Universidad Autónoma de Tlaxcala

Posgrado en Ciencias Biológicas

Contribución de las interacciones intracamada al desarrollo de diferencias individuales en fisiología y conducta en el conejo doméstico

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTORA EN CIENCIAS BIOLÓGICAS

Presenta

Yesenia Fernández Pérez

Director
Dr. Amando Bautista Ortega

Tlaxcala, Tlax. Octubre, 2024

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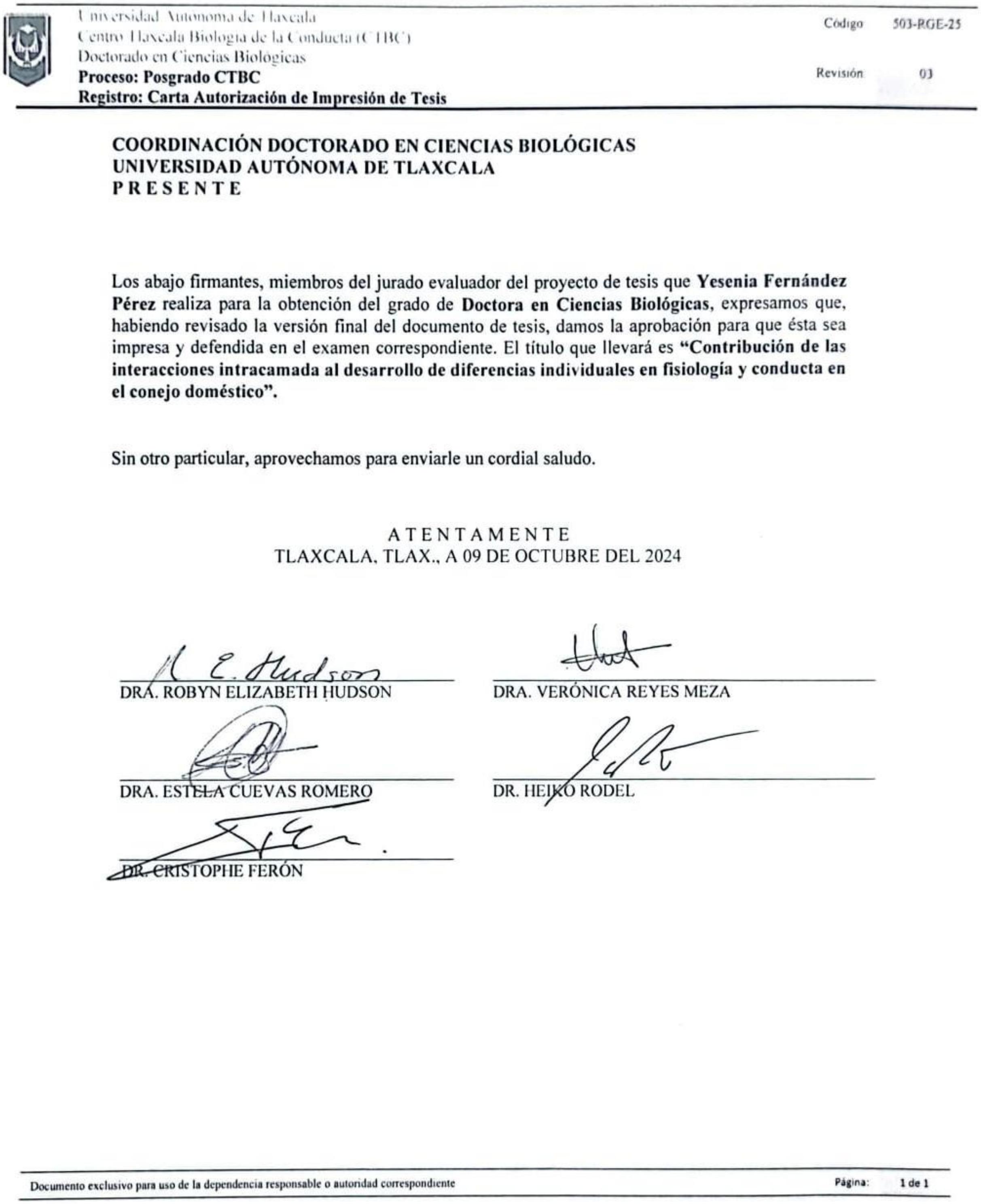

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Los abajo firmantes, miembros del jurado evaluador del proyecto de tesis que **Yesenia Fernández Pérez** realiza para la obtención del grado de **Doctora en Ciencias Biológicas**, expresamos que, habiendo revisado la versión final del documento de tesis, damos la aprobación para que ésta sea impresa y defendida en el examen correspondiente. El título que llevará es **"Contribución de las interacciones intracamada al desarrollo de diferencias individuales en fisiología y conducta en el conejo doméstico".**

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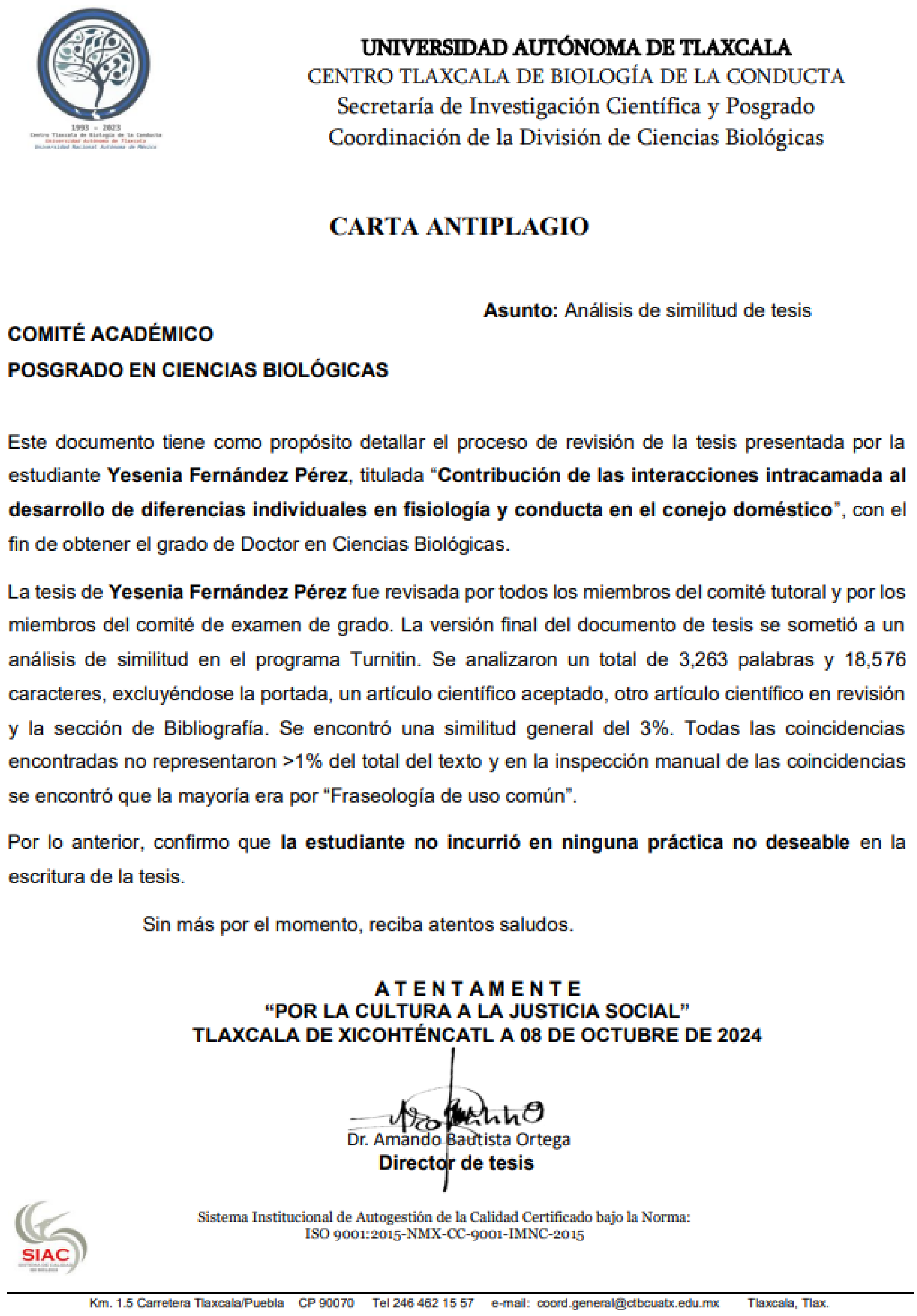

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Este documento tiene como propósito detallar el proceso de revisión de la tesis presentada por la estudiante **Yesenia Fernández Pérez**, titulada "**Contribución de las interacciones intracamada al desarrollo de diferencias individuales en fisiología y conducta en el conejo doméstico**", con el fin de obtener el grado de Doctor en Ciencias Biológicas.

La tesis de **Yesenia Fernández Pérez** fue revisada por todos los miembros del comité tutoral y por los miembros del comité de examen de grado. La versión final del documento de tesis se sometió a un análisis de similitud en el programa Turnitin. Se analizaron un total de 3,263 palabras y 18,576 caracteres, excluyéndose la portada, un artículo científico aceptado, otro artículo científico en revisión y la sección de Bibliografía. Se encontró una similitud general del 3%. Todas las coincidencias encontradas no representaron >1% del total del texto y en la inspección manual de las coincidencias se encontró que la mayoría era por "Fraseología de uso común".

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Sin más por el momento, reciba atentos saludos.

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Resumen

Las diferencias individuales en el fisiología y conducta ("personalidad") son de gran interés para los biólogos del comportamiento. Desde el punto de vista de la ontogenia, las preguntas importantes incluyen cuán temprano en la vida surgen tales diferencias, qué factores influyen en su aparición, y si se mantienen estables a lo largo del desarrollo de los individuos.

Dada la naturaleza exigente de los estudios longitudinales, hay una falta de información sobre estas cuestiones en mamíferos. El objetivo en el presente estudio es investigar el efecto de las interacciones entre los compañeros de camada durante el período postnatal temprano sobre el desarrollo de diferencias individuales en el inicio de la endotermia en crías del conejo doméstico, una función esencial para el mantenimiento de la homeostasis en endotermos homeotérmicos. Así como en la conducta de marcaje del mentón (chinning) en el conejo doméstico, una parte notable del sistema de comunicación química de esta especie.

Capítulo 1. Deferencias individuales en el desarrollo de la endotermia entre compañeros de camada del conejo doméstico.

La regulación de la temperatura corporal es una función esencial para el mantenimiento de la homeostasis en endotermos homeotermos. Sin embargo, los mamíferos altriciales recién nacidos son incapaces de mantener su temperatura corporal de manera independiente, debido a que carecen de una capa aislante de pelo, tienen un área relativamente grande de exposición a la pérdida del calor en relación con su volumen y un control vasomotor inmaduro. Por lo que al nacimiento las crías altriciales son consideradas poiquilotermas ectotermas, pero en un periodo más avanzado del desarrollo estos individuos alcanzan la endotermia homeotérmica. No obstante, se desconoce si existen diferencias individuales intracamada en el inicio de la endotermia y qué factores influyen en su aparición. Por lo tanto, el objetivo de este trabajo fue determinar el efecto de las interacciones tempranas intracamada sobre las diferencias individuales en el inicio de la endotermia homeotérmica en crías del conejo doméstico. Evaluamos en 54 individuos de 10 camadas la capacidad de mantener la temperatura corporal estable ante la exposición individual al frío durante los primeros 15 días postnatales y lo relacionamos con diferencias individuales en crecimiento y conducta durante el periodo postnatal temprano. Los resultados sugieren que las crías más pesadas dentro de la camada no solo logran un crecimiento más rápido y una mejor posición dentro del agrupamiento que forman con sus hermanos para termorregular conductualmente, sino que también desarrollan la endotermia homeotérmica más rápidamente. Además, el crecimiento del pelo es mayor en las crías con mayor masa corporal inicial lo que refuerza su capacidad para mantener una temperatura corporal estable, reduciendo sus costos energéticos. Concluimos que las interacciones tempranas entre compañeros de camada en función de la masa corporal relativa de los individuos que la conforman son determinantes para la adquisición de capacidades termorreguladoras individuales, las cuales podrían representar una medida de individualidad con posibles consecuencias en supervivencia, aptitud y fenotipo metabólico a mediano y largo plazo.

Capítulo 2. Desarrollo de diferencias individuales en la frecuencia de la conducta de marcaje del mentón en el conejo doméstico.

El conejo doméstico (*Oryctolagus cuniculus*) despliegan la conducta de marcaje del mentón, que consiste en frotar repetidamente su glándula submandibular en objetos llamativos. En el despliegue de este comportamiento, los conejos muestran diferencias individuales muy estables en su frecuencia. Probamos repetidamente la frecuencia de marcaje del mentón desde el destete hasta la madurez sexual en 63 conejos de raza chinchilla (35 hembras, 28 machos) de 14 camadas. Encontramos consistencias significativas a lo largo del tiempo, es decir, las diferencias en la frecuencia de marcaje del mentón entre hermanos de camada se mantuvieron estables en ambos sexos todo el período posterior al destete hasta la madurez sexual. Inesperadamente, no encontramos asociación con las variables morfológicas, fisiológicas o conductuales que se sabe que forman un complejo de desarrollo temprano bien correlacionado en esta especie. Concluimos tentativamente que, en el conejo, las diferencias individuales en la frecuencia de marcaje del mentón tienen poca relación con aspectos previamente estudiados de las trayectorias de desarrollo individuales. El origen y significado funcional de las diferencias individuales en la frecuencia de marcaje, ya sea en contextos reproductivos u otros contextos sociales, es en gran parte desconocido y requiere más investigación.

Palabras clave: Diferencias individuales, Ontogenia, Hermanos, *Oryctolagus cuniculus*

Índice

I. Introducción general

1.1 Diferencias individuales

La personalidad animal, definida como las diferencias individuales en fisiología y conducta que son estables en el tiempo y consistentes entre contextos (Gosling 2001), son importantes para entender cómo responden los organismos a los retos ambientales y las interacciones sociales. Tales diferencias han sido ampliamente estudiadas, tanto en vertebrados como en invertebrados (Stamps y Groothuis 2010). No obstante, la mayoría se centran en la historia evolutiva y el valor adaptativo (Roche y cols. 2016), proporcionando información sobre por qué tales diferencias individuales pueden haber surgido y persistido en el tiempo. Sin embargo, las causas próximas, como los mecanismos neurobiológicos que subyacen a un comportamiento particular, ayudan a explicar por qué los individuos pueden mostrar diferentes respuestas fisiológicas o tendencias de comportamiento. Además, entender la ontogenia, origen y desarrollo, de estas diferencias, es crítico para comprender preguntas de tipo causal (función y evolución) (Stamps y Groothuis 2010, Groothuis y Trilmich 2011).

El origen de las diferencias individuales es en parte genético (Dingemanse y cols. 2002, Sinn y cols. 2006). Por ejemplo, se ha demostrado que la variabilidad genética puede influir en la predisposición a ciertos comportamientos, como la agresividad o la sociabilidad (Martín-Villuendas 2021). Así mismo, metaanálisis revelan asociaciones significativas entre marcadores genéticos y rasgos conductuales, particularmente en estudios de personalidad animal (Bubac y cols. 2020).

Si bien las influencias genéticas son clave en el origen y desarrollo de las diferencias individuales, es esencial reconocer que los factores ambientales también juegan un papel crítico en la configuración de estas diferencias. En particular, el entorno social temprano puede contribuir al desarrollo de diferencias en conducta y la fisiología. Por ejemplo, en humanos, las interacciones tempranas entre madre e hijo son determinantes en el establecimiento del vínculo afectivo, que a su vez influye en el desarrollo psicosocial del infante. Según Palacios-Hernández (2016), la respuesta emocional de la madre juega un papel mediador en este proceso, y cualquier alteración en este vínculo puede tener consecuencias significativas en áreas como la cognición y la salud mental del niño. En mamíferos no humanos, se ha demostrado que el entorno social en el que viven las hembras gestantes es influye en el desarrollo de su descendencia, ya que la exposición al estrés prenatal puede afectar el desarrollo conductual, disminuir la función inmunológica, reducir la propensión a la interacción social, y el desarrollo de ejes hormonales de los vástagos tanto a corto como a largo plazo (Götz y Stefanski 2007).

De igual forma, otro factor importante que tiene un impacto significativo en el desarrollo de diferencias individuales en mamíferos, es la presencia de hermanos durante los primeros días postnatales. Esta tiene el potencial de moldear las diferencias individuales, afectando el desarrollo morfológico, fisiológico y conductual de los individuos durante diferentes etapas de la vida, prenatal y postnatal (Hudson y Trillmich 2008, Hudson y cols. 2011).

Prenatalmente, en mamíferos de patrón politoco, se ha descrito que existe una variación en la masa corporal al nacimiento entre los individuos de la camada, asociado al sitio de implantación a lo largo del cuerno uterino. Por ejemplo, en el conejo doméstico (*O. cuniculus*) aquellos individuos implantados en los extremos, ya sea cerca del ovario o cerca del cérvix, son más pesados al nacimiento con respecto a los que se implantaron en el centro (Bautista y cols. 2015a). Así mismo, se sabe que la masa corporal de las crías al nacer es inversamente proporcional al número de individuos que la conforman, y que estas diferencias en masa se mantienen al menos hasta el destete (revisado en Hudson y cols. 2011).

Aunado a lo anterior, el tamaño de camada juega un papel muy importante en el desarrollo y supervivencia de los individuos que la conforman, otorgándoles tanto ventajas como desventajas. En mamíferos altriciales, es decir que nacen con parpados cerrados, con los conductos auditivos cerrados, prácticamente sin pelo y con sistema locomotor inmaduro, tener un mayor número de hermanos les proporciona una ventaja térmica pues al nacimiento las crías no son capaces de termorregular por sí mismas, necesitan de la presencia de hermanos para mantener su temperatura corporal (Bautista y cols. 2003). Al estar en contacto con otros individuos, su área corporal expuesta al frío disminuye y, por lo tanto, disminuye el gasto energético utilizado para minimizar la pérdida de calor, permitiéndoles así reasignar la energía ahorrada a otras funciones, como el crecimiento (Bautista y cols. 2003). No obstante, a mayor número de hermanos, la competencia por recursos, tanto en mamíferos altriciales, como precociales, es mayor (revisado en Bautista y cols. 2005). Por ejemplo, en conejillos de indias (*Cavia aperea f. porcellus*) los individuos pertenecientes a camadas más grandes muestran tasas de crecimiento más bajas y mayores niveles de estrés, ya que tienen que competir por tetinas limitadas (Fey y Trillmich 2008). Así mismo, en leones (*Panthera leo*), se ha observado que los cachorros que nacen en camadas más grandes tienden a crecer más lentamente en comparación con aquellos de camadas más pequeñas, lo que sugiere que la competencia por recursos es un factor determinante en el desarrollo de los individuos (Packer y cols. 2011). Esto indicaría que a los individuos les conviene tener menor número hermanos, porque representa menor competencia y mayor proporción de leche para éstos. Sin embargo, en el caso de los mamíferos altriciales, específicamente en el conejo silvestre (*O. cuniculus*), se ha descrito que los beneficios térmicos que proporciona tener mayor número de hermanos de camada, en condiciones más frías de temperatura del suelo, superan las consecuencias negativas de la competencia por leche materna, de manera que el tamaño óptimo de la camada para el crecimiento también depende de las condiciones ambientales en las que se desarrollan los individuos (Rödel y cols. 2008b).

Como ha sido señalado anteriormente, durante el desarrollo postnatal temprano, el entorno juega un papel crucial en el desarrollo de los mamíferos. Factores como la disponibilidad de alimento, la presencia de depredadores y las condiciones climáticas pueden afectar la forma en que los individuos crecen y se desarrollan con consecuencias irreversibles que pueden afectar su sobrevivencia y reproducción (Lindström 1999).

En el caso específico del conejo doméstico (*O. cuninulus*), un modelo de estudio que ha sido utilizado por más de 20 años para investigar el efecto de las interacciones entre hermanos sobre el desarrollo de las diferencias individuales en fisiología y conducta, se sabe que las diferencias en la masa corporal al nacimiento predicen diferencias individuales en crecimiento, desarrollo y supervivencia (Bautista y cols. 2015b), debido a que los individuos con mayor biomasa al nacimiento son más exitosos en la competencia por recursos limitados, como la leche materna y sitios térmicamente ventajosos dentro del agrupamiento, que forman los integrantes de la camada para termorregular dentro del nido. Por lo tanto, muestran tasas de crecimiento y supervivencia más altas que sus hermanos más ligeros (Rödel y cols. 2008a, Bautista y cols. 2015b). Además, las interacciones tempranas dentro de la camada se asocian a diferencias individuales en conducta a mediano y largo plazo (Reyes-Meza y cols. 2011), las crías más ligeras al nacimiento y que generalmente ocupan la periferia del agrupamiento, muestran un estilo conductual más proactivo, en comparación con sus hermanas más pesadas en la etapa adulta (Reyes-Meza y cols. 2011).

Estos datos sugieren que las interacciones tempranas entre compañeros de camada, podrían modular diferencias individuales de rasgos fundamentales, como lo son la termorregulación y la comunicación entre individuos de una misma especie. Ello debido a que los primeros días postnatales son cruciales en la supervivencia de mamíferos altriciales, como lo es el conejo doméstico, se ha demostrado que el agruparse con los compañeros de camada es una estrategia vital para mantener alta la temperatura corporal pero son los más pesados dentro de la camada quienes son ocupan posiciones centrales en del agrupamiento, generalmente, tienen mayor acceso a los pezones de la madre durante el amamantamiento y mayor temperatura corporal (Zepeda y cols. 2019). De modo que, aparentemente tienen una ventaja sobre las crías ligeras, pues no invierten energía en la activación del tejido adiposo pardo para tratar de mantener su temperatura corporal, pero sí en obtener mayor ganancia en masa (Bautista y cols. 2013) y probablemente en el desarrollo de sus capacidades termorreguladoras (Figura 1).

En cuanto a las diferencias individuales en comunicación entre los individuos, se sabe que los conejos adultos muestran diferencias estables en una de las conductas de señalización química más notorias en esta especie, el marcaje por frotamiento del mentón (chinning) (Arteaga y cols. 2008). Sin embargo, se desconoce cómo y cuándo durante el desarrollo surgen tales diferencias entre individuos, es probable que las interacciones entre hermanos de camada contribuyen tales diferencias observadas en la etapa adulta (Figura 1).

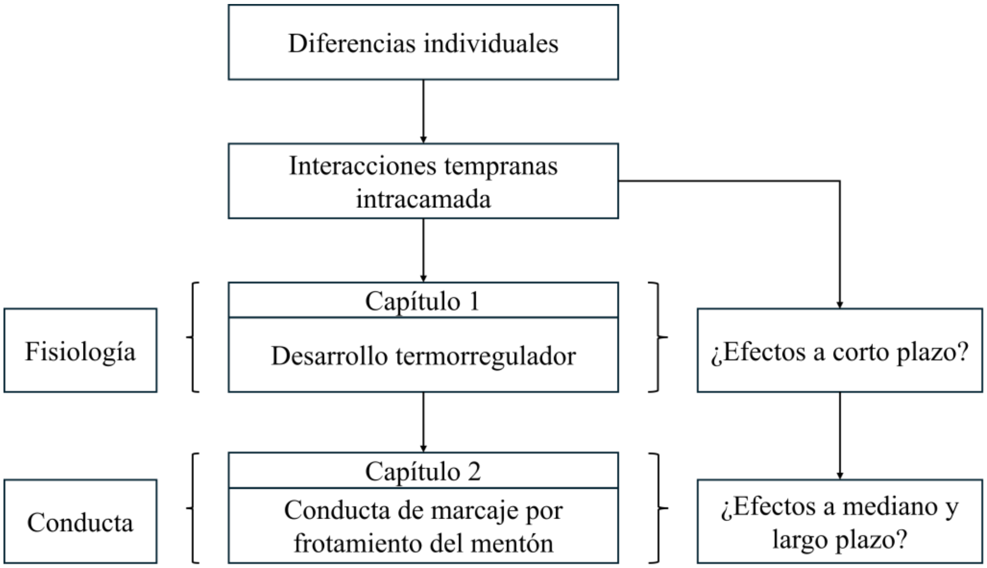

Figura 1. Esquema del objetivo principal del estudio: El desarrollo de diferencias individuales en crías de conejo doméstico (*Oryctolagus cuniculus*) en función de las interacciones tempranas dentro de la camada. Se destacan las dos áreas de interés: el desarrollo termorregulador, que explora las diferencias en el inicio de la endotermia homeotérmica a corto plazo, y la conducta de marcaje por frotamiento del mentón, observada como un indicador de interacción social y establecimiento territorial, a mediano y largo plazo.

1.2 Temperatura corporal y termorregulación

La temperatura corporal se define como la medida del calor interno del organismo, que refleja el equilibrio entre la producción y la pérdida de calor, indicando el gasto relativo de procesos físicos y químicos (revisado en Barraza-Gómez 2023).

Los vertebrados se clasifican generalmente como ectotermos y endotermos. En los individuos ectotermos, la variación térmica del ambiente determina la temperatura corporal del organismo (poiquilotermos) y afecta termodinámicamente su metabolismo, por lo que regulan su temperatura corporal conductualmente y por modulación cardiovascular (Seebacher 2005).

A diferencia de los ectotermos, los individuos endotermos, pueden regular su temperatura corporal internamente. Por ello, mantienen su temperatura relativamente estable, mediante ajustes en la producción de calor metabólico, a través de una variedad de respuestas termorregulatorias como la termogénesis con tiriteo, la termogénesis sin tiriteo, la vasomoción cutánea, la sudoración y la piloerección, en respuesta a la variación de la temperatura ambiental (endotermos homeotermos; Seebacher 2009).

La termorregulación es un proceso neural que integra información acerca del ambiente externo y responde de manera apropiada para optimizar el ambiente térmico interno mediante el sistema termorregulatorio (Seebacher 2009). En los mamíferos, el incremento en la producción de calor resultante de la tasa metabólica y del desacoplamiento del transporte de electrones de la fosforilación oxidativa mantiene la temperatura corporal estable (Seebacher 2009). Sin embargo, las crías altriciales recién nacidas de muchos mamíferos de patrón politoco son incapaces de mantener su temperatura corporal cuando son separados del nido y del asilamiento provisto por sus compañeros de camada, y expuestos al frío (revisado en Gilbert y cols. 2007). Por ello, al nacimiento las crías altriciales son consideradas poiquilotermas, pero en un periodo más avanzado del desarrollo estos individuos alcanzan la endotermia homeotérmica (Geiser 2008).

El desarrollo de la endotermia homeotérmica, se ha descrito, está influenciada por una variedad de factores, incluyendo la maduración del tejido muscular, productor de calor (Visser y Ricklefs 1993), el desarrollo de plumaje o pelaje aislante, y un incremento en su masa corporal que confiere una disminución de la relación superficie-volumen (O'Connor 1975, Visser y Ricklefs 1993), el tamaño de la nidada (Andreasson y cols. 2016), el tipo y ubicación del nido (Morton y Carey 1971) y los riesgos de depredación específicos de la etapa de desarrollo temprano (Cheng y Martin 2012). Por lo tanto, no sería sorprendente que el inicio de la endotermia esté también modulado por las interacciones tempranas entre hermanos de camada, como, las diferencias en masa corporal al nacimiento, la cantidad de leche ingerida, la posición ocupada dentro del agrupamiento y el crecimiento postnatal temprano, generando posibles diferencias individuales en el inicio y desarrollo de la endotermia homeotérmica entre individuos de una misma camada.

1.3 Comunicación química

La comunicación química es un rasgo importante que se produce en los diferentes niveles de organización biológica, incluyendo la regulación entre células, tejidos, órganos, sistemas, e individuos (Agosta 1992), el código común entre el emisor y el receptor son señales químicas.

A nivel de individuos, éstas son secreciones corporales, las cuales contienen moléculas odoríferas que son transportadas por el aire o depositadas sobre objetos en el ambiente, y detectadas en forma de olor por individuos de la misma especie a través de los sistemas olfatorios, principal y accesorio, desencadenando una cascada de señales químicas en el organismo receptor (Coombes y cols. 2018), provocando diversos efectos en los individuos que las perciben, tanto fisiológicos como conductuales (Hart 1985) con repercusiones en el comportamiento social y las interacciones ecológicas entre los individuos.

Dichas señales olfatorias, son emitidas por diversas glándulas corporales y pueden ser transmitidas a través de dos formas, de manera pasiva, mediante liberación de moléculas odoriferas, tal es el caso de las feromonas mamarias y las secreciones de la glándula inguinal, en mamíferos (Hudson y Distel 1983, Melo y González-Mariscal 2010). O bien, de manera activa por medio de despliegues conductuales, por ejemplo, la micción, la defecación (Bogoni y cols. 2017) o la conducta de marcaje por frotamiento del mentón (Mykytowycz 1972).

Esta última, la conducta de marcaje, ha mostrado gran consistencia como rasgo de individualidad, por lo tanto, como se mencionó anteriormente, las diferencias individuales en frecuencia de marcaje (Arteaga y cols. 2008), podrían estar moduladas por interacciones tempranas entre compañeros de camada, tanto prenatales como postnatales.

II. Objetivo

Explorar la influencia de la presencia de los hermanos de camada en el desarrollo de diferencias individuales en fisiología y conducta en crías del conejo doméstico, específicamente, a corto plazo en diferencias individuales intracamada en el desarrollo termorregulatorio, y a mediano y largo plazo en diferencias individuales en la frecuencia de la conducta del marcaje del mentón.

III. Capítulo 1

Deferencias individuales en el desarrollo de la endotermia entre compañeros de camada del conejo doméstico.

La endotermia es un rasgo fundamental para los mamíferos cuyo establecimiento tiene consecuencias directas sobre la tasa metabólica y por ende en las historias de vida de los individuos con posibles efectos tanto a corto como a largo plazo sobre su supervivencia y adecuación.

En este capítulo evaluamos el efecto de las interacciones tempranas entre hermanos de camada, particularmente, diferencias individuales en crecimiento y conducta durante el periodo postnatal temprano, sobre la capacidad de los individuos de mantener la temperatura corporal estable ante la exposición individual al frío durante los primeros 15 días postnatales.

Individual differences in the development of thermoregulation among littermates of the domestic rabbit

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Abstract

The tight regulation of body temperature is essential for the maintenance of homeostasis in homeothermic endotherms. However, newborn altricial mammals are limited in their ability to maintain body temperature independently because they usually lack an insulating layer of fur, they have a relatively large body surface to volume ratio, and they have immature vasomotor control. Thus, altricial young are generally considered ectothermic poikilotherms at birth, but rapidly develop homeothermic endothermy. However, in polytocous species it is not known whether there are differences among littermates in the temporal development of endothermy and if so, what might account for this. Our aim was to determine the contribution of interactions among littermates to possible individual differences in the onset of homeothermic endothermy in altricial pups of the domestic rabbit. In 10 litters we evaluated individual differences in pups' position within the huddle on differences in their ability to maintain a stable body temperature when exposed individually to an acute daily cold challenge across the first 15 postnatal days. Greater relative body mass at birth was associated with greater body mass gain, occupancy of central positions in the litter huddle, and more rapid growth of fur, which together were associated with the earlier development of homeothermic endothermy. We conclude that early interactions among littermates associated with relative differences in initial body mass contribute to individual differences in the development of endothermy, with possible long-term consequences for individuals' fitness and metabolic phenotype.

Keywords Thermoregulation · Development · Siblings · Infrared thermography · Fur · *Oryctolagus cuniculus*

Introduction

Mammals are homeothermic endotherms, that is, they are able to endogenously maintain their body temperature within a very narrow range despite wide fluctuations in environmental conditions. This is vital to sustaining a wide range of biochemical and physiological processes essential for survival, and represents a major evolutionary adaptation of mammals, together with birds (Crompton et al. 1978). The importance of this ability is reflected in the distribution of homeothermic endotherms across the whole planet, and in the wide range of mechanisms they employ to achieve this major homeostatic goal. In mammals, these include morphological adaptations such as the development of fur, physiological mechanisms such as laying down a layer of subcutaneous fat, and/or behavioral adaptations such as huddling together with conspecifics and seeking out or constructing various kinds of shelters (Angilletta et al. 2010).

However, newborn altricial mammals have difficulty maintaining body temperature independently because they often lack an insulating cover of fur, they have a relatively large body surface exposed to heat loss relative to their body volume, and have limited autonomic control of vasodilation and vasoconstriction (Geiser 2008). They thus depend for survival during early development on being born into a nest or den, being brooded by the mother or other care givers, and on means of behavioral thermoregulation such as the warmth and insulation provided by huddling together with littermates (Vaughan et al. 2011). Because of their dependence on external sources of warmth, altricial young are usually considered ectothermic poikilotherms at birth (but see Blumberg and Sokoloff 1998 and Hill 2022 for caution in the use of this concept). Nevertheless, they rapidly develop homeothermic endothermy, essential by the time they leave the nest or den and are no longer brooded, and must embark on an independent life. The establishment of endothermy thus marks an important stage in the physiological independence of the offspring, but which at the same time implies a trade-off between energy available for growth and energy needed to maintain thermal homeostasis (Brown and Downs 2002).

Although in polytocous altricial species there has been increasing interest in individual differences among littermates in growth, survival, early social interactions, and the contribution of these to individual differences in physiological and behavioral phenotype (review in Hudson and Trillmich 2008) to our knowledge it is still not known whether there are individual differences among littermates in the development of endothermy, and if so, what might account for these.

The European rabbit (*Oryctolagus cuniculus*) provides a particularly good opportunity to investigate this. The rabbit has a pattern of unusually limited maternal care, in which after giving birth the mother immediately leaves the nest and returns just once for a few minutes approximately every 24 hours to nurse the young (Zarrow et al. 1965; Lincoln 1974; Hudson and Distel 1982; Rödel et al. 2012). This means that during early development the pups grow up almost exclusively in the company of their littermates (Bautista et al. 2003; Gilbert et al. 2007, 2012; Hudson et al. 2011). Consequently, in this altricial polytocous species, relations among littermates contribute importantly to the early development of individual differences in morphological, physiological and behavioral phenotype (review in Hudson et al. 2011). For example, during the early postnatal period, critical for offspring survival (Coureaud et al. 2000; Drummond et al. 2000; Rödel et al. 2009; Belabbas et al. 2023), heavier young at birth typically obtain more milk, are more efficient at converting this into body mass, show more rapid motor development, and occupy more central, better insulated positions in the litter huddle (Drummond et al. 2000; Bautista et al. 2008; Rödel et al. 2008a; Muciño et al. 2009; Hudson et al. 2011), that is, heavier pups relative to their littermates are in direct body contact with a greater number of littermates within the huddle, resulting in them having to invest less energy to maintain an adequate body temperature (reviewed in Bautista et al. 2015a, b).

Thus, our aim in the present study was to investigate whether previously reported individual differences in morphology, physiology and behavior in rabbit pups during early development are also associated with differences in the development of endothermy, the establishment of which has direct consequences for metabolic rate and with possible short- and long-term effects on survival and reproductive performance (reviews in Lovegrove 2016; Polymeropoulos et al. 2018). Specifically, we expected that lighter littermates at birth, which typically occupy peripheral positions in the litter huddle and have lower growth rates and lower body temperatures (reviews in Bautista et al. 2017; Zepeda et al. 2019), will show slower development of endothermy relative to their heavier littermates.

Methods

Study animals

We obtained data from chinchilla-strain domestic rabbits bred and maintained at the Centro Tlaxcala de Biología de la Conducta, Tlaxcala, Mexico. We used 10 litters (adjusted at birth to the eight heaviest pups) from 10 different multiparous females, each mated with one of eight different males. A total of 54 pups (27 females, 27 males) surviving to the end of the study were included in the final analysis. The mothers were between 20 and 38 months old. They were kept in individual stainless-steel cages (90 x 60 x 40 cm height), under fluorescent lights and on a 16:8 h light/dark cycle (lights on at 06:00 h) to approximate conditions at the height of the summer breeding season for rabbits in Europe. Ambient temperature was maintained between 18 and 20 °C, and water and food (Albapesa, México, Cre-C conejos® rabbit chow) were permanently available. Two days before parturition females were given a closed top wooden nest box (40 x 35 x 30 cm height) with a 20 x 15 cm access door and 25 g of meadow hay tied to a cage wall to allow the naturalistic behavior of hay gathering and nest building.

Procedures

Postnatal day (PND) 0. Day of birth. The body mass of each pup was recorded using electronic scales, resolution 0.1 g, and each litter adjusted to eight pups by culling or cross-fostering. For individual identification, pups were numbered on their back using non-toxic white correcting fluid (Aqua Kores®, Mexico; chinchilla-breed pups are dark gray) and on the abdomen using a permanent marker pen. They were then returned to the mother's cage and left without human disturbance for one day.

PND 1. At 08:00 h, after the pups had been nursed once overnight, they were taken in an open top wooden box (40 x 35 x 15 cm height) to a room with continuous fluorescent light and controlled ambient temperature between 23.0 ± 1.2 °C (below the critical thermoneutral zone of approximately 35 °C for newborn rabbits; Hull 1965; Pacheco-Cobos et al. 2003). Based on previous findings (Bautista et al. 2003), this temperature was chosen to induce pups to huddle but to limit offspring mortality and well above temperatures recorded in rabbit nest burrows in the field (Rödel et al. 2008b). To observe huddling behavior, the nest material was removed from the box, the floor was lined with flannel, and a 28 cm-diameter wire mesh hoop was placed in the center of the box to prevent pups becoming trapped in the corners (Bautista et al. 2008, 2013). Each pup was remarked with its identification number and weighed. To eliminate drafts, the box containing the pups was placed under a transparent acrylic box (45 x 45 x 70 cm height), with a closed-circuit video camera mounted in its ceiling and connected to a computer outside the cold room and programmed to record intra-litter behavioral dynamics at 09:00 h for 15 min (see below for details of how the video recordings were analyzed; Reyes-Meza et al. 2011; review in Zepeda et al. 2019). Video recordings continued for 15 min at 12:00, 14:00, 16:00, 18:00 and 23:00 h (Fig. 1).

At 13:30 h (several hours after being nursed in order to minimize thermogenic effects of postprandial digestion), pups were placed in random order in individual 12 x 8 x 6.5 cm height open transparent plastic boxes for 30 min at 23 ± 0.7 °C. An infrared shot was taken 30 cm above each pup with a thermographic camera (FLUKE® Ti25 IR Fusion Technology, Everett, WA, USA; emissivity 0.95). This was repeated every five minutes resulting in a total of seven thermographs for each pup (Fig. 2a). Although surface body temperature is not representative of core body temperature, it is a reliable measure of relative differences among individuals in their thermoregulatory response to cold stress across age, which was the main interest of the present study (Blumberg and Sokoloff 1998; Bautista et al. 2003, 2013). It also has the advantage when working with newborn altricial mammals of being noninvasive. The pups were then returned to their experimental nest box to regroup, and to continue with the video recording protocol (Fig. 1).

At 17:15 h. To evaluate fur growth and to determine the gain in thermal insulation provided by this (see below; Fig. 6), each day during the first 15 postnatal days three hairs, complete from root to tip, were plucked from the lumbar region of each pup and mounted with adhesive tape on a glass slide labeled with the pup's identification.

PND 2 to PND 15. The same procedure as for PND 1 was followed except that after the 09:00 h filming, we placed the mother beside the box so that she could jump in to nurse the pups. Nursing occurred for approximately three minutes, as is usual for rabbits (Zarrow et al. 1965; Hudson and Distel 1982; Rödel et al. 2012). When the mother jumped out of the box, indicating the end of nursing, she was returned to her cage. From PND 2, the 15-minute video recordings were at 01:00, 03:00, 05:00, 07:00, 09:00, 12:00, 14:00, 16:00, 18:00 and 23:00 h (Fig.1b). For the individual cold exposure test, since rapid fur growth prevented recording skin temperature with the thermographic camera, starting at PND 2 a 2 x 2.5 cm area was shaved on the lumbar region (the rump) of each pup, and for use in a later study, on a somewhat larger area of the interscapular region (Fig. 2b, c).

Position in the litter huddle

On PND 1 to 7, the period when pups are sparsely furred and depend critically on body contact with littermates to maintain an adequate and stable body temperature (Bautista et al. 2003; Gilbert et al. 2007), we quantified individual within-litter differences in position within the huddle by recording the number of neighbors with which each pup was in body contact with at least 10% of its body surface. To quantify each pup´s number of neighbors, we analyzed four frames from each video recording at 0, 5, 10, and 15 minutes, a total of 40 measures per pup/day. For each pup in each frame, we counted the number of littermates (neighbors) with which it was in body contact (Bautista et al. 2015a, b; Reyes-Meza et al.

2011). From this, we obtained the mean number of neighbors for each pup during the first seven postnatal days.

The quantification of each pup's number of neighbors was done by the same experimenter (YF), experienced in counting the number of littermates in body contact, using D-Link, D-View Cam software (D-Link®, Taipei, Taiwan). Inter-rater reliability was assessed using 280 frames (10%) pseudo-randomly selected from the first seven postnatal days and independently reanalyzed by a second experimenter. The comparison of the scores of the two raters using the R package *rptR* (Stoffel et al. 2017) with a Poisson distribution showed significant inter-rater agreement ($R = 0.89$, $P < 0.001$).

Ability to maintain body temperature

The change in surface skin temperature during individual pups' exposure to cold and the slope of temperature decrease was calculated using the average values of the temperature of the shaved rump area during the thermal challenge test (see below; also Fig. 2d). For this, thermographs were analyzed using Fluke® Smart View version 4.3 software for a circle 12.4 mm in diameter (20 pixels; cf. Gilbert et al. 2012) in the center of the shaved rump area.

Growth of fur

Using a digital microscope (Fotgear®, Guandong, China) and the software MICAM, version 3.0 (https://micam.software.informer.com, Marien van Westen), each slide with the three hairs sampled was photographed on a white background together with a 10 mm ruler to subsequently determine the length of each hair. The images were analyzed using ImageJ software version 1.53 (Schneider et al. 2012). For this, on the scale attached to the hair microphotograph, a 10 mm reference line was drawn and sequentially, the Analyze > Set Scale > Known distance commands were applied, where the scale distance was recorded: 10, Unit of length: mm. Once the scale was set, a line was drawn over the length of the hair from tip to base, not including the root, and the Analyze > Measure commands were used to obtain the length in mm of each hair. A daily average of hair length was obtained for each pup from the three hairs collected. To determine hair growth, the length on day t-1 was subtracted from day t.

Thermal insulation provided by fur

To determine the degree of insulation provided by the increasing growth of fur, each thermal image was transformed into a .csv data file, in which each cell contained the temperature value of each pixel of the thermal image (Lecorps et al. 2016). Measurements were performed using the R program version 4.2.0 (R Core Team 2022). We applied a script using the *image* function that allows viewing the thermal image in the R program and the *locator* function to select different points to extract the temperature values. Because of distortion of the thermal image of the shaved rectangle due to changes in pups' body posture, the four most extreme corners of the image that formed the shaved area of the rump were selected to define the shaved area. These points were also used to trace five frames, each one pixel (0.62 mm) in width around the shaved area and used to measure the insulating properties of the fur across the first 15 postnatal days (see below; Fig. 7). At the most central point of the shaved area a circle, 20 pixels in diameter (Gilbert et al. 2012), was draw to record the temperature of the shaved area.

Statistical analysis

Statistical analyses were performed using the program R, version 4.2.2 (R Core Team 2022). Only pups that survived to postnatal day 15 ($N = 54$ individuals) were included in the study. For all models we included litter identity ($N = 10$ litters) as a random intercept factor and sex as a fixed factor. The homogeneity of variances and goodness of fit of the liner mixed models used (LMMs) were tested by plotting the residuals against the fitted values. The normality of the model residuals was ensured by checking the normal probability plots (Faraway 2005). P values for the LMMs were calculated using F-tests based on the Satterthwaite approximation (Bolker et al. 2009). At the intra-litter level, we tested the effect of initial ranked body mass at PND 1 on the relative increase in ranked body mass at PND 15 and the position within the litter huddle ($N = 54$ pups from 10 litters). We applied LMMs based on restricted maximum likelihood estimates using the *lme4* package (Bates et al. 2015).

To investigate the change in surface temperature of the rump during the cold challenge, we applied an LMM using the *lme4* package (Bates et al. 2015). Likewise, we evaluated the interaction effect between the time course of the temperature challenge and postnatal day. Then, to determine the change in thermoregulatory performance of the pups with age, we evaluated the relation between postnatal day and the daily decrease in the slope of temperature decrease during the thermal challenge, using a non-linear mixed effects model (NLMM) based on the likelihood ratio test using the *nlme* package (Pinheiro et al. 2023). The calculation of the individual slopes of the association between the skin temperature of the rump and the time of the exposure to cold was performed daily for each individual pup (P value for all slopes < 0.05).

We subsequently analyzed the effect of relative starting mass at PND 1 on thermoregulatory performance, also using an LMM, with litter of origin as random factor, and using as response variable the residuals of the decrease in the steepness of slopes of the decline in temperature. As predictor variables we used the intra-litter rank of starting mass, where the heaviest pup of each litter received a value of 1 and the lightest pup a value of 0 and the remaining pups' values > 0 and < 1, as well as the number of neighbors in the huddle and the interaction between these variables and postnatal day.

Using an LMM, with litter of origin as random factor, we assessed the daily fur growth by a 5th order polynomial regression, using postnatal day as a covariate. We also tested the effect of relative starting mass and the number of neighbors in the litter huddle on the average fur growth from PND 1 to 8, the period for which the greatest daily growth of fur was observed (Fig. 5a). The thermal insulation provided by the fur was determined by comparing the difference in temperature between the unshaved area and the first pixel frame bordering the shaved area across the first 15 postnatal days (covariate). For this we implemented a 4th order polynomial effect in our LMM. We used the same method to evaluate the difference in temperature between the center of the shaved area and five successive areas of the unshaved area at 1, 2, 3, 4 and 5-pixels distance from the border using post hoc tests.

Finally, using an LMM, with litter of origin as random factor, we analyzed the effect of pups' relative starting mass and the number of neighbors in the litter huddle on the differences in temperature between the shaved and unshaved areas.

Results

Association between initial body mass, growth, and huddle position

Within litters, there was a significant and positive association between the pups' ranked starting body mass at PND 1 and their relative increase in body mass at PND 15 (LMM: $F_{1,801} = 121.026$, ${}_{marginal}R^2 = 0.693$, $\beta = 0.837$, 0.817 – 0.842 $CI_{95\%}$, $P < 0.001$); pups with a relatively greater starting body mass compared to their littermates had a significantly greater relative increase in body mass during the following two postnatal weeks. There was not a significant difference between females and males ($F_{1,801} = 0.472$, $\beta = -0.104$, -0.114 – -0.093 $CI_{95\%}$, $P = 0.495$). Also, relatively heavier pups were in physical contact with significantly more littermates within the huddle (PND 1–7) than lighter ones (LMM: $F_{1,807} = 265.387$, ${}_{marginal}R^2 = 0.247$, $\beta = 0.497$, 0.494 – 0.499 $CI_{95\%}$, $P < 0.001$), thus occupying more central positions. Again, there was not a significant difference between females and males ($F_{1,807} = 2.343$, $\beta = 0.093$, 0.088 – 0.097 $CI_{95\%}$, $P = 0.126$).

Change in skin temperature during the cold challenge

Skin temperatures of the rump decreased significantly and (almost) linearly during the 30-min thermal challenge (LMM: $F_{1,5603} = 1174.504$, ${}_{marginal}R^2 = 0.201$, $\beta = -0.452$, -0.452 – -0.451 $CI_{95\%}$, $P < 0.001$; Fig. 3a). There was a significant interaction between the time course of the temperature challenge and postnatal day ($F_{1,5603} = 72.406$, $\beta = 0.091$, 0.090 – 0.091 $CI_{95\%}$, $P < 0.001$), indicating that the slope of the decrease in rump temperature during the challenge test became less steep with pups' increasing age (Fig. 3b). There was not a significant difference between females and males ($F_{1,5603} = 0.739$, $\beta = -0.063$, -0.064 – -0.061 $CI_{95\%}$, $P = 0.395$).

Individual differences in the development of endothermy

To assess individual pups' development of endothermy, we used the slopes from the association between the skin temperature of the rump during the cold challenge (Fig. 3). More negative slopes indicate stronger decreases in the rump temperature. According to this measure, thermoregulatory performance increased significantly over the first 15 postnatal days, following a non-linear asymptotic function (NLMM: ${}_{marginal}R^2 = 0.243$, $P < 0.001$; Fig. 4a). It reached a plateau at around PND 7, indicating a rather stable thermoregulatory performance by this age.

Based on the residuals resulting from this non-linear regression, we analyzed the association between pups' relative starting mass (ranked within litters) and their thermoregulatory performance (ability to maintain a stable body temperature). This analysis revealed a significant interaction between ranked starting mass and postnatal day (LMM: $F_{1,805} = 13.077$, $\beta = -0.051$, -0.051 – -0.050 $CI_{95\%}$, $P < 0.001$); that is, during early postnatal days (for example Fig 4b, PND 2) thermoregulatory performance increased strongly with increasing relative starting mass. However, during later postnatal days (for example Fig. 4b, PND 13) there was no longer a notable effect of relative starting mass.

There were no significant effects of sex ($P > 0.10$).

Growth of fur

The growth of fur during the first 15 postnatal days was significantly described by a non-linear function (5th order polynomial: $F_{5,740} = 14.996$, ${}_{marginal}R^2 = 0.090$, $P < 0.001$), characterized by an initial increase and with a maximum growth at around PND 9 (Fig. 5a).

The average daily growth of fur during this initial increase until PND 8 (see gray shading in Fig. 5a) was significantly and positively associated with pups' ranked relative starting mass ($F_{1,43} = 4.998$, ${}_{marginal}R^2 = 0.072$, $\beta = 0.163$, 0.141 - 0.184 $CI_{95\%}$, $P = 0.031$; Fig. 5b); that is, relatively heavier siblings initially showed significantly greater average fur growth than their lighter littermates. Furthermore, average daily fur growth during the initial increase phase (PND 1–8) was significantly and positively associated with pups' number of neighbors in the litter huddle ($F_{1,51} = 10.101$, ${}_{marginal}R^2 = 0.149$, $\beta = 0.085$, 0.077 - 0.092 $CI_{95\%}$, $P = 0.002$; Fig. 5c); that is, pups which occupied more central positions in the huddle during early postnatal life showed faster fur growth.

There were no significant differences in fur growth between sexes ($P > 0.10$).

Thermal insulation provided by fur

The difference in temperature between the center of the shaved area and the surrounding fur-covered area at a distance of 1 pixel increased notably with age and was well described ($marginalR^2 = 0.521$) by a non-linear function with a strong initial increase until PND 8 (4th order polynomial: $F_{4,749} = 262.078$, $P < 0.001$; Fig. 6); that is, after an initial steep increase in the temperature difference, this measure reached a rather stable plateau from PND 9 on, i.e., an age when the rate of fur growth reached its maximum.

We also analyzed the difference between the shaved and adjacent unshaved areas at 1, 2, 3, 4, 5 pixel distance at PND 1, 7 and 15. At PND 1 ($F_{5,265} = 35.887$, $P < 0.001$), PND 7 ($F_{5,265} = 165.800$, $P < 0.001$), and PND 15 ($F_{5,265} = 300.600$, $P < 0.001$), there were significant differences between these six areas. On all of these days, there were significant decreases in temperature with increasing distance from the shaved area, which increased with increasing age and growth of fur (post hoc comparisons in Fig. 7a–c).

Discussion

For altricial mammals, typically considered ectothermic poikilotherms at birth as they are limited in their ability to maintain a stable body temperature (Geiser 2008), achieving homeothermic endothermy represents a major developmental landmark. Although this is energetically costly, it has advantages such as faster offspring development and greater long-term fitness (Gavrilove 2013; Brashears et al. 2017; McClelland et al. 2017; Robertson et al. 2019). Consistent with our previous findings on the development of individual differences during early development among littermates of newborn rabbits (e.g. Bautista et al. 2015b, 2017; García-Torres et al. 2015; Hudson et al. 2011; Zepeda et al. 2019), and in other altricial polytocous mammals (Bautista et al. 2010; Raihani et al. 2014; Zepeda et al., 2018), we found a positive association between relative differences in body mass among littermates at birth, greater body mass gain, occupancy of more central positions in the litter huddle, and now in the present study, earlier development of endothermy as measured by individual differences in the ability of littermates in the age at which they could defend their body temperature when exposed to a cold challenge. Under the conditions of the present study, pups were generally able to maintain a stable body temperature when exposed to a 30-minute cold challenge by around postnatal day nine (Fig. 4a), although with notable individual differences among siblings in the age of achieving this. Relatively heavier siblings were able to do so at a significantly earlier age than their lighter littermates (Fig. 4b).

Additionally, we now report that individual differences in the growth of an insulating coat of fur also contributed to individual differences in achieving endothermy; heavier littermates showed significantly quicker fur growth across early postnatal development than their lighter littermates (Fig. 5b). Surprisingly, given the functional significance of fur, the development of this has received little attention in studies of early mammalian development. The present results now provide evidence of the importance of the rapid growth of fur during the early postnatal period, particularly during the first postnatal week (Fig. 6; cf. Bautista et al. 2003), and how even small increments in this can contribute importantly to thermoregulation and to achieving endothermy by altricial young. In the present study this was most clearly seen in the rapid change in the gradient in surface body temperature between the naked, shaved rump area and its furred surround with the increase in insulation provided by the growth of fur across the first two postnatal weeks (Fig. 7).

However, the production of a fur coat covering the entire body presumably comes at a considerable energetic cost. Studies in several mammalian species, including kangaroos (Dawson and Maloney 2017) and South American camelids (Gerken 2010) have examined the contribution of a coat of fur to thermoregulation and also to understanding the energetic costs associated with coat production. Additionally, in a study of juvenile Siberian hamsters (*Phodopus sungorus*) the energy trade-off between growth and thermoregulation was investigated, as well as the possible adaptive role of fur in maintaining energy balance during growth (Batavia et al. 2010). Although the results of that study showed that fur was not necessary for normal growth, i.e., body mass and length of individuals were not affected by the presence or absence of fur, the energetic costs associated with thermoregulation were increased, as shaved individuals needed to consume substantially more food to maintain a stable body temperature and normal growth.

In the case of newborn rabbits, despite the thermoregulatory benefit provided by rapid fur growth, not all litter members appear to have the energetic resources to invest equally in this, given the struggle to maintain a thermally advantageous position in the litter huddle (Bautista et al. 2008, 2013, 2017; cf. Robertson et al. 2019), and the struggle to obtain sufficient milk in the short period available each day (Bautista et al. 2005). Therefore, based on the present findings we propose that in addition to previously reported advantages, heavier pups at birth have greater energetic resources to invest in fur growth, which helps them to reduce the energetic costs of maintaining an adequate body temperature and increases their probability of early survival compared to their lighter littermates. This, in turn, contributes to what is emerging as the interplay of an increasingly complex network of morphological, physiological and behavioral factors associated with early sibling interactions and the development of individual phenotypic variability (Drummond et al. 2000; Bautista et al. 2013, 2015b, 2017; Muciño et al. 2009; Zepeda et al. 2019).

As in our previous studies on the emergence of individual differences among siblings during early development, a question arising from the present study is whether individual differences in attaining endothermy contribute to possible medium- or long-term differences in metabolic phenotype and ability to meet the challenge of thermally hostile environments in later life? This seems plausible given reports of the long-term effects of early ambient temperature on individuals' later metabolic rate and their ability to maintain a stable body temperature under thermally challenging conditions in various species (Morrison et al. 2002; Nord and Giroud 2020) including in humans (Gorbunov et al. 2020;

Guilbert et al., 2022). Such effects include the influence of temperature on the rate of biochemical reactions, on the rate of blood flow, on metabolic rate, the rate of heat production and of ion pumping (Tattersall et al. 2012). For example, in fat-tailed dunnarts (*Sminthopsis crassicaudata*), permanent exposure to warm or cold temperatures from early life has been shown to irreversibly influence thermoregulatory mechanisms; cold-reared individuals had longer episodes of lethargy and lower resting metabolic rates at low temperatures in adulthood compared to warm-reared individuals (Riek and Geiser 2012).

In a longitudinal study of individual differences in the metabolic phenotype of rabbit littermates from birth to adulthood we are currently investigating whether this is also the case for relatively lighter versus heavier littermates, experiencing relatively cooler or warmer environmental temperatures in the litter huddle, respectively.

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Author contributions AB conceived and directed the study and helped design the figures; YF conducted the experiments, collected and analyzed the data and helped write the paper; HGR and CF gave statistical advice and contributed substantially to data analysis; VRM provided equipment, helped with thermal image analysis and discussion of results; RH obtained funding and directed preparation of and largely wrote the paper. All authors read and gave critical comments on draft versions of the paper.

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Data availability Data used for preparing figures and performing statistical tests and analyses are provisionally provided for the use of reviewers at https://drive.google.com/drive/folders/1tdDM9Vy69xITMkCdWI8kACfXFkt4Rghn?usp=sharing Upon acceptance of the paper we will make this information publicly available at the same site.

Code availablity Not applicable.

Declarations

Ethics approval The maintenance of animals and experimental protocols complied with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA, with the National Guide for the Protection, Care and Use of Laboratory Animals, Mexico (Norma Oficial Mexicana NOM-062– 200–1999) and with the regulations of the Institutional Commission for the Use and Care of Laboratory Animals (IIB, UNAM, CICUAL, protocol ID 6311).

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Figure legends

Fig. 1 Timelines of the experimental procedure. a) Across the 15 postnatal day period of the study. b) Across each postnatal 24-h day. REC = 15-min video recording of pups' relative position in the litter huddle

Fig. 2 Thermographic measurement of surface body temperature. a) Method of recording dorsal surface body temperature of individual littermates during the cold exposure challenge. b) Dorsal schematic of a rabbit pup. Rectangles indicate the shaved area of the rump (2 x 2.5 cm) used in this study, and of the interscapular area (used in a later study). c) Thermographic image at PND 7, where red represents the highest dorsal body surface temperature grading to green which represents the lowest. Note the difference in temperature between shaved and unshaved areas, indicating the insulation provided by the fur against heat loss. d) Example of the decline in rump temperature of a pup on PND 7 across the 30-min cold challenge

Fig. 3 Decline in rump temperature during the daily cold challenge. a) Each point represents the mean and 95% *CI* for the 15 days of testing of 54 pups from 10 litters. b) Linear regressions showing the reduction in the decline in rump temperature during the cold challenge across postnatal days 1, 3, 5, 7, 9, 11, 13, and 15, of 54 pups from 10 litters

Fig. 4 Development in the response during the daily cold challenge. a) Developmental increase in thermoregulatory performance. Increasing defense of body temperature across the first 15 postnatal days. A value approaching 0 indicates less heat loss during the thermal challenge. Each point represents the mean and 95% *CI* of 54 pups from 10 litters. b) Regression lines show predicted values including 95% *CI* (gray shading), based on estimates provided by a LMM (see text). The graphs for PND 2, PND 7, and PND 13 illustrate the residuals of skin temperature and pup relative starting body mass (0 indicates relatively lightest pups and 1 relatively heaviest pups per litter). Analysis based on 5670 observations from 54 pups from 10 litters

Fig. 5 Fur growth during the first 15 postnatal days. a) Each point represents the mean and 95% *CI* of 54 pups from 10 litters. Note the steeper slope in the growth of fur after PND 7 as shown by the difference in the regression values for PND 1 – 7 and PND 8 – 15 (see text). b, c) Significant and positive associations between individual pups' growth of fur at PND 7 and their relative body mass and relative position in the litter huddle according to the mean number of littermates ("neighbors") with which they were in direct body contact from PND 1 to PND 7 (fewer neighbors = peripheral pups, more neighbors = central pups). See text for statistics. As indicated by the positive slopes of the regression lines, relatively heavier pups and central pups tended to have faster fur growth. Each point represents data for one pup; 54 pups from 10 litters

Fig. 6 Differences in temperature between the shaved and non-shaved area (px1 distance) during the first 15 postnatal days. Each point represents the mean and 95% *CI* of 54 pups from 10 litters

Fig. 7 Increased insulation by fur growth with age. Change in surface temperature of the rump of pups between the shaved area and the area bordering it during the individual cold challenge on PND 1, PND 7 and PND 15. Note the increasing contrast in temperature between the shaved center and the furred surround across the three panels indicating the increased insulating effect of the fur with age. Each point represents the mean and 95% *CI* for 54 pups from10 litters. The insert on PND 15 shows where the measures indicated on the horizontal axes of the three panels were taken. See text for details of the distances between measures. Different letters above the points indicate significant differences as shown by post hoc tests

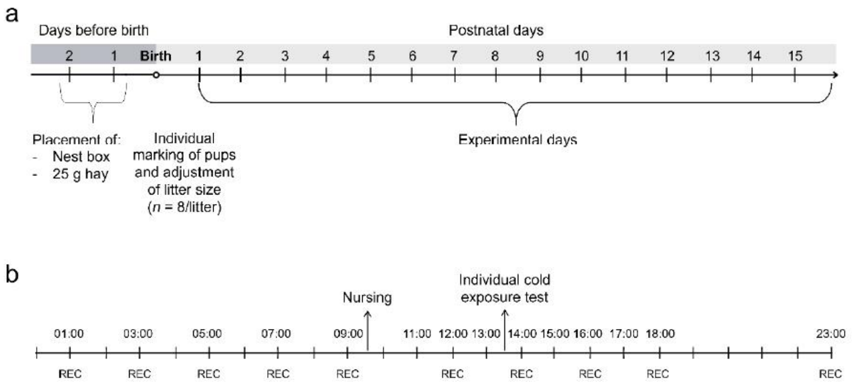

Figure 1 Fernández et al.

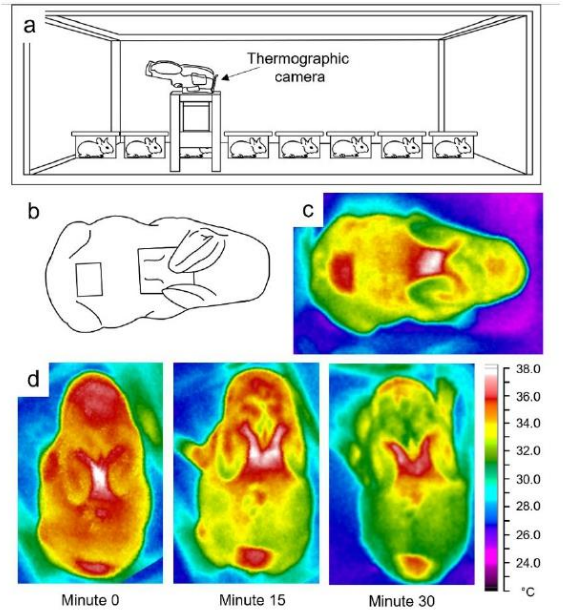

Figure 2 Fernández et al.

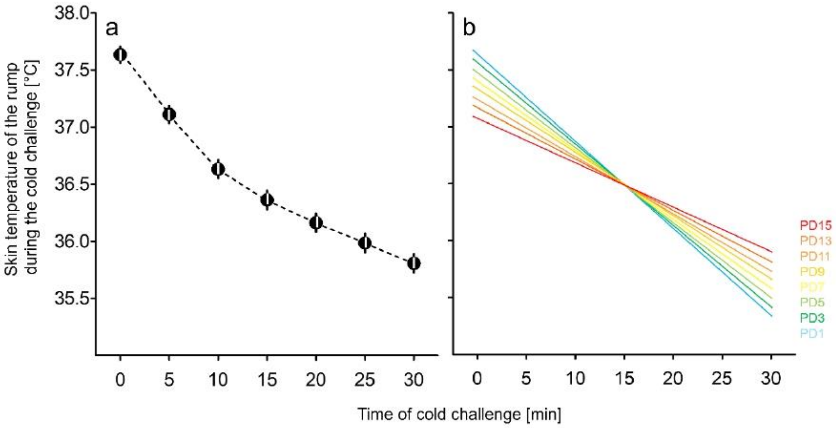

Figure 3 Fernández et al.

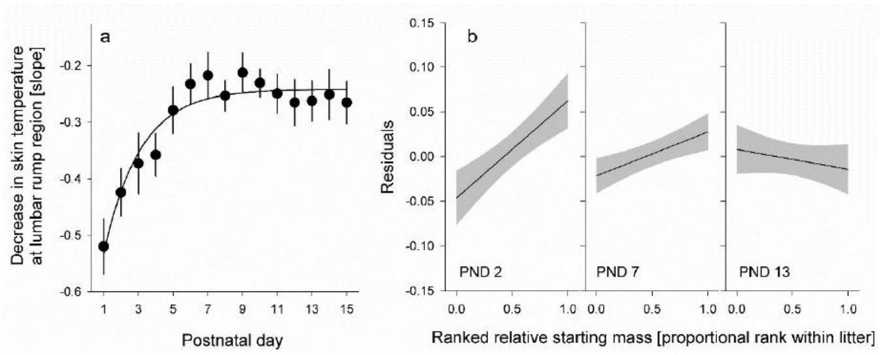

Figure 4 Fernández et al.

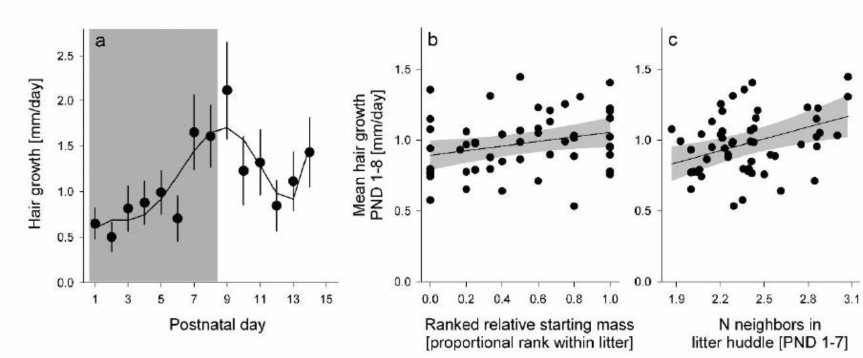

Figure 5 Fernández et al.

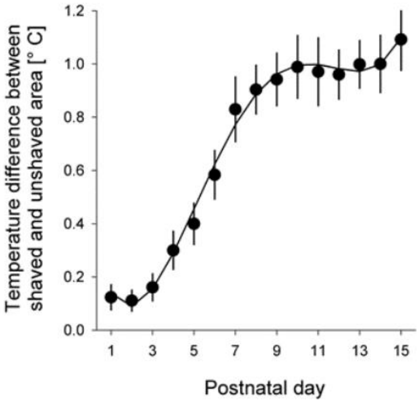

Figure 6 Fernández et al.

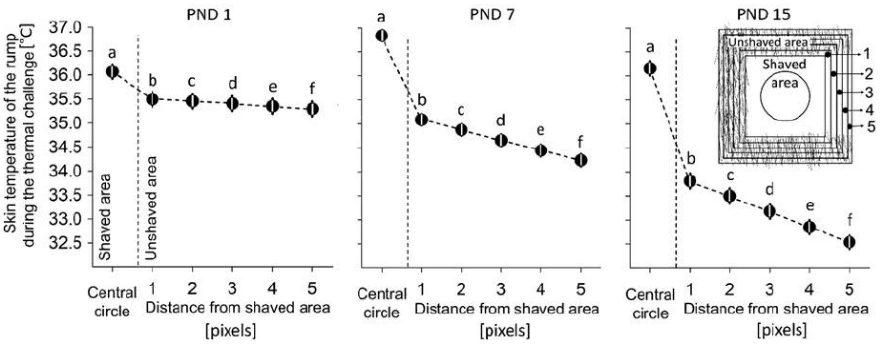

Figure 7 Fernández et al.

IV. Capítulo 2

Desarrollo de diferencias individuales en la frecuencia de la conducta de marcaje del mentón en el conejo doméstico.

Las diferencias individuales en el comportamiento son un tema importante de la biología del comportamiento, en el que influyen factores genéticos, ambientales y de desarrollo. La investigación pone de relieve cómo surgen y evolucionan estas diferencias a lo largo del tiempo, lo que permite comprender la compleja interacción entre naturaleza y crianza.

En este capítulo estudiamos las diferencias individuales en el desempeño de un comportamiento en el conejo europeo (*Oryctolagus cuniculus*), el marcaje por frotamiento del mentón, una parte importante del sistema de comunicación química en esta especie.

Investigamos la estabilidad de diferencias individuales entre los compañeros de camada en la frecuencia de marcaje y relacionamos esto con las interacciones entre los compañeros de camada durante el período postnatal temprano.

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RESEARCH ARTICLE

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Stable individual differences in the frequency of chin-marking behavior across development in the domestic rabbit

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Abstract

Individual differences in behavior ("personality") are of considerable interest to behavioral biologists. Important questions include how early in life such differences emerge, what factors influence their emergence, and whether they remain stable across development and into adult life. Given the demanding nature of longitudinal studies, there is a lack of information regarding these questions in mammals. Our aim in this study was to investigate the development of individual differences in chin-marking behavior (chinning) in the domestic rabbit, a notable part of this species' system of chemical communication, and to relate this to individual differences in growth and behavior among littermates during the early postnatal period. We tested repeatedly the frequency of chinning movements from weaning to sexual maturity in 63 chinchilla-strain rabbits (35 females, 28 males) from 14 litters. Within litters, we found significant consistencies over time in this behavior, that is, in both sexes inter-individual differences among litter siblings in the frequency of chinning movements remained stable across the postweaning period until sexual maturity. Unexpectedly, however, we found no significant associations with the morphological, physiological, or behavioral variables known to form a well-correlated early developmental complex in this species. We tentatively conclude that in the rabbit, individual differences in the frequency of chinning have little relation to other previously studied aspects of individual developmental trajectories. The origin and functional significance of individual differences in chinning frequency, whether in reproductive or other social contexts is largely unknown and requires further investigation.

KEYWORDS
behavioral syndromes, chemical communication, *Oryctolagus cuniculus*, personality, repeatability, siblings

1 | INTRODUCTION

Stable individual differences in behavior, broadly defined as consistency across time and/or contexts (also sometimes referred to as personality, temperament, coping style, or behavioral phenotype) have attracted considerable attention among behavioral biologists. Such differences have been found in a wide range of taxa (Bell et al., 2009; Briffa & Weiss, 2010; Kralj-Fišer & Schuett, 2014; Stamps & Groothuis, 2010) and are thought to represent important adaptive strategies with consequences for individual fitness (Bergmüller & Taborsky, 2010; Dall et al., 2012; Montiglio et al., 2013; Wolf & Weissing, 2012). Suites of correlated, repeatable behavioral and/or physiological traits are sometimes referred to as syndromes (Dosmann et al., 2015; Sih et al., 2004), and are thought to contribute significantly to evolutionary processes (Dochtermann & Dingemanse, 2013). Such differences may appear early in life (Trillmich & Hudson, 2011), but may also change across life stages and are not necessarily stable across an individual's life span (Stamps & Biro, 2016; Trillmich et al., 2015). Thus, longitudinal studies are needed to investigate when and how individual differences in behavioral and associated morphological and physiological traits emerge during development and to what extent they persist (Cabrera et al., 2021; Fawcett & Frankenhuis, 2015; Groothuis & Trillmich, 2011; Trillmich & Hudson, 2011). Due to the demanding nature of longitudinal studies and the challenge of testing animals across ontogenetic change, there is still little information on the development and persistence of individual differences in behavioral and physiological phenotypes in mammals (Bell et al., 2009; Cabrera et al., 2021; Stamps & Groothuis, 2010; Trillmich & Hudson, 2011).

One aspect of the early environment that can importantly influence individual developmental trajectories is interaction among siblings, whether of the same or different age (Hudson & Trillmich, 2008). In the European rabbit *Oryctolagus cuniculus*, consistent individual differences have been found in a range of correlated behavioral, morphological, and physiological variables among litter siblings during the nest period, with some evidence that these differences have consequences for fitness in later life. These variables include intra-uterine position, body mass at birth, success in competing for milk and for thermally advantageous positions within the litter huddle, and growth and survival to weaning and beyond (laboratory rabbit: Bautista et al., 2015a,2015b; Reyes-Meza et al., 2011; summary in Zepeda et al., 2019; wild rabbit: Rödel et al., 2015, 2017). In this study, we were interested in extending these findings to the investigation of an additional behavior of the rabbit that can be readily followed across development and into adult life, chin marking.

Chin marking (chinning) is a conspicuous part of the rabbit's complex system of chemical communication, performed by both sexes in the laboratory and in the wild. In females, it is thought to advertise sexual receptivity and willingness to mate, and in males to indicate dominance and to be associated with territorial defense (laboratory rabbit: Black-Cleworth & Verberne, 1975; González-Mariscal et al., 1990, 1992, 1993; Hudson et al., 1990, 1994; Hudson & Vodermeyer, 1992; Melo et al., 2008; Soares & Diamond, 1982; wild rabbit: Bell, 1980; Hayes et al., 2002a,2002b; Mykytowycz, 1962, 1965). It consists in the animal rubbing its chin on objects in the environment, presumably to deposit secretions from its chin glands (Figure 1). It is readily performed in the laboratory under experimental conditions and is easy to quantify by scoring the frequency of chinning movements. Animals already start chinning around weaning at one month of age and continue with increasing frequency across postweaning development until sexual maturity (González-Mariscal et al., 1992). In both sexes, the frequency of chinning by adults is strongly influenced by gonadal steroids. It is

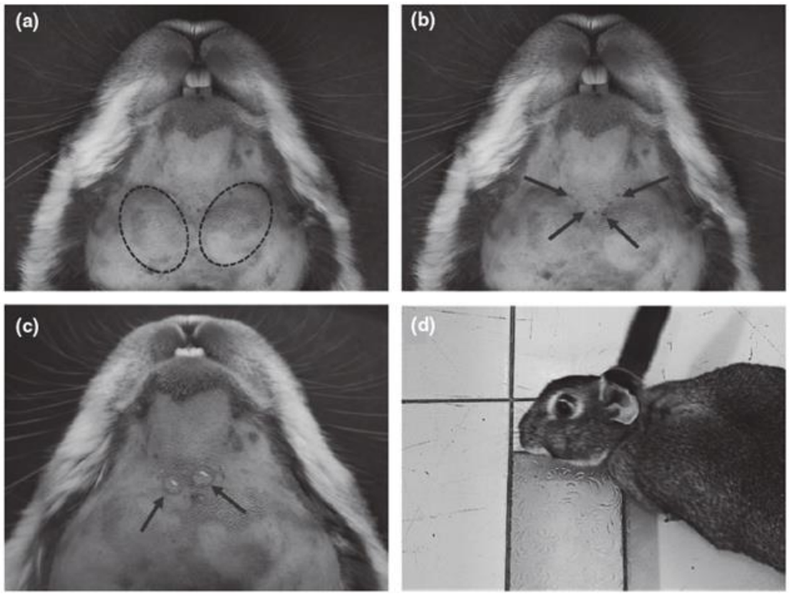

FIGURE 1 (a) Chin of an adult male rabbit shaved to show the approximate area of the chin glands beneath the skin indicated by the broken lines. (b) The same animal showing the typical "V"-shaped array of ducts to the surface, several marked by arrows. (c) The same animal with secretion, indicated by arrows, expressed from two of the chin-gland ducts. (d) An adult female chinning the glass stimulus brick used to test individual differences in the frequency of chinning. See Video S1 for an example enhanced by naturally circulating levels or by the administration of these (González-Mariscal et al., 1992, 1993; Hudson et al., 1990; Melo et al., 2008) and is reduced or eliminated in both sexes following gonadectomy (Chirino et al., 1993; González-Mariscal et al., 1992; Hudson et al., 1990, 1994; Hudson & Vodermeyer, 1992; Martínez-Gómez et al., 1997). Particularly relevant to this study, consistent individual differences have been reported in the frequency of chinning in both sexes in a range of studies (Arteaga et al., 2008; González-Mariscal et al., 1992; Hudson & Vodermeyer, 1992; Martínez-Gómez et al., 1997).

It was therefore our aim to investigate the emergence of individual differences among litter siblings of both sexes in the frequency of chinning across development and the possible relation of this to other well-documented aspects of individual differences in morphology, physiology, and behavior in this species during early life. We expected that stable individual differences in the frequency of chinning would emerge across development and that these would be associated with other previously documented aspects of individual differences during the nest period. Specifically, we expected that heavier littermates at birth, which typically occupy thermally more advantageous central positions in the litter huddle and have higher growth rates (reviewed in Bautista et al., 2015a,2015b; Zepeda et al., 2019), would show greater frequencies of chinning as a possible indicator of their better body condition.

2 | METHODS

2.1 | Study animals

We used 14 unculled litters of 5–10 pups of chinchilla-breed domestic rabbits from 14 different females each mated with one of 12 different stud males bred and maintained at the Centro Tlaxcala de Biología de la Conducta (CTBC). A total of 63 pups, 35 females, 28 males surviving to the end of the study, were included in the final analysis. Eighteen females and 26 males from 13 litters died before the end of the study and were not included in the analysis beyond PD0. We note that mortality across development is common in domestic rabbits, both in the laboratory and under conditions of husbandry. Consistent with previous reports, deaths occurred mainly during the first postnatal week (>50%), apparently due to differences among females in the quality of maternal care such as lactational performance, as well as competition among littermates to obtain sufficient milk during the short, once-daily nursing episodes (Coureaud et al., 2000; Drummond et al., 2000; Rashwan & Marai, 2000; Schlolaut et al., 2013).

Breeding females were kept in standard individual stainless steel cages 90 × 60 × 40 (height) cm under fluorescent lights set to a 16:8 h light:dark cycle, which approximates conditions at the height of the summer breeding season for rabbits in Europe. Ambient air temperature was maintained between 17°C and 24°C, and fresh water and food (Purina® rabbit chow,) were permanently available. Animals were kept and treated according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA, to the National Guide for the Protection, Care and Use of Laboratory Animals, Mexico (Norma Oficial Mexicana NOM-062-200-1999) and to the regulations of the Bioethics and Academic Integrity Committee of the CTBC (CICUAL-ID-6311).

2.2 | Experimental procedure

Except for recording chinning frequency (see below), the procedures followed and the preweaning measures taken were basically the same as in our previous studies of individual differences among litter siblings in postnatal behavior, growth, and survival in the rabbit (e.g., Bautista et al., 2008, 2013,2015a,2015b).

2.2.1 | Postnatal treatment

The day of birth was denoted postnatal day 0 (PD 0). On PD 1 at 09:00 h, after litters had been nursed once, they were removed in their wooden, open-top nest box from the mother's home cage. Pups were individually weighed and were numbered on the back and sides (dark gray skin in this breed) with white nontoxic correcting fluid for individual identification (Bautista et al., 2008,2013; Reyes-Meza et al., 2011); after PD 7 when the pups had fur, individual identification numbers were registered in their ears using a marker pen (Sharpie®). The nest material was then removed from the nest box to allow observation of the pups' behavior, and the box was lined with flannel. To keep the pups in the center of the box and prevent them from becoming trapped in the corners, we placed them inside a 28 cm diameter wire mesh hoop (Bautista et al., 2008,2013). The box with the pups was placed in a cold room in continuous light with the temperature set at 25°C. This temperature, below the approximately 35°C critical thermo-neutral temperature for newborn rabbits (Hull, 1965; Pacheco-Cobos et al., 2003; Satinoff et al., 1976), induces them to huddle but without compromising pup survival (Bautista et al., 2003). In nature, the ambient temperature surrounding the nest chamber can drop below pups' thermo-neutral range and well below the temperatures maintained here (see below; Rödel et al., 2008). To eliminate possible drafts, the nest box was placed beneath a transparent acrylic cover (45 × 45 × 70 cm height), with a closed-circuit video camera mounted in the roof of the box and connected to a computer outside the cold room to record the pups' behavior and to quantify pups' number of neighbors in the litter huddle (Reyes-Meza et al., 2011; review in Zepeda et al., 2019). Litters were filmed for 15 min at 1:00, 03:00, 05:00, 07:00, 09:00, 12:00, 14:00, 16:00, 18:00, and 23:00 h (see below for details of how the videos were analyzed).

From PD 2–7, at 10:00 h we removed the acrylic cover, removed the mesh hoop without disturbing the huddle, and as in previous studies (review in Zepeda et al., 2019) placed the mother beside the box so she could jump in for the approximately 3 min once-daily nursing typical of the rabbit (Apel et al., 2020; Hudson & Distel, 1982; Rödel et al., 2012; Zarrow et al., 1965). Immediately after the mother jumped out of the box, indicating the end of nursing, we weighed the pups individually and placed them back in the box inside the hoop, and placed the box under the acrylic cover.

To ensure the continued thermal importance of the litter huddle despite pups' increase in body volume, growth of fur (Bautista et al., 2003; Gilbert et al., 2007), and decline in their critical thermoneutral temperature to approximately 32°C by PD 4 (Hull, 1965), the temperature of the cold room was decreased by 1°C at 18:00 h each day (Bautista et al., 2013).

On PD 7 at 16:15 h, the pups were returned permanently in their nest box, together with the original nest material, to their mother's cage and were weighed daily at 11:00 h. After weighing and nursing on PD 35, we checked the sex of the young, tattooed them in the ears for permanent individual identification, and separated them permanently from their mother. Littermates of the same sex were placed together in standard stainless-steel cages (91 × 60 × 42 cm, height) with free access to water and to the same dry food as their mother. On PD 70, they were rehoused in individual stainless-steel cages (45 × 60 cm × 42 cm height) where they remained until sexual maturity and final testing on PD 126.

To quantify pups' number of neighbors in the litter huddle, we analyzed four frames from each video—at 0, 5, 10, and 15 min—giving a total of 40 frames per day/litter. For each pup in each frame, we counted the number of littermates (neighbors) with which it was in contact with an estimated 10% or more of its body surface. From this, we obtained the mean number of neighbors with which each pup was in contact during the first seven postnatal days (Bautista et al., 2015a,2015b; Reyes-Meza et al., 2011). We then calculated the relative within-litter differences among littermates in the number of huddling partners as the % deviation from the mean number of huddling partners averaged over the values of all pups for each litter.

Given the modest number of subjects (35 females, 28 males), in the analysis we used to evaluate the contribution of developmental variables to individual differences in chinning frequency in our animals as adults (see below), we were limited in the number of variables we could include in the models. Since past studies have repeatedly shown a positive association between body mass at birth and number of neighbors in the huddle on the one hand, and milk ingestion and body temperature on the other (reviewed in Zepeda et al., 2019), we took body mass at birth and number of neighbors (position in the huddle) as proxies for the latter two variables.

Daily maintenance and cleaning of cages was completed by 10:00 h and testing was from 11:00–13:00 h. The order of testing animals within each litter was determined using the online random-order software random.org.

2.2.2 | Chinning tests

On PD 17, 18, and 19 at 11:30 h, the litters were taken to an adjacent test room where they were weighed and marked on the head with colored acrylic paint (Vinci Acrilica®) for individual identification in the video recordings during testing. They were placed together in an enclosed wooden start box (42 × 35 × 32 height cm) attached to a test arena (100 × 150 × 85 height cm) with opaque acrylic walls. If they did not leave the start box after 1 min, they were gently pushed into the arena and the door of the start box was closed behind them for 10 min habituation to the environment used for subsequent tests of chinning on PD 21, 22, and 23.

To test the frequency of chinning movements, the animals were transported individually by hand from their cage in the colony room to an adjacent room containing only the test arena and no other animals. Translocating the animals took about 30 s. The same two experimenters, one male and one female, conducted all tests of the same litter, the man for four litters and the woman for ten litters.

The animals were placed individually in the start box, and if necessary, after 1 min they were gently pushed into the arena and the door of the start box was closed behind them. They were then left for 10 min in the presence of a brick of rough textured glass (23.5 × 12 × 6 height cm; Figure 1d). Behavior of the animals was video recorded (Canon Vixia HF G-20, Japan) for later analysis of the number of chinning movements directed to the brick, and as occasionally occurred, to other parts of the arena (Video S1). After each test, the experimenters washed their hands with soap and water, and the arena and brick were wiped with soap and water followed by isopropyl alcohol. This resulted in an interval of approximately 10 min between testing each animal.

Frequency of chinning was tested at three ages: preweaning on PD 21, 22, 23; juvenile on PD 74, 75, 76; and adult on PD 124, 125, 126, resulting in nine repeats for each animal. Single chin marks were defined and counted as an animal making physical contact with the glass brick or other part of the arena with its chin to the moment it raised its head and broke physical contact with the substrate. Before each block of tests, the animals were again given three days of 10-min habituation to the test environment, as for the preweaning trials except that the animals were habituated individually.

The frequency of chinning movements was scored from the video recordings by the same experimenter (YF), experienced in the observation of chinning, using CyberLink PowerDVD 15 software. Inter-rater reliability was assessed by having videos of 54 trials (10%) pseudo-randomly selected for males and females from each of the three age classes, independently reanalyzed by another experimenter (LA), also experienced in scoring chinning. A comparison of the scores of the two raters using the R package *rptR* (Stoffel et al., 2017) with a Poisson distribution showed highly significant agreement ($R = .98$, $p < .001$).

2.3 | Statistical analysis

Statistical analyses were performed using the program R, version 3.6.3 (R Core Team, 2020). To test for the consistency of individual differences in the frequency of chinning movements, we calculated the repeatability of this behavior using the relative value (within-litter variance calculated as individuals' % deviation from the litter mean) of the frequency (number of marks/10 min) for each individual on the nine days of testing. Repeatability, also known as the intra-class correlation index, describes the relative division of variance within and between groups (Nakagawa & Schielzeth, 2010). A high repeatability value, close to 1, indicates a high level of consistency between measurements. Repeatability estimation based on general linear mixed-effects models (GLMM) was performed using the *rptR* package (Nakagawa & Schielzeth, 2010) with a Poisson distribution. Repeatability was calculated separately within each age class (preweaning, juvenile, and sexual maturity; Table 1, see Figure S2). Similarly, across-age repeatability was calculated using the relative values of the sum of the frequency of the three test days for each age class (see Table 2; Figure S3). For each within-age and across-age repeatability analysis, the litter of origin was considered a random factor. Confidence intervals (95%) were estimated using bootstrapping with 1000 resamplings, and statistical significance (*P* values) were calculated based on 1000 Monte Carlo permutations.

Then, to investigate which factors influenced the occurrence of individual differences in chinning, we calculated the association between the variables based on linear mixed-effects models (LMM) calculated using the *nlme* package (Pinheiro & Bates, 2022) for non-normally distributed data. In order to estimate the effect of the predictor variables on chinning frequency at sexual maturity, we investigated the associations with sex, body mass at birth, and individuals' position in the litter huddle. The variable sex was transformed to 1 and −1 for females and males, respectively. For the variables body mass at birth and position in the huddle, and chinning frequency at sexual maturity, relative within-litter values were calculated as individuals' % deviation from the litter mean. The litter of origin was considered a random intercept factor (Zepeda et al., 2019).

3 | RESULTS

Only 21 of the 63 subjects (33%,14 females, 7 males) chinned the stimulus brick when tested before weaning. Post weaning, however, most animals (73%) of both sexes chinned the brick at least a few times in each session, in general showing an increase with age (Figure S1). Notable were the large individual differences in the frequency of chinning both within and between litters. In litter 6, for example, whereas animal number 1 (female) chinned as an adult on average more than 50 times per session, her sibling number 5 (male) did not mark at all (Figure S1). Similarly, whereas three of the four siblings of litter 8 (2 females, 1 male) showed high frequencies of chinning as adults, all six members of litter 2 (4 females, 2 males) showed low frequencies (Figure S1).

There was no significant difference in the frequency of chinning at sexual maturity between the sexes (LMM with 1000 permutations: $\beta = -41.82 \pm 36.33$ SE, $p = .45$).

TABLE 1 Summary of repeatabilities (*R*) within age classes expressed as the percentage deviation from the litter mean

	FEMALES						MALES					
	Preweaning		Juvenile		Sexual Maturity		Preweaning		Juvenile		Sexual Maturity	
AGE	PD 22	PD 23	PD 75	PD 76	DP 125	DP 126	PD 22	PD 23	PD 75	PD 76	DP 125	DP 126
Preweaning												
PD 21	0	0.536***					0	0				
PD 22		0						0				
Juvenile												
PD 74			0.768***	0.826***					0.891***	0.803***		
PD 75				0.500***						0.790***		
Sexual Maturity												
PD 124					0.873***	0.843***					0.835***	0.737***
PD 125						0.824***						0.737***

Note: Significant values are highlighted in bold and with asterisks (*** $p < .001$). The first block shows the repeatability in chinning frequency for females ($N = 35$) and the second block shows the same for males ($N = 28$) for each age class.

Abbreviation: PD, postnatal day.

TABLE 2 Summary of repeatabilities (R) for inter-age classes based on the percentage deviation from the litter mean

	FEMALES		MALES	
AGE	Juvenile	Sexual Maturity	Juvenile	Sexual Maturity
Preweaning	**0.363****	0.125	0	0
Juvenile		**0.696*****		**0.448*****

Note: Significant values are highlighted in bold and with asterisks ($^{**}p < .01$, $^{***}p < .001$). The first block shows the repeatability in chinning frequency for females ($N = 35$) and the second block shows the same for males ($N = 28$) for each age class.

3.1 | Consistency of individual differences in chinning frequency

3.1.1 | Consistency within age classes

Individual differences in chinning frequency for females were significantly repeatable within each of the three age classes, (preweaning, juvenile, and sexual maturity), and repeatability within age classes increased at each developmental stage. However, males only showed significant repeatability in the frequency of chinning during the juvenile and the adult stage (Table 1; Figure S2).

3.1.2 | Consistency across age classes

In females, there was low but significant repeatability of individual differences in chinning between the preweaning and juvenile stages, and high repeatability between the juvenile and sexual maturity stages. In males, however, significant consistency was only found between the juvenile and sexual maturity stages (Table 2; Figure S3). The repeatability of individual differences in chinning between the juvenile and the sexual maturity stages did not differ significantly between females ($R = .67$, $CI_{95\%} = [0.48, 0.83]$) and males ($R = .45$, $CI_{95\%} = [0.11, 0.69]$), as evidenced by the overlapping 95% confidence intervals.

3.2 | Association between early intra-litter variables and individual differences in the frequency of chinning at maturity

We evaluated whether early sibling interactions modulated individual differences in chinning frequency of littermates as adults. In general, the results did not show significant effects of relative starting body mass (LMM with 1000 permutations: $\beta = -0.24$, ± 0.13 SE, $p = .064$) or position in the litter huddle ($\beta = -0.079$, ± 0.13 SE, $p = .537$) on chinning at sexual maturity. However, a statistically significant association was found between body mass at birth and position in the litter huddle; lighter pups relative to their littermates occupied more peripheral positions ($\beta = +0.56$, ± 0.12 SE, $p < .001$).

4 | DISCUSSION

In this study, we report two new findings: evidence for stable individual differences among litter siblings in rabbits across development in the frequency of chin marking (chinning) from an early age, particularly in females, and an apparent dissociation of this from other, well-documented individual differences in morphology, physiology, and behavior among litter siblings during early development and into adult life. This is one of the few longitudinal studies in a medium size mammal to track the development of individual differences in a highly evolved and ethologically relevant behavior using replications within and across age classes (three replications for each of three age classes, a total of nine replications for each individual; cf. Cabrera et al., 2021). In a continuing line of work (reviewed in Zepeda et al., 2019), we have focused on how relations among litter siblings in polytocous altricial mammals, mainly in the European rabbit but also in various rodent and feline species, can contribute to the development of individual differences from an early age. In this study, we predicted that in rabbits, litter siblings would show consistent individual differences in the frequency of chinning—a notable part of the rabbit's complex system of chemical communication (Bell, 1980; Hayes et al., 2002a,2002b; Mykytowycz, 1962, 1965; Schalken, 1976)—across development, and that this would be associated with early individual differences in litter siblings' morphology, physiology, and behavior. Specifically, we predicted that larger, faster growing siblings occupying the better-insulated central positions in the litter huddle (reviewed in Zepeda et al., 2019) would show higher frequencies of chinning, possibly as an advertisement of their better physical condition or fitness. However, although we found the same associations between body mass at birth, position in the litter huddle and by extrapolation, also with milk intake and weight gain, as in previous studies, we failed to find significant associations between these measures and individual differences in chinning frequency among litter siblings across development. This dissociation between the development of individual differences in chinning frequency and other measures of early development was the case despite the results of chinning frequency corresponding well in several respects to previous studies. González-Mariscal et al. (1992) also reported the early emergence of chinning in juveniles and obtained similar values for males and females at sexual maturity. Large and stable individual differences in adults were also found by Arteaga et al. (2008), González-Mariscal et al. (1990), Hudson and Vodermeyer (1992), and Martínez-Gómez et al. (1997).

Nevertheless, the mechanisms underlying the emergence during development of individual differences in the frequency of chinning and the persistence of such differences into adult life are not clear. Chinning frequency and the size, histological characteristics and secretory activity of the chin glands in adults of both sexes are strongly influenced by the endogenous secretion, and experimental reduction or experimental administration, of steroid hormones (chinning frequency: Chirino et al., 1993; González-Mariscal et al., 1990,1992,1993; Hudson et al., 1990; Hudson & Vodermeyer, 1992; Martínez-Gómez et al., 1997; chin gland morphology: Cerbón et al., 1996; Wales & Ebling, 1971). However, how the action of steroid hormones relates to the emergence of enduring individual differences in juveniles of either sex well before sexual maturation is presently unclear. An additional consideration is that in this induced ovulator, adult females kept under standard breeding conditions as was the case in the present study (see *2.1*), remain in a state of persistent estrus (Bakker & Baum, 2000; Hudson & Vodermayer, 1992; Myers & Poole, 1962).

As has been noted in the literature, the performance of chinning is affected also by psychological factors such as familiarity with the test situation or the presence of scent marks of conspecifics, not necessarily related to the action of steroids (Black-Cleworth & Verberne, 1975; González-Mariscal et al., 1990; Hudson & Vodermeyer, 1992; Martínez-Gómez et al., 1997). This suggests the need to look for broader, more general underlying mechanisms such as individual differences in arousal and the response to stressful situations and associated physiological responses such as activation of the hypothalamic-pituitary-adrenal and/or the hypothalamic-pituitary-gonadal axes in accounting for individual differences in chinning or indeed other behaviors (cf. Koolhaas et al., 2010). Also needing future consideration is whether the number of chin marks is the best or sufficient measure of an individual's chin marking performance. The manner in which animals mark, such as the strength, duration and angle of the chin when placing each mark, and the chemical composition of individuals' secretions—the "quality" of the secretions—is also likely to be important (Hudson & Vodermayer, 1992).

We conclude that despite the large body of information on chin-marking behavior in the rabbit and in relation to underlying hormonal mechanisms, an understanding of the developmental origins and functional significance of the stable and sometimes considerable individual differences in the performance of this behavior requires further investigation.

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CONFLICT OF INTERESTS

None.

ETHICAL APPROVAL

Animals were kept and treated according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA, to the National Guide for the Protection, Care and Use of Laboratory Animals, Mexico (Norma Oficial Mexicana NOM-062–200–1999), and the regulations of the Bioethics and Academic Integrity Committee of the CTBC (CICUAL-ID-6311).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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V. Discusión general

En este trabajo hemos investigado el desarrollo de las diferencias individuales en crías de conejo doméstico, pues gracias al robusto patrón de conducta maternal, en donde la madre está prácticamente ausente de manera natural y las crías crecen casi exclusivamente en compañía de sus compañeros de camada (Hudson y cols. 2011), proporcionan un modelo adecuado para evaluar si las interacciones tempranas con sus compañeros de camada, al ser parte importante de su ambiente temprano, influyen sobre rasgos conductuales y fisiológicos de los individuos, a corto, mediano y largo plazo.

Específicamente, en el presente trabajo evaluamos el efecto de la presencia de los hermanos sobre el desarrollo de diferencias individuales en dos rasgos fundamentales en esta especie. Primero, evaluamos si las interacciones tempranas entre compañeros de camada podrían modular el inicio de la endotermia homeotermica, ya que como se ha descrito anteriormente, el desarrollo e inicio de la endotermia homeotermica varía en función de distintos factores (Andreasson y cols. 2016, Robertson y cols. 2019). La regulación de la temperatura corporal es una función esencial para el mantenimiento de la homeostasis, en particular para las crías altriciales, ya que carecen de una capa aislante de pelo, tienen un área relativamente grande de exposición a la pérdida del calor en relación a su volumen y un control vasomotor inmaduro (revisado en Blumberg y Sokoloff 1998). Además, cuando las crías altriciales experimentan temperaturas debajo de su zona termoneutral, se enfrentan a la disyuntiva de invertir energía para mantener su temperatura corporal o derivarla al crecimiento (Bautista y cols. 2003, 2013).

Nuestros hallazgos muestran que las interacciones tempranas entre hermanos de camada explican en parte el origen de las diferencias individuales en las capacidades termorreguladoras individuales. Encontramos que las diferencias individuales en masa corporal al día postnatal 1 modulan la habilidad de las crías para mantener la temperatura corporal estable cuando son expuesta de manera individual a temperaturas por debajo de su zona termoneutral, sobre todo los primeros siete días postnatales, pues a medida que crecen, el efecto de la masa corporal van disminuyendo, probablemente debido al desarrollo del tejido muscular que permitiría a los individuos mantener su temperatura corporal estable por medio de mecanismos como la termogénesis por tiriteo, asi como la presencia de una capa aislante de pelo.

Estos resultados indican que las crías ligeras de la camada en el día postnatal 1, desarrollan más tardíamente sus capacidades termorreguladoras, lo cual podría indicar que el retraso en el inicio de la endotermia homeotérmica en las crías ligeras les proporciona un beneficio en términos de energéticos, puesto que el establecimiento de la endotermia marca la independencia fisiológica de la cría, pero a su vez, implica un *trade-off* entre la energía destinada a crecimiento y la que se canaliza para la homeostasis térmica (Brown y Downs 2002).

Asimismo, encontramos que las crías que muestran mayor masa corporal en el día postnatal 1, ocupan posiciones centrales dentro del agrupamiento, y tienen mayor ganancia en más corporal, también muestran un mayor crecimiento del pelo, que les proporciona mayor aislamiento térmico (revisado en Liwanag y cols. 2012).

Estos resultados indican que las interacciones tempranas entre compañeros de camada, en función de su masa corporal relativa, influyen en el desarrollo de sus capacidades termorreguladoras individuales, las cuales podrían representar una medida de individualidad con posibles consecuencias a mediano y largo plazo sobre la supervivencia, y el fenotipo metabólico de los individuos (Juárez-Ramírez y cols. En preparación).

De igual manera, evaluamos el efecto de las interacciones tempranas entre compañeros de camada sobre las diferencias individuales en la conducta de marcaje del mentón, una parte del sistema de comunicación química en el conejo (Arteaga y cols. 2008) que permite a los individuos transmitir información sobre su estado social y reproductivo (Mykytowycz 1968, González-Mariscal y cols. 1990) a través de moléculas odoríferas que depositan sobre objetos que encuentran en el ambiente (Hayes y cols. 2003).

Sorpresivamente, nuestras variables predictoras, tamaño de camada, masa corporal al nacimiento, posición en el agrupamiento y ganancia en peso al destete, no explicaron las diferencias individuales en la frecuencia de marcaje. Esto posiblemente se debe a que tal conducta comienza a establecerse como rasgo de individualidad a medida que los individuos alcanzan la madurez sexual. Además, al ser parte de la comunicación química, podrían, en un contexto social (Kent y cols. 2008), estar asociadas a experiencias de vida más tardías o bien, a la función biológica propia de la conducta, que hasta ahora no ha sido claramente descrita.

Adicionalmente, los resultados obtenidos, replican el efecto de la posición intrauterina y el tamaño de camada sobre la masa corporal al nacimiento (Bautista y cols. 2015a). A su vez, los resultados muestran que la masa corporal al nacimiento condiciona la posición que ocupan las crías dentro del agrupamiento que forman para termorregular (ver introducción), a mayor masa corporal las crías ocupan posiciones más centrales y ello les confiere ventajas en la competencia por recursos sobre las crías que ocupan posiciones periféricas (Bautista y cols. 2015b), dando como resultado mayor ganancia en masa (Bautista y cols. 2013). Estos resultados confirman y replican hallazgos previos de la importancia de las interacciones entre hermanos de camada en la formación de diferencias individuales en la masa corporal al destete (Zepeda y cols. 2019). En resumen, hasta ahora nuestros hallazgos nos permiten conocer algunos de los factores del ambiente de desarrollo temprano, asociados a las interacciones entre compañeros de camada, que predicen las trayectorias en las historias de vida de los individuos con posibles efectos tanto a corto como a largo plazo sobre su supervivencia y adecuación.

VI. Conclusión

El estudio del desarrollo de diferencias individuales en fisiología y conducta de los mamíferos, particularmente en crías de conejo doméstico, revela la influencia significativa de las interacciones entre hermanos de camada. La masa corporal al nacimiento y la posición en el agrupamiento son determinantes en el desarrollo individual, con implicaciones en la competencia por recursos y en el crecimiento.

Estos resultados no solo amplían nuestra comprensión sobre las trayectorias de vida de las crías del conejo doméstico, sino que también subrayan la importancia de la presencia de los hermanos durante el desarrollo temprano en la formación de características individuales con repercusiones a corto, a mediano y a largo plazo.

VII. Perspectivas

Capítulo 1

1. Determinar las diferencias individuales en la zona termoneutral de las crías de conejo doméstico y relacionarlo con interacciones tempranas entre hermanos de camada.
2. Correlacionar las diferencias individuales en el inicio de la endotermia homeotérmica con posibles diferencias individuales en la tasa metabólica de los individuos a mediano y largo plazo.

Capítulo 2

1. Evaluar cómo las diferencias individuales en conducta de marcaje del mentón se correlacionan con la supervivencia y reproducción de los individuos.
2. Evaluar cómo se correlacionan las posibles diferencias individuales en la intensidad del marcaje del mentón con las diferencias individuales estables en la frecuencia de marcaje.

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IX. Participaciones en congresos

Factores que modulan las diferencias individuales en el inicio de la endotermia en crías del conejo doméstico: efectos a nivel intracamada y poblacional. Fernández Y, Reyes-Meza V, García-Torres E, Hudson R, Bautista A. XXV Curso Internacional de Bases Biológicas de la Conducta. Tlaxcala, México. Octubre 2020 (Poster).

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