ha informado que los perros de tamaño pequeño tienden a perder calor rápidamente porque tienen una superficie mayor en relación con el volumen (Rigotti *et al*. 2015); por lo tanto, requieren una mayor producción de calor para mantener su termoestabilidad, además, se puede atribuir a la amplia variabilidad de las características de las razas caninas (Kwon y Brundage, 2019). Por ejemplo, perros de pelo corto de razas como Pinscher miniatura o cruzas de Pitbull presentaron una temperatura superficial más alta de aproximadamente 2°C más que las razas de pelo largo como Shih-tzu, Schnauzer miniatura, cruzas de Labrador o Pomerania. Debido a esto, la temperatura del ambiente donde viven los cachorros podría tener un efecto favorable o desfavorable en su bienestar (Jordan *et al.* 2022). Veronesi *et al.* (2022) mencionan que el tamaño corporal de una raza también influye en la viabilidad de los cachorros recién nacidos, donde los cachorros de tamaño pequeño pueden tener los niveles más altos de angustia, pero mayores posibilidades de supervivencia en comparación con animales de gran tamaño.

El peso al nacer es esencial para la supervivencia de los recién nacidos y es uno de los más importantes factores que influyen en la pérdida de temperatura ya que el índice de masa corporal (IMC) determina la capacidad de termorregulación en recién nacidos como los zorros (Harri *et al*. 1991). Respecto al peso de la perra, este también influye en el peso al nacer de los recién nacidos (Indrebø *et al.* 2007). Además, de acuerdo con Mila *et al.* (2015), el peso al nacer está influenciado de manera estadísticamente significativa por el tamaño de la camada, presentando un mayor número de cachorros con bajo peso al nacer en camadas grandes en comparación con camadas pequeñas. Según los datos obtenidos en este estudio, se observó que cuanto mayor sea el tamaño de la camada y cuanto mayor sea el peso de los cachorros al nacer, más fácilmente se estabiliza la termorregulación en los cachorros que provienen de camadas más grandes y tienen mayor peso al nacer.

Se ha visto que las ventanas térmicas de más utilidad son la carúncula lagrimal, ojo, oreja, flancos del tórax, área apendicular y cara (Travain *et al.* 2015; Casas-Alvarado *et al*. 2022; Reyes-Sotelo *et al.* 2022). Sin embargo, también se han sugerido otras ventanas térmicas en casos patológicos, como la glándula mamaria y la ventana ventral para casos de cáncer de glándula mamaria y cuello para casos de hipertiroidismo en gatos (Nitrini *et al.* 2021; Waddell *et al.* 2015).

Los cambios termorreguladores más notables en los cachorros recién nacidos fueron las bajas temperaturas en las regiones distales, es decir, en las ventanas térmicas de los miembros pélvicos y torácicos (MTM y MPF), y las temperaturas más altas en las ventanas térmicas A, T y PSI. Estos hallazgos se pueden explicar por medio de la circulación periférica y central, es decir, las estructuras relacionadas con el metabolismo y las funciones vitales se encuentran en la zona torácica, abdominal y regiones

craneales (Casas-Alvarado *et al*. 2022). Las ventanas apendiculares presentaron las temperaturas más bajas. Esto puede explicarse por la locomoción limitada de las especies altriciales y su incapacidad para caminar o ponerse de pie inmediatamente después del nacimiento. Sin embargo, los cachorros recién nacidos pueden gatear y tener actividad o movimiento de las extremidades, aunque estos movimientos son mínimos y pueden no promover cambios térmicos significativos porque los recién nacidos tienen presión arterial baja y, por tanto, el flujo sanguíneo en la periferia es menor que el del sistema circulatorio central (Mota-Rojas *et al*. 2021a). Lo anterior se puede explicar de mejor manera con el esquema de la **figura 18.**

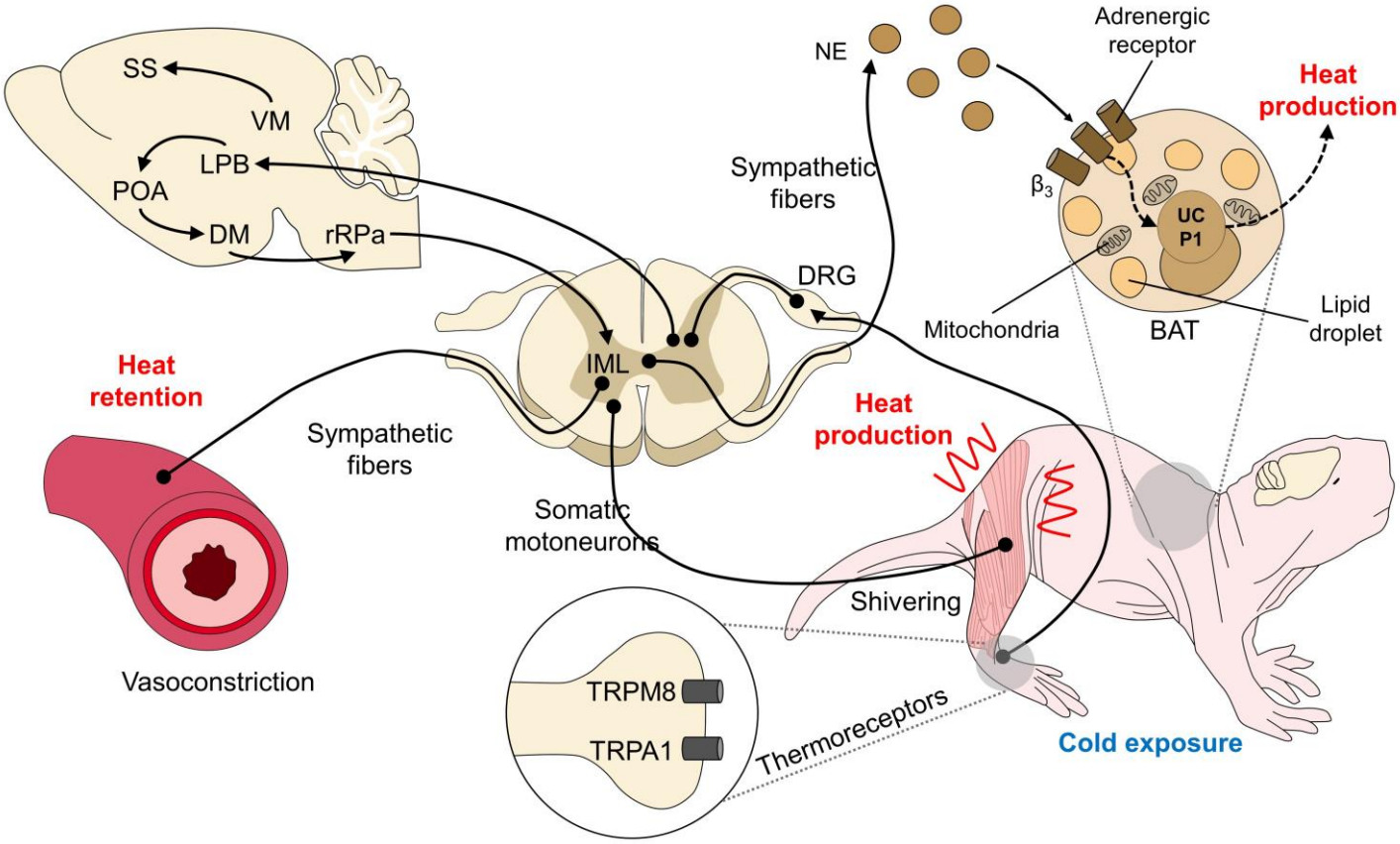

Figura 18. Mecanismos compensatorios neonatales frente a la hipotermia. En los recién nacidos, ilustrado por un *Heterocephalus glaber* en esta figura, cuando se activan los receptores periféricos responsables de la sensación térmica del frío (p. ej., TRPM8 y TRPA1), se genera una respuesta neuronal que involucra estructuras espinales (DRG) y el cerebro. En el cerebro, el centro termorregulador (POA) recibe la señal del LPB. El POA tiene conexiones con el DM, y este, a su vez, con las neuronas rRPA y IML. Una vez en la médula espinal, se producen dos respuestas a través de eferentes simpáticos. En primer lugar, la inervación de los vasos sanguíneos genera vasoconstricción y retención de calor; por otro lado, la liberación simpática de NE actúa sobre los receptores adrenérgicos BAT para producir calor. Una respuesta adicional es el titiriteo, que es un proceso generador de calor que depende de las motoneuronas somáticas y sus terminales en la médula espinal. Estos mecanismos promueven la producción o retención de calor para proteger al cuerpo de las consecuencias de la hipotermia. BAT: tejido adipocitario marrón; DM: hipotálamo dorsomedial; GRD: ganglio de la raíz dorsal; IML: núcleo intermediolateral; LPB: núcleo parabraquial lateral; NE: noradrenalina; POA: área preóptica del hipotálamo; rRPa: rafe pálido rostral; SS: corteza somatosensorial; UCP1: proteína desacopladora 1; VM: hipotálamo ventromedial (Lezama-García *et al.* 2022c).

Al considerar los cambios termográficos en la piel de perros recién nacidos, es importante resaltar el grado de desarrollo neurológico, el cual es responsable del mecanismo de modulación térmica. Los recién nacidos dependen de la termogénesis sin temblores mediante la lipólisis de tejido adiposo marrón y oxidación de ácidos grasos en las mitocondrias a través de la síntesis de ATP (Asakura, 2004). Se sabe que los músculos neonatales (músculos esqueléticos) son inmaduros al nacer, por lo que la termogénesis por titiriteo es ineficaz para producir calor (Asakura, 2004). En especies altriciales, la termoestabilidad se alcanza hasta

el día 18 de vida (Pineda *et al*. 2008), y la autonómica se encuentra completamente desarrollada al final de la cuarta semana (Lawler, 2008).

Según los resultados del presente estudio, independientemente del peso de la madre, los cachorros mostraron una disminución en la temperatura desde el tiempo seco, hasta antes de ser calostrados, en las regiones anatómicas de MTBB, MTM, MTC, MPF y N y esto podría no solo estar asociado con la caída fisiológica de la temperatura en los recién nacidos, sino también a los recursos energéticos limitados que pueden compensarse mediante la ingesta de calostro (Le Dividich y Noblet, 1981). La disminución de la temperatura observada en los cachorros depende de sus limitadas reservas de energía inmediatamente después del nacimiento (Mugnier *et al.* 2021). Los recién nacidos tienen reservas mínimas de glucógeno para mantener niveles estables de glucosa y el hígado inmaduro es ineficiente para generar energía para termorregularse cuando las reservas de glucógeno se vacían, ya que no son capaces de producir calor por escalofríos, y el tejido adiposo marrón en los recién nacidos no está muy desarrollado (Münnich y Küchenmeister 2014). El aumento adicional de la temperatura superficial de todas las ventanas térmicas después de la ingesta de calostro y a los 30 min AB confirman la fuente de energía proporcionada y el impacto en la temperatura. Este efecto puede verse agravado por dificultades en la ingesta de calostro (Mugnier *et al.* 2021) y puede disminuir la temperatura central y, en consecuencia, la temperatura superficial evaluada por TIR.

Mila *et al*. (2015) afirman que el calostro es una fuente de energía ya que puede proporcionar 1300-1800 kcal/L y también tiene un alto contenido de IgG (Chastant-Maillard *et al.* 2017). Sin embargo, la variabilidad de las propiedades del calostro depende del orden de nacimiento y el tiempo de lactancia (Mila *et al*. 2015), factores no evaluados en este estudio y que pueden ser sugeridos para futuras investigaciones.

En general, los cachorros recién nacidos tienen un requerimiento energético diario de aproximadamente 20-26 kcal/100 g de peso (Lawler, 2008) y necesitan una ingesta promedio de calostro de 12 ml por 100 g de peso corporal para cubrir estos requisitos (Chastant-Maillard *et al.* 2017). Además, en terneros (Buhler *et al.* 1998), cerdos (Burrin *et al.* 1992) y perros (Schwarz y Heird, 1994), el calostro contribuye a la correcta maduración y función del sistema digestivo y a la absorción de nutrientes (Reyes-Sotelo *et al.* 2022). La cantidad de glucosa que aporta el calostro a los cachorros es crucial porque sólo el 1,3% del contenido de grasa corporal está disponible en los recién nacidos, y la hipoglucemia y la hipotermia son causas importantes de mortalidad neonatal (Mila *et al*. 2015). Curiosamente, el calostro de perras de raza pequeña (menos de 10 kg) aporta un 10% más de energía que el calostro de hembras de razas grandes (Chastant-Maillard *et al*. 2017), y la ingestión de calostro podría proporcionar un 10% en recuperación de peso durante las primeras 24 h después del nacimiento, asegurando la supervivencia del cachorro recién nacido (Reyes-Sotelo *et al.* 2022). Esta podría ser la explicación del por qué las temperaturas de los cachorros a las 24 h AB alcanzaron una cierta termoestabilidad entre los grupos después de la ingesta de calostro, independientemente del peso de la madre, aunque también otro factor que podría intervenir es la temperatura ambiental.

Al contrario del descenso de temperatura descrito en la mayoría de las ventanas térmicas, en T, A, y PSI, hubo un aumento en la temperatura desde el tiempo seco hasta antes de ser calostrados. La ubicación anatómica de estas regiones y la irrigación vascular podrían explicar este efecto. Como ha sido reportado por varios estudios, la temperatura ocular evaluada ha mostrado una correlación positiva con la temperatura central del cuerpo en comparación con otras regiones anatómicas (Bartolomé *et al*. 2013; Church et al. 2014). Lo anterior podría deberse a la proximidad de la órbita ocular al cerebro y al amplio suministro de sangre proporcionado por las arterias supra e infraorbitarias (Stewart *et al*. 2007; Sutherland *et al*. 2020; Kim y Cho, 2021).

A lo largo de los siete tiempos de evaluación, las diferencias significativas de temperatura encontradas desde el tiempo húmedo hasta 24 h AB en las regiones evaluadas, reflejan la alteración de la estabilidad térmica de los cachorros recién nacidos y la activación de los mecanismos termorreguladores. En el ambiente intrauterino, una transferencia de calor por la circulación materna mantiene el feto con una temperatura de 0,3 a 0,5°C superior a la temperatura corporal de la madre. Sin embargo, los cachorros maternalmente dependientes experimentan una rápida pérdida de temperatura al nacer debido a una

exposición al ambiente frío extrauterino y pérdida de calor por evaporación de la superficie dérmica húmeda por la presencia del líquido amniótico (Asakura, 2004).

La hipotermia ocurre inmediatamente después del nacimiento como mecanismo de protección para prevenir el daño hipóxico en el recién nacido y reducir la tasa metabólica para mejorar su supervivencia en las primeras horas (Lawler, 2008; Lezama-García *et al.* 2022a). Este efecto se pudo observar en los cachorros recién nacidos del presente estudio en los primeros tres momentos de evaluación (húmedo, seco y calostrado), donde se registraron las temperaturas superficiales más bajas.

La termorregulación neonatal implica mecanismos bioquímicos, anatómicos, fisiológicos y endocrinos que desencadenan cambios respiratorios y vasculares, los cuales activan el metabolismo para producir energía (Hillman *et al.* 2012; Reyes-Sotelo *et al.* 2021). Como se mencionó que la ingesta de calostro es esencial para que los recién nacidos prevengan las consecuencias de la hipotermia y reflejen los cambios térmicos en la temperatura superficial evaluada por TIR.

Otro elemento que influye en las diferencias de temperatura es la importante evaporación de calor neonatal a través de la superficie dérmica húmeda, ya que los animales nacen mojados (Asakura, 2004). Esto sucede porque la función de aislamiento y protección que proporciona el pelaje no es eficiente ya que el agua (líquido amniótico en este caso) tiene una alta conductividad térmica, lo que genera una hipotermia más significativa. Además, otros factores que contribuyen incluyen la disminución de la grasa corporal, la edad y la falta de aclimatación al medio ambiente (Mallet, 2002; Oncken *et al*. 2001; Dwyer *et al.* 2016; Kozat, 2018). De esta manera, es posible explicar que la temperatura corporal aumentó al pasar del tiempo húmedo al seco, ya sea por la disminución de la pérdida de calor por evaporación o la disminución de la convección favorecida por las corrientes de aire. Otro factor crítico es el efecto de frotamiento en los cachorros, que estimula la microcirculación vascular dérmica de los vasos sanguíneos periféricos (vasodilatación periférica), aumentando la hiperemia microvascular a nivel dérmico. Del mismo modo, un factor que debe ser considerado es el hecho de que la gran variabilidad de razas, tamaños, tipo, densidad y color del pelaje de los animales monitoreados térmicamente puede ocasionar variaciones en el registro de las temperaturas superficiales medidas por medio de la TIR. Esto debido a que esta tecnología capta la intensidad de la radiación infrarroja que emiten los cuerpos.

La termoestabilidad observada a las 24 h AB en los cachorros de todos los grupos podría ser atribuido a la eficacia de la respuesta termorreguladora de los recién nacidos independientemente del peso inicial de la perra, como se muestra en los valores en todas las ventanas térmicas, donde los cuatro grupos tuvieron un aumento promedio similar. Diversos factores pueden influir en esta respuesta, tales como las reservas de glucógeno, la ingesta de calostro, el aumento de la digestión, la presencia de la madre y miembros de la camada. Esto se observa en los resultados presentados, y a las 24 h AB, todos los cachorros alcanzaron la termoestabilidad después del secado de su pelaje, la ingesta de calostro y la transferencia de calor de la temperatura de la madre por convección.

Estos comportamientos termorreguladores son importantes durante los primeros días de los recién nacidos, ya que los cachorros no pueden mantener su temperatura corporal cuando se exponen a ambientes fríos hasta los seis días después del nacimiento (Münnich y Küchenmeister, 2014).

Por lo tanto, los resultados sugieren que, durante las primeras horas de vida de los cachorros recién nacidos, sus mecanismos de termorregulación son deficientes por razones anatómicas y factores metabólicos que pueden reducirse mediante la ingesta de calostro, manteniendo una adecuada temperatura corporal y por medio de los mecanismos para conservar o disipar el calor en el medio evaluado.

Una de las principales limitaciones de este estudio fue que, dado que los nacimientos no siempre ocurrían en el mismo lugar, las temperaturas ambientales no se pudieron controlar y estandarizar para todos los casos. Otro punto importante es la gran diversidad de razas y tamaños en esta especie, por lo que la forma más práctica de agrupar a las hembras podría ser por su peso y no por su raza. Sin embargo, sería interesante evaluar las diferencias en las temperaturas al comparar diversas razas y si la raza pudiera influir en la capacidad para termorregular de maneras más eficientes.

En algunos estudios, realizados en humanos, existe una correlación positiva entre la obesidad materna y la incidencia de complicaciones de alto riesgo. En este sentido, el índice de masa corporal (IMC) previo al embarazo es un factor determinante durante la gestación (Nelson *et al.* 2010). Además, se ha visto en humanos que la obesidad de la madre durante el embarazo podría predisponer al desarrollo de obesidad en el niño entre 3 y 5 años (Griffiths *et al.* 2010). Por lo tanto, considerar el IMC en perras antes y durante la gestación y su asociación con los parámetros térmicos en cachorros recién nacidos podría ser un campo para futuras investigaciones.

Fase 4. Relación entre el peso del cachorro recién nacido y su equilibrio térmico

De acuerdo con los resultados, hubo una asociación entre la respuesta térmica de los cachorros y su peso, registrando la temperatura más alta en el cuartil Q_4 en todas las ventanas térmicas en todos los momentos de evaluación. Además, aunque las temperaturas en los tiempos húmedo y seco difirieron entre los cuatro grupos (Q_1, Q_2, Q_3 y Q_4), ambos (húmedo y seco) tuvieron los valores más bajos en todos los grupos. Curiosamente, a las 24 h AB, la temperatura de los cachorros en cada ventana térmica fue parecida entre los grupos, mostrando una capacidad termorreguladora similar independiente de su peso. Sin embargo, seguía siendo el más bajo en grupo Q_1 y más alto en el Q_4. Estos resultados difieren de los de otro estudio realizado por este grupo de trabajo (Lezama-García *et al.* 2022b), donde los grupos fueron clasificados según el peso de la madre y se encontró que, en los grupos de perras con mayor peso, la termorregulación de los cachorros se estabilizó a las 24 h AB, mostrando correlaciones positivas a mayor peso de las madres, debido a una mejor termorregulación de sus cachorros.

Los presentes resultados muestran el efecto del peso de los cachorros sobre su temperatura superficial. Los recién nacidos con bajo peso al nacer tuvieron valores de temperatura más bajos para todas las ventanas térmicas en cada momento medido, en comparación con los cachorros más pesados. Según Harri *et al*. (1991), el peso al nacer es importante para la supervivencia de los recién nacidos y es uno de los factores más importantes que influyen en la pérdida de temperatura, ya que el IMC determina la capacidad de termorregulación en los recién nacidos, como ocurre con los zorros. Sin embargo, el tamaño de la camada también influye significativamente en el peso de los recién nacidos. En este sentido, es común tener un mayor número de cachorros con bajo peso al nacer en camadas grandes, en comparación con las camadas pequeñas (Mila *et al.* 2015). Este efecto se puede observar también en lechones, donde camadas grandes pueden tener lechones más pequeños con mayores tasas de mortalidad, por hipotermia o traumatismo generado por la madre al ser muy débiles para moverse (Gómez-Prado *et al.* 2022).

Esta dependencia termorreguladora del peso y el tiempo también se ha descrito en cachorros que no pueden estabilizar su temperatura durante varios días después del nacimiento y logran estabilizarla hasta las seis semanas AB (Piccione *et al.* 2009). Es importante considerar que la temperatura del ambiente en el que viven los cachorros podría tener un efecto favorable o desfavorable sobre su bienestar (Jordan *et al.* 2022). Sin embargo, en este estudio, la temperatura del ambiente no estaba controlada pero en todos los casos hubo condiciones similares (34–40°C) y se colocaron tapetes de fomi en el lugar del parto para evitar pérdidas de temperatura por convección. En este estudio, se han observado diferencias significativas a lo largo de los tiempos evaluados, desde húmedo hasta 24 h AB, en las regiones evaluadas. Cuando los fetos todavía están en el útero de la perra mantienen una temperatura de 0.3 a 0.5°C superior a la temperatura corporal de la perra. Sin embargo, al nacer, los cachorros experimentan un cambio térmico importante y una pérdida de temperatura por evaporación ya que nacen mojados, debido al líquido amniótico (Asakura, 2004). Esto sucede porque la función de aislamiento y protección que proporciona el pelaje no es eficiente, ya que el líquido amniótico tiene una alta conductividad térmica lo que genera mayor hipotermia. Además de esto, otros factores que contribuyen son la escasa cantidad de grasa corporal, la edad y falta de aclimatación al medio ambiente (Mallet, 2002; Oncken *et al.* 2001). Por lo tanto, el aumento de la temperatura corporal al pasar del tiempo húmedo al seco puede explicarse bien por la disminución de la pérdida de calor por evaporación, o la disminución de la convección favorecida por las corrientes de aire. Otro factor crítico fue el hecho de frotar a los cachorros, lo cual estimuló la microcirculación vascular dérmica de vasos sanguíneos periféricos (vasodilatación periférica), con lo que se produjo un aumento de la hiperemia microvascular a nivel dérmico.

En el presente estudio se observó hipotermia en los cachorros recién nacidos en los primeros tres tiempos de evaluación (húmedo, seco y calostrado), en los cuales se registraron las temperaturas superficiales más bajas.

La termoestabilidad observada a las 24 h AB en cachorros de todos los grupos podría atribuirse a la eficacia de la respuesta termorreguladora de los recién nacidos independientemente de su inicial peso, como se muestra en los valores de TIR en todas las ventanas térmicas, en los cuatro grupos, Varios factores pueden influir en esta respuesta, como son las reservas de glucógeno, la ingesta de calostro, el aumento de la digestión, la presencia de la madre y los miembros de la camada. Las ventanas torácicas demuestran un dato importante: como lo demostraron otros estudios realizados con otras especies, la temperatura tiende a aumentar después del consumo de calostro. En este estudio se puede mostrar que inmediatamente después del consumo de calostro, la temperatura tiende a aumentar en los cuatro cuartiles en la ventana térmica T. Como se observa en los resultados presentados, a las 24 h AB todos los cachorros alcanzaron la termoestabilidad luego de que se secó su pelaje y luego de la ingesta de calostro y transferencia de calor de la temperatura de la madre por convección. Según Münnich y Küchenmeister (2014), estos comportamientos termorreguladores son importantes durante los primeros días de los recién nacidos, ya que los cachorros no pueden mantener su temperatura corporal cuando se exponen a ambientes fríos. Según los resultados reportados en este estudio, el factor termorregulador más notable fueron las bajas temperaturas registradas en las regiones distales, es decir, en las ventanas térmicas de MTM y MPF y las altas temperaturas registradas en las ventanas térmicas A, T y PSI. Esto podría deberse a que las estructuras más importantes relacionadas con el metabolismo y las funciones vitales se encuentran en las regiones torácica, abdominal y craneal (Casas-Alvarado *et al.* 2020; Mota-Rojas *et al.* 2022b). Las ventanas apendiculares presentaron las temperaturas más bajas, lo que se puede explicar, en el caso de cachorros recién nacidos, debido a que aún no presentan locomoción, al ser una especie altricial presentan un bajo grado de desarrollo neurológico.

Los recién nacidos dependen de la vasoconstricción periférica y derivaciones arteriovenosas a nivel de la piel para reducir la pérdida de calor, pero también utilizan la termogénesis sin titiriteo mediante la lipólisis del BAT y la oxidación de los ácidos grasos en la piel y mitocondrias a través de la síntesis de adenosin trifosfato (ATP) ya que los músculos esqueléticos neonatales son inmaduros al nacer. Es precisamente este punto el que proporciona la explicación de por qué la termogénesis por titiriteo en cachorros recién nacidos no es tan efectiva (Asakura, 2004).

La investigación adicional que podría realizarse en base a los presentes hallazgos incluye el estudio de la influencia de factores como la temperatura ambiental, el tamaño de la camada y la calidad del cuidado materno sobre la termoestabilidad de cachorros recién nacidos durante las primeras horas de vida. Es importante mencionar que una de las principales limitaciones de este estudio fue que, como los nacimientos no siempre ocurrieron en el mismo lugar, las temperaturas ambientales no se pudieron controlar y estandarizar para todos los casos.

10. CONCLUSIONES

La monitorización electrónica fetal y uterina es una herramienta que debe implementarse en las perras en todas las clínicas veterinarias, hospitales y criaderos de perros, para asegurar el bienestar de las perras gestantes y de los recién nacidos, así como para ayudar a disminuir los elevados índices de mortalidad perinatal en esta especie, ya que, además, se trata de una técnica eficaz, no invasiva, fácil de utilizar y accesible en la mayoría de los casos.

Se ha observado que el peso, tanto de las madres como de los recién nacidos, es un valor que puede afectar la vitalidad de los recién nacidos y la dinámica uterina de las perras, mostrando diferencias en la frecuencia, intensidad y duración de las contracciones miometriales. Esto es, a mayor peso de las perras, surgen más cambios en la dinámica uterina, observándose que las contracciones más intensas y frecuentes ocurrieron en las madres primíparas más pesadas, lo que aumentó el intervalo de expulsión entre cachorros en las perras primíparas más livianas y lo disminuyó en las multíparas más pesadas. La duración de la fase de expulsión, así como el número de nacidos muertos, fue mayor en las primíparas más pesadas. De manera similar, los cachorros más pesados nacieron de las primíparas más pesadas. El mayor número de mortinatos (17 mortinatos) se observó en hembras primíparas del grupo G_4, con un total de 72 crías mortinatas (23,6%).

De acuerdo con lo observado, se concluye que los cachorros machos recién nacidos fueron significativamente más pesados que las hembras recién nacidas, y el peso al nacer también difirió significativamente entre los cachorros recién nacidos de diferentes grupos de peso de la madre. Sin embargo, no se encontraron diferencias significativas en el intervalo de expulsión entre hembras y machos. Según los resultados también se puede sugerir que el sexo del recién nacido no interfiere con su supervivencia.

Por otro lado, tras analizar los datos obtenidos, se observó que el peso de los recién nacidos se encuentra correlacionado con el grado de tinción de meconio, presentándose más casos de tinción de meconio severa en los cachorros del grupo de mayor peso al nacer. Además, el peso de los recién nacidos se correlacionó con las alteraciones en las variables sanguíneas, mostrando los casos más severos de acidosis metabólica, hipoxia, hipoglucemia y mayor mortinatalidad en los cachorros del cuartil Q_4. Por el contrario, no se encontraron correlaciones estadísticamente significativas entre el peso de los recién nacidos y su vitalidad. Sin embargo, se pudo observar que los cachorros más vigorosos se encontraron en el cuartil Q_1, pero en el minuto 60 post nacimiento todos los cachorros en los cuatro cuartiles estandarizaron sus puntuaciones de vitalidad. De acuerdo con estos datos, la hipótesis de que los recién nacidos más grandes tendrían puntuaciones más bajas en vitalidad y un mayor número de alteraciones en el perfil sanguíneo se confirmaron al observarse en ellos mayores alteraciones y más casos de tinción de meconio que en los cachorros más pequeños.

En el presente estudio, la lectura de la temperatura superficial por medio de TIR de cachorros recién nacidos mostró que el peso de la madre parece influir en la termoestabilidad neonatal, es decir, que los cachorros nacidos de perras de mayor peso presentaron las temperaturas más altas desde el nacimiento hasta las 24 h después del mismo.

En cuanto a las regiones del cuerpo donde se evaluaron las temperaturas, los hallazgos sugieren que las ventanas térmicas con temperaturas más altas fueron la A, T, N y PSI, y las de temperaturas más bajas fueron las obtenidas en las ventanas térmicas MTBB, MTC, MTM y MPF. En MTC hubo interacción entre los grupos ($p< 0.0001$) y entre los grupos y tiempos, específicamente en G_4 donde hubo superposición de los valores de temperatura para el tiempo 4 h y 24 h después del nacimiento, así como para el tiempo 1 h después del nacimiento y el tiempo de calostrado; las demás ventanas térmicas no presentaron interacciones.

Uno de los datos más interesantes es que, a diferencia de otros estudios realizados en otras especies, en el presente se observó un aumento significativo en las temperaturas de los cachorros en las ventanas térmicas A, T y PSI inmediatamente después de ingerir calostro, lo que podría ser un área para futuras investigaciones.

En cuanto a cómo el peso de los cachorros influye en su termorregulación, los resultados también sugieren una correlación positiva entre el peso del cachorro y su capacidad para lograr termoestabilidad, lo cual se observó continuamente en todas las ventanas térmicas evaluadas. Los cachorros nacidos con pesos inferiores (Q_1, 126-226 g) presentaron las temperaturas más bajas, mientras que los cachorros con pesos más altos (Q_4, 388-452 g) presentaron las temperaturas más altas. Asimismo, el efecto tiempo del registro de temperatura mostró cambios relevantes en ellos; las temperaturas más bajas se obtuvieron cuando los cachorros aún estaban húmedos y las más altas a las 24 h después del nacimiento. De igual forma, se ha observado que, durante las primeras horas de vida de los cachorros, su mecanismo de termorregulación es deficiente debido a factores anatómicos y metabólicos, los cuales también pueden verse afectados por la ingesta de calostro, el mantenimiento de una temperatura corporal adecuada y sus mecanismos para conservar o disipar el calor en las ventanas térmicas evaluadas. Es por ello que, la respuesta térmica de los cachorros recién nacidos puede ayudar a identificar reducciones drásticas de temperatura o deficiencias de la compensación termorreguladora durante las primeras horas de vida, previniendo las consecuencias graves que conduzcan a hipotermia o incluso su muerte.

11. APLICACIONES PRÁCTICAS

Un aspecto importante para destacar es que el uso de las ventanas térmicas utilizadas en este trabajo en cachorros recién nacidos no había sido reportado previamente, por lo que se podrían considerar estas ventanas térmicas en esta especie y en otras en futuros estudios.

La evaluación de la vitalidad por medio del puntaje de obtenido en la escala Apgar, actualmente, representa el método más fácil y simple, no invasivo, confiable y fácilmente realizable en cualquiera de las condiciones de la práctica clínica, para la evaluación de la viabilidad de los cachorros recién nacidos, así como para proporcionar un pronóstico de supervivencia a corto plazo, tanto en perros como en diversas especies.

Por lo que, la utilización de tecnologías tales como el MEF, la evaluación del perfil sanguíneo, la escala de vitalidad y la evaluación térmica de los cachorros recién nacidos pudiera y debiera implementarse en la clínica y práctica médica diaria, ya que ayudan a mejorar el bienestar de los animales, mejorando su calidad de vida y su tasa de supervivencia, disminuyendo con ello la mortalidad y las pérdidas emocionales y económicas que esto conlleva.

12. PERSPECTIVAS

El compromiso veterinario en obstetricia de caninos y felinos domésticos tiene varios objetivos. Dentro de los más importantes se puede mencionar el hecho de incrementar el número de cachorros nacidos vivos, minimizar la morbilidad y mortalidad materna y promover un aumento en la supervivencia de los recién nacidos durante la primera semana de vida.

Al utilizar la MEF en medicina veterinaria, tenemos en nuestras manos una herramienta que podría facilitar el cuidado perinatal, mejorando así la supervivencia de las crías, favoreciendo el bienestar de los animales, ahorrando tiempo valioso para tomar decisiones de vital importancia y ayudando a reducir los costos de producción por pérdidas o muertes. En este sentido, el uso de la evaluación y la correcta interpretación de las desaceleraciones de los latidos cardiacos fetales puede ayudar a prevenir a tiempo si una perra requerirá o no una cesárea, y con ello, reducir las tasas de mortalidad. Aunque existen diversas ventajas de la utilización de esta herramienta, algunos autores como Alfirevic et al. (2013), mencionan que no es tan útil para prevenir la parálisis cerebral y otros trastornos del neurodesarrollo. Por lo tanto, se podría decir que lo ideal sería utilizar varias técnicas que combinadas ayuden a realizar la evaluación fetal y materna lo más completa posible (Lezama-García et al. 2023). Es decir, hacer uso del MEF mediante cardiotocografía, pero también anexando la aplicación de termografía (Lezama-García *et al.* 2022b, 2022a), la evaluación de la vitalidad del recién nacido (APGAR) (Veronesi *et al.* 2009b, 2022; Trujillo-Ortega *et al.* 2011; Veronesi 2016) y la gasometría (Reyes-Sotelo *et al*. 2021, 2022).

Por todo lo anterior y de acuerdo con Groppetti *et al*. (2010) y Forsberg (2010), una de las principales perspectivas que se pueden inferir con este estudio, es que la aplicación de la monitorización uterina y fetal para realizar un seguimiento antes, durante y después del parto, es una herramienta valiosa para ayudar a predecir casos de hipoxia, asfixia, distocia e inercia uterina, que podrían provocar partos con complicaciones y, por tanto, un aumento de la tasa de mortalidad así como una disminución de la vitalidad de los recién nacidos (Plavec *et al.* 2022; Uchańska *et al.* 2022; Bienboire-Frosini *et al.* 2023; Szenci 2023).

La investigación adicional que podría realizarse con base a los presentes hallazgos incluye el estudio de la influencia de factores tales como la temperatura ambiental, el tamaño de la camada y la calidad del cuidado materno sobre la termoestabilidad de cachorros recién nacidos durante las primeras horas de vida. En este contexto es importante mencionar que una de las principales limitaciones de este estudio fue que, como los nacimientos no siempre ocurrieron en el mismo lugar, las temperaturas ambientales no se pudieron controlar y estandarizar para todos los casos, por lo que llama la atención el hecho de poder realizar estas evaluaciones tratando de tener control de estas variables. Asimismo, sería interesante aplicar la TIR para evaluar la asociación que pudiera haber entre el porcentaje de cachorros machos y su termoestabildad, ya que se ha observado que el porcentaje de cachorros machos se correlaciona positivamente con la lactancia lateral, la cual es una posición que proporciona a los cachorros un acceso más fácil a la leche. En este sentido, se ha visto que, desde el punto de vista evolutivo, las madres prestan más atención a los cachorros machos porque es más conveniente en términos de aptitud física (Ogi *et al*. 2021) y esto podría alterar la respuesta térmica periférica de neonatos.

Otro factor que resulta importante considerar en futuras investigaciones, es el efecto de la raza en la presentación de cambios térmicos. Lo anterior debido a la gran variedad de colores, densidad y tipos de pelaje que las diversas razas pueden presentar. De igual forma, el efecto que tiene la raza de las perras en la presentación de las diferentes conductas maternas y las variaciones que pudieran registrarse en la dinámica uterina y en su desempeño reproductivo, pudieran ser datos que ayudarían a los criadores a determinar que razas pudieran ser más sencillas de reproducir en este sentido.

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