TESIS DOCTORAL

Repercusiones de la restricción alimentaria durante el primer tercio de gestación sobre los parámetros fisiológicos y los rendimientos de la vaca nodriza y su descendencia

AGUSTÍ NOYA CLAVÉ

Diciembre 2019

TESIS DOCTORAL

REPERCUSIONES DE LA RESTRICCIÓN ALIMENTARIA DURANTE EL PRIMER TERCIO DE GESTACIÓN SOBRE LOS PARÁMETROS FISIOLÓGICOS Y LOS RENDIMIENTOS DE LA VACA NODRIZA Y SU DESCENDENCIA

Memoria presentada por **Agustí Noya Clavé** para optar al grado de Doctor con Mención Internacional por la Universidad de Zaragoza

Diciembre 2019

CERTIFICACIÓN DE LAS DIRECTORAS DE TESIS

Albina Sanz Pascua e **Isabel Casasús Pueyo**, Doctoras en Veterinaria e Investigadoras de la Unidad de Producción y Sanidad Animal del Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón,

HACEN CONSTAR

Que **Agustí Noya Clavé** ha realizado bajo nuestra dirección los trabajos correspondientes a su Tesis Doctoral titulada "**Repercusiones de la restricción alimentaria durante el primer tercio de gestación sobre los parámetros fisiológicos y los rendimientos de la vaca nodriza y su descendencia**", que corresponde con el proyecto de Tesis aprobado por la comisión de Doctorado, y que cumple con los requisitos exigidos para optar al grado de Doctor con Mención Internacional por la Universidad de Zaragoza, por lo que autorizan su presentación por compendio de publicaciones para que pueda ser juzgada por el Tribunal correspondiente.

Lo que suscribimos como directoras del trabajo, en Zaragoza, a 20 de diciembre de 2019

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Esta Tesis Doctoral se ha realizado en el Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón en el marco del siguiente proyecto de investigación:

"Efectos de la alimentación materna sobre el desarrollo embrionario y la descendencia: implicaciones en la eficiencia productiva de la vaca nodriza". Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, RTA2013-00059-C02-01). Investigadora responsable: Albina Sanz.

El doctorando ha disfrutado de un contrato concedido por el CITA el 5 de noviembre de 2014 en el marco del proyecto de investigación "Caracterización zootécnica, genética y calidad de la canal y de la carne de la población bovina Serrana de Teruel. FITE 2013" (ref. CITA: 451-A), y de un contrato predoctoral financiado por el INIA el 10 de mayo de 2016 asociado al proyecto "Efectos de la alimentación materna sobre el desarrollo embrionario y la descendencia: implicaciones en la eficiencia productiva de la vaca nodriza", publicado en la convocatoria del 13 abril de 2015 (BOE 30 abril de 2015).

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JUSTIFICACIÓN DE LA CONTRIBUCIÓN DEL DOCTORANDO A LAS PUBLICACIONES DE LA TESIS

Albina Sanz Pascua e **Isabel Casasús Pueyo**, Doctoras en Veterinaria e Investigadoras de la Unidad de Producción y Sanidad Animal del Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón, como Directoras de la Tesis Doctoral titulada "Repercusiones de la restricción alimentaria durante el primer tercio de gestación sobre los parámetros fisiológicos y los rendimientos de la vaca nodriza y su descendencia",

INFORMAN de que:

- El doctorando Agustí Noya Clavé ha contribuido al desarrollo metodológico, la recogida y análisis de datos y muestras, el análisis estadístico, la elaboración y discusión de los resultados, y la redacción de las Publicaciones 1, 2, 4 y 5 de la presente Tesis Doctoral.

- El doctorando Agustí Noya Clavé ha contribuido al desarrollo metodológico, la recogida y análisis de datos y muestras, la elaboración y discusión de los resultados, y la redacción de la Publicación 3 de la presente Tesis Doctoral.

Lo que suscribimos como directoras del trabajo, en Zaragoza, a 20 de diciembre de 2019

Albina Sanz Pascua

Isabel Casasús Pueyo

Esta Tesis Doctoral es la suma de cada una de aquellas pequeñas piedras que, colocadas con esmero, han ido dibujando el camino para poder llegar hasta aquí. Un camino de largo recorrido, sinuoso, no exento de avatares y donde la línea recta, no siempre ha sido la distancia más corta entre dos hitos. Pero precisamente la perseverancia y el esfuerzo requeridos realza el trabajo de todos aquellos que, en menor o mayor medida, han contribuido a su fin. En estas líneas es donde de forma simbólica, me gustaría dejar constancia de mi agradecimiento.

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Resumen / Summary

RESUMEN

Esta Tesis Doctoral se planteó para evaluar las repercusiones a corto, medio y largo plazo de una restricción alimentaria en vacas nodrizas durante el primer tercio de gestación. Para ello, se analizaron los efectos de la subnutrición sobre los parámetros productivos y reproductivos de las vacas y su descendencia en las razas Parda de Montaña y Pirenaica. Durante las fases de gestación y la siguiente lactación se estudiaron los rendimientos del conjunto vaca-ternero hasta el destete. Posteriormente se procedió a la recría de las hembras y se analizaron sus rendimientos hasta el final de su primera lactación.

Al inicio del ensayo se sincronizaron e inseminaron 115 vacas adultas que estaban criando a un ternero, y se dividieron en dos grupos en función de la alimentación que recibirían durante los primeros 82 días de gestación. El grupo control recibió una dieta que cubrió el 100% de sus necesidades de gestación, lactación y mantenimiento, mientras que el grupo subnutrido recibió una dieta que cubrió únicamente el 65% de sus necesidades. Finalizado el primer tercio de gestación, se destetaron los terneros y todos los animales implicados en el ensayo se alimentaron con una dieta que cubría el 100% de sus necesidades.

Al finalizar el periodo de restricción alimentaria, las vacas subnutridas presentaron menor peso y condición corporal que las vacas del grupo control. La subnutrición disminuyó el crecimiento de los terneros que estaban criando y alteró los perfiles metabólicos y endocrinos de las vacas. Se observó un incremento en la concentración plasmática de ácidos grasos no esterificados (AGNE) y una reducción de la concentración del factor de crecimiento similar a la insulina 1 (IGF-1), especialmente en la raza Pirenaica, que mostró una mayor sensibilidad a la restricción alimentaria. Sin embargo, la subnutrición no afectó a los parámetros reproductivos de las vacas, posiblemente debido a la óptima condición corporal que presentaban los animales en el momento de la inseminación artificial. No hubo diferencias en la tasa de fertilidad, ni en los parámetros relacionados con el reconocimiento materno-fetal y el mantenimiento de la gestación, como la expresión de genes estimulados por el interferón tau y las concentraciones plasmáticas de progesterona y proteína específica de preñez B (PSPB).

Las vacas subnutridas presentaron un peso al parto y una duración del anestro postparto similares a los de las vacas del grupo control. No obstante, las vacas subnutridas tuvieron una menor condición corporal al parto, hecho que repercutió en la mayoría de los parámetros productivos de las vacas y los terneros durante la lactación. La subnutrición materna durante la gestación temprana tuvo consecuencias a largo

plazo en el fenotipo de la descendencia. Los terneros procedentes de madres subnutridas sufrieron una alteración de sus parámetros fisiológicos al nacimiento, con una mayor concentración plasmática de cortisol, una menor concentración de IGF-1 y un retraso en la maduración del sistema hematopoyético. No obstante, no hubo diferencias en el peso y la vitalidad de los terneros al nacimiento. La restricción alimentaria disminuyó la concentración de inmunoglobulinas G en el calostro de las vacas Pirenaicas, pero no afectó a la transferencia de inmunidad pasiva, ya que todos los terneros presentaron concentraciones plasmáticas de inmunoglobulinas G y M similares. A pesar de no haber diferencias en la producción de leche entre las vacas Pirenaicas al inicio de lactación, los terneros Pirenaicos procedentes de madres subnutridas tuvieron una menor capacidad de ingestión que sus homólogos del grupo control. No se encontraron diferencias en el crecimiento de estos terneros durante la primera semana de vida, pero al destete los terneros Pirenaicos de madres subnutridas tuvieron un peso un 19% inferior al de sus homólogos del grupo control. Los parámetros metabólicos y endocrinos de los terneros también se vieron afectados por la subnutrición materna, destacando una menor concentración plasmática de IGF-1 durante los dos primeros meses de lactación en los terneros Pirenaicos procedentes de madres subnutridas.

Tras el destete a los 4 meses de lactación, las hembras se recriaron para sincronizarlas e inseminarlas a los 16 meses de edad. Las diferencias en el crecimiento observadas durante la lactación desaparecieron a partir de los 12 meses de edad (edad media de entrada a la pubertad para todos los grupos). Esto indicó que las terneras procedentes de madres subnutridas aceleraron su crecimiento en los meses previos a la entrada a la pubertad, comprometiendo su estado metabólico con mayores concentraciones plasmáticas de AGNE, colesterol y urea a los 12 meses de edad. La subnutrición materna pudo haber afectado el desarrollo embrionario de las gónadas durante la gestación temprana, ya que las novillas que procedían de madres subnutridas presentaron un menor recuento de folículos antrales en el momento de la inseminación. No obstante, no se encontraron diferencias en la tasa de fertilidad, ni en el peso al nacimiento y crecimiento de sus terneros durante la siguiente lactación. En todo caso, serán necesarios estudios posteriores para determinar las repercusiones de la subnutrición materna en los rendimientos productivos y reproductivos de estas novillas en su vida adulta.

La restricción alimentaria durante el primer tercio de gestación no solo repercutió en los parámetros productivos de la madre, sino que también afectó a la fisiología y a los rendimientos de la siguiente generación. A la vista de los resultados obtenidos en esta Tesis Doctoral, es necesario garantizar una alimentación adecuada a las necesidades de la vaca nodriza durante las primeras etapas de la gestación.

SUMMARY

The aim of this PhD Thesis was to analyze the short-, medium- and long-term effects of a nutritional restriction during the first third of gestation in suckler cows. To this purpose, the effects of undernutrition on productive and reproductive traits of cows and their progeny were analyzed in Parda de Montaña and Pirenaica breeds. The performance of the cow-calf pairs was assessed during the gestational period and the following lactation. After that, heifers were reared and their performance until the end of their first lactation period was analyzed.

At the beginning of the study, 115 adult cows rearing a calf were synchronized and inseminated and, after that, they were distributed into two nutritional groups during the first 82 days of pregnancy. The control group was fed a diet that met 100% of the gestation, lactation and maintenance requirements, whereas the undernourished group received a diet that only met 65% of its requirements. After the first third of gestation, the calves were weaned and all the animals involved in our study were fed a diet to meet 100% of their requirements.

At the end of the nutritional treatment, cows from the undernourished group had lower weight and body condition score than cows from the control group. Undernutrition decreased the growth rate of the reared calves and affected the metabolic and endocrine profiles of cows. Undernourished cows increased non-esterified fatty acids (NEFA) and decreased plasma insulin-like growth factor 1 (IGF-1) concentrations, especially in the Pirenaica breed, which showed a greater sensitivity to a nutritional restriction. However, undernutrition had no effects on the cow reproductive traits, possibly due to the optimal body condition score of cows at artificial insemination. No differences were found between groups in fertility rate or indicators related to maternal recognition and maintenance of pregnancy, such as interferon-tau stimulated gene expression and progesterone and pregnancy-specific protein B (PSPB) concentrations.

Undernourished cows had similar live weight at calving and postpartum anestrus length than control cows. However, undernourished cows had lower body condition score at calving, which affected most of the cow-calf parameters throughout the lactation period. Early maternal undernutrition had long-term effects on the progeny phenotype. Calves from undernourished cows had altered physiological response at birth, with higher cortisol and lower plasma IGF-1 concentrations, and a later maturation of their hematopoietic system. However, no differences were found in live weight and vitality of calves at birth. Undernutrition decreased the colostrum concentration of immunoglobulins (Ig) G in Pirenaica cows. Despite this, the passive transfer of immunity was not affected because all calves had similar plasma concentrations of Ig G and M. Whereas no differences in milk yield were found between Pirenaica cows at the

beginning of lactation, Pirenaica calves from undernourished cows had lower milk intake capacity than their control counterparts. No differences were found regarding the calf growth during the first week of life, but Pirenaica calves from undernourished cows had a 19%-reduction in their weaning weight in comparison to their control counterparts. Metabolic and endocrine parameters of calves were also affected by undernutrition, particularly reflected in the lower plasma IGF-1 concentration in Pirenaica calves from undernourished cows during the first two months of lactation.

After calf weaning at 4 months of age, female calves were reared to be synchronized and inseminated at 16 months of age. The growth differences observed in calves during lactation disappeared from 12 months of age (mean age of onset of puberty for all groups). This indicated that heifers from undernourished cows accelerated their growth rates during the months before puberty, compromising their metabolic profiles with higher NEFA, cholesterol and urea concentrations at 12 months of age. Maternal undernutrition could have affected the embryonic development of the heifers' gonads, as heifers from undernourished cows had a reduced antral follicle count at artificial insemination. However, no differences were found in fertility rate neither in birth weight and growth rate of heifers' progeny. Further research is needed to assess the long-term effects of maternal undernutrition on productive and reproductive performance of these heifers during their adult life.

A nutritional restriction during the first third of pregnancy not only affected the cow productive performance, but it also influenced the physiology and the performance of the progeny. From the results obtained in this study, it is necessary to guarantee an adequate nutrition to meet the suckler cow requirements during the first stages of the gestation.

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PUBLICACIÓN 5. Effects of developmental programming caused by maternal nutrient intake on postnatal performance of beef heifers and their calves.

LISTA DE ABREVIATURAS

ADF	Acid detergent fiber
ADG	Average daily gain
ADL	Acid detergent lignin
ADN	Ácido desoxirribonucleico
AGNE	Ácidos grasos no esterificados
AI	Artificial insemination
a.s.l.	Above sea level
BCS	Body condition score
BW	Body weight
CC	Condición corporal
CITA	Centro de Investigación y Tecnología Agroalimentaria
CONTROL	Relativo a las vacas que fueron alimentadas al 100% de sus necesidades durante el 1er tercio de gestación
CP	Crude protein
CV	Coefficient of variation
d	Day / día
DM	Dry matter
DNA	Deoxyribonucleic acid
ECM	Energy corrected milk
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EM	Energía metabolizable
FTAI	Fixed-time artificial insemination
GLM	General linear model
GnRH	Gonadotropin-releasing hormone / hormona liberadora de gonadotropina
h	Hora
ha	Hectárea
IA	Inseminación artificial
IATF	Inseminación artificial a tiempo fijo
IFN-τ	Interferón tau
Ig	Immunoglobulin / inmunoglobulina
IGF-1	Insulin-like growth factor 1 / factor de crecimiento similar a la Insulina 1
INIA	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria
ISGs	Interferon-stimulated genes / genes estimulados por el interferón
LH	Luteinizing hormone / hormona luteinizante
LSMEANS	Least-square means / medias mínimas cuadráticas

LW	Live weight
MAPA	Ministerio de Agricultura, Pesca y Alimentación
MBW	Mature body weight
ME	Metabolizable energy
min	Minute / minuto
MIXED	Mixed linear model
MS	Materia seca
m.s.n.m.	Metros sobre el nivel del mar
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
P	Probabilidad de error
PA	Parda de Montaña
PAG	Pregnancy-associated glycoproteins / glicoproteínas asociadas a la preñez
PB	Proteína bruta
PBMC	Peripheral blood mononuclear cells / células mononucleares de sangre periférica
PGF_{2a}	F_{2a} prostaglandin / prostaglandina F_{2a}
PI	Pirenaica
PMSG	Pregnant mare serum gonadotropin / gonadotropina sérica de yegua preñada
PPA	Postpartum anestrus
PRID	Progesterone releasing intravaginal device / dispositivo intravaginal liberador de progesterona
PSPB	Pregnancy-specific protein B / proteína específica de preñez B
PV	Peso vivo
r	Coeficiente de correlación de Pearson
RSD	Residual standard deviation
SAGAS	Grupo de investigación en Sistemas Agroganaderos Sostenibles
SED	Standard error of the difference
SUBNUT	Relativo a las vacas que fueron alimentadas al 65% de sus necesidades durante el 1^{er} tercio de gestación
TAI	Timed artificial insemination
UE	Unión Europea
UEECA	Unión de Entidades Españolas de Ciencia Animal
vs	Versus

1. Introducción

1. INTRODUCCIÓN

1.1. Situación actual del bovino de carne

En España el sector vacuno de carne representa un 17,5% de la producción final ganadera, únicamente por detrás del sector porcino. Desde 2015, el número de cabezas sacrificadas en España ha ido aumentando, reflejando una mejora de los índices productivos en el país. En 2018, se exportaron un 26% más de animales vivos respecto al año anterior, no obstante, el número de animales vivos importados aumentó un 2,2% respecto al 2017 (MAPA, 2019a). A pesar de estos buenos indicadores productivos, sigue siendo necesaria la importación de animales procedentes de otros países para satisfacer las necesidades de mercado. Por otro lado, el coste de la alimentación de los animales sigue una tendencia creciente, con un incremento global del 0,6% respecto a noviembre del 2015 (MAPA, 2019b).

Actualmente el censo de vacuno en España es de 6.660.168 animales, correspondiendo el 34% a vacas nodrizas mayores de 24 meses (MAPA, 2019a). La tasa de fertilidad media de la vaca nodriza en 2017 fue del 71%, con un intervalo entre partos de 440 días. En ese año, el 2% de las vacas nodrizas mayores de 4 años todavía no había registrado ningún parto (MAPA, 2018), resultados que distan del objetivo técnico de poder destetar un ternero por vaca y año.

Estos datos permiten concluir que España tiene una gran capacidad de producción de vacuno de carne, lo que permite destinar parte de su producción a las exportaciones, no obstante, necesita importar animales procedentes de otros países. La necesidad de importar animales, junto con el incremento continuo del coste de la alimentación y los bajos rendimientos reproductivos de la vaca nodriza, ponen de manifiesto la necesidad de implementar mejoras para tecnificar el sector y maximizar su eficiencia.

1.2. Las razas autóctonas del Pirineo

La Parda de Montaña y la Pirenaica son dos razas autóctonas de vacuno de carne mayoritarias en el Pirineo aragonés, adaptadas al sistema de ganadería semiextensiva utilizada tradicionalmente en esta zona. La Parda de Montaña es una raza que proviene de la selección para producción cárnica de la antigua Parda Alpina, mientras que la Pirenaica es una raza rústica autóctona de los Pirineos caracterizada por su adaptación a las condiciones de montaña y su conformación cárnica. Estas dos razas tienen una conformación parecida, con un peso adulto aproximado de 580 kg (Casasús et al., 2002). La Parda y la Pirenaica tienen unos rendimientos productivos

similares en animales adultos o de reposición (Casasús et al., 2004) o durante el cebo (Blanco et al., 2009), pero tienen diferencias interraciales, como es el menor peso al nacimiento de los terneros Pirenaicos (Casasús et al., 2002), la mayor producción de leche de las vacas Pardas (Sanz et al., 2003) o los mayores rendimientos durante la lactación y pesos al destete de los terneros Pardos (Villalba et al., 2000). Hay estudios que describen diferencias entre estas dos razas en cuanto a los mecanismos de adaptación y respuestas fisiológicas establecidas cuando son sometidas a diferentes tipos de manejo (Garcia-Belenguer et al., 1996; Álvarez-Rodríguez et al., 2010). Estas diferentes estrategias fisiológicas podrían condicionar también, en función de la raza y su base genética, las consecuencias que puede tener una restricción alimentaria durante la gestación en el feto y el futuro ternero (Fontes et al., 2019).

1.3. Manejo actual de las vacas nodrizas en las zonas de montaña

La adaptación del sector a las Políticas Agrarias impuestas por la UE junto con el objetivo de reducir los elevados costes de alimentación ha hecho que las explotaciones adopten cada vez condiciones más extensivas para el manejo del ganado.

En un sistema semiextensivo, basado en el aprovechamiento de recursos pastorales, los animales van a permanecer largos periodos de tiempo de forma no estabulada, ya sea aprovechando los pastos de alta montaña en los puertos durante los meses de verano, los pastos intermedios en zonas boscosas durante los meses de otoño y primavera, o las praderas de fondo de valle en los meses más fríos (Figura 1). Debido a la marcada estacionalidad climatológica a lo largo del año, diferentes factores ambientales influirán tanto en la cantidad como en la calidad de los recursos pastorales. En la mayoría de casos, la disponibilidad de alimento en el medio será la única fuente de alimentación, siendo frecuentes periodos de subnutrición o malnutrición que condicionarán los rendimientos del animal a lo largo de su ciclo productivo.

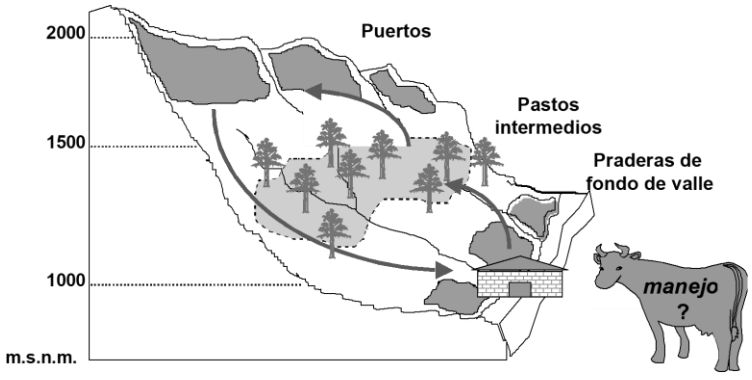

Figura 1. Manejo de los rebaños de vacas nodrizas en las zonas de montaña.

En Aragón, la mayoría de los partos de las vacas nodrizas se registran durante los meses de marzo – abril (MAPA, 2018), lo que significa que la gestación se habrá iniciado durante los meses de junio - julio del año anterior, cuando la mayoría de estos animales se encuentra en los puertos de alta montaña y su alimentación dependerá exclusivamente de la cantidad de pasto disponible.

1.4. La nutrición en el ciclo productivo de la vaca nodriza

Como se ha señalado anteriormente, la productividad de los rebaños de vacas nodrizas está muy condicionada al manejo alimentario que reciben los animales a lo largo de su ciclo productivo. Estudiar y conocer las repercusiones de una restricción alimentaria es fundamental para mejorar sus índices de producción.

Los efectos de la subnutrición a partir de la segunda mitad de gestación o durante la lactación han sido ampliamente estudiados en el vacuno de carne (Shell et al., 1995; Spitzer et al., 1995; Martin et al., 1997; Sanz et al., 2004). En parámetros reproductivos, un periodo de subnutrición durante la gestación tardía o el periparto afectará a la duración del anestro postparto de la vaca (Meikle et al., 2004; Sanz et al., 2004), la tasa de fertilidad en la siguiente época de reproducción, así como la calidad del embrión y su viabilidad (Diskin et al., 2003). El estado metabólico de un animal, reflejado en su peso y condición corporal (CC), juega un papel clave en su fisiología reproductiva (Diskin et al., 2003; D'Occhio et al., 2019), pudiendo afectar al desarrollo del folículo dominante, la calidad del oocito y a la secreción de hormona luteinizante (LH) que desencadenará la ovulación. Además, durante el último tercio de gestación se produce el 75% del crecimiento fetal. Una restricción alimentaria en esta fase podrá tener consecuencias en el desarrollo corporal del feto, pudiendo afectar al peso del ternero al nacimiento (Greenwood y Cafe, 2007; LeMaster et al., 2017) y a su crecimiento durante la lactación (Barker, 2007; Nathanielsz et al., 2007).

Sin embargo, a causa de la baja demanda metabólica que implica el desarrollo y mantenimiento del embrión o feto durante la gestación temprana, junto con la dificultad y complejidad de analizar sus repercusiones en el ternero recién nacido o durante su vida postnatal, pocos estudios se han centrado en analizar las consecuencias que puede tener la subnutrición peri-implantacional sobre los parámetros fisiológicos de la madre y la descendencia a corto, medio y largo plazo. La gestación temprana coincidirá en la mayoría de los casos con la fase de lactación, en la que se producirá una importante movilización de reservas corporales para hacer frente a la alta demanda metabólica que se produce durante este periodo. Los nutrientes y su energía irán destinados al mantenimiento del propio animal, a la producción de leche y al

desarrollo de la placenta y del embrión o feto. En esta fase se producirán las primeras etapas del desarrollo embrionario, con cambios sustanciales que condicionarán la diferenciación celular del embrión y que serán determinantes para su vida postnatal (Rhind, 2004; Reik, 2007).

1.4.1. Implicaciones de la subnutrición durante el primer tercio de gestación sobre el reconocimiento materno y el mantenimiento de la gestación

El primer tercio de gestación es un periodo crítico para el desarrollo y viabilidad del feto en el que una situación de estrés puede alterar los parámetros fisiológicos de la madre y tener consecuencias nefastas para el correcto desarrollo de la gestación. En este aspecto, una restricción alimentaria después de la ovulación podría alterar el microambiente del oviducto y del útero, el reconocimiento materno y el mantenimiento de la gestación, incrementando el riesgo de pérdidas embrionarias (Block et al., 2011).

En el ganado bovino, la implantación del concepto (embrión y sus membranas adyacentes) y el reconocimiento materno de la gestación se producen aproximadamente a partir de la tercera semana post-concepción, siendo dos puntos críticos para el mantenimiento de la gestación y la supervivencia del embrión (Hopper, 2015).

Durante el periodo peri-implantacional, el embrión anuncia su presencia en el lumen uterino mediante señales moleculares para evitar el comienzo de un nuevo ciclo estral, inhibiendo la secreción de prostaglandina $F_{2\alpha}$ ($PGF_{2\alpha}$) y la lisis del cuerpo lúteo. El mantenimiento de los niveles de progesterona implica la regulación de la expresión de ciertos genes y la activación de diferentes mecanismos celulares para estimular la receptividad uterina y modular los mecanismos inmunitarios para la correcta implantación y supervivencia del embrión (Spencer et al., 2016). Una restricción alimentaria podría disminuir la concentración plasmática de progesterona secretada por el cuerpo lúteo, incrementando el riesgo de pérdidas embrionarias (Sanz et al., 2004). Niveles más bajos de progesterona durante los primeros días post-concepción han sido asociados con embriones de menor tamaño y, por tanto, con menor capacidad para emitir señales moleculares para evitar la luteolisis (Mann y Lamming, 2001).

Las células trofoblásticas del embrión secretarán a partir de la segunda semana de gestación el interferón tau (IFN-τ), que inducirá en la madre cambios temporales en el propio endometrio y en los tejidos periféricos (Pugliesi et al., 2014; Ruhmann et al., 2017). La secreción de IFN-τ desencadenará la expresión de genes (ISGs) en diferentes tejidos maternos, como por ejemplo en las células sanguíneas. La cuantificación de la

expresión de estos genes en células mononucleares de sangre periférica (PBMC) de la madre se ha establecido como un buen biomarcador temprano para determinar, no solo el estado de preñez, sino también la viabilidad del embrión (Ahmad Sheikh et al., 2018). Las células trofoblásticas del embrión también secretarán durante toda la gestación un conjunto de glicoproteínas específicas de la preñez (PAG) (Serrano et al., 2009). Dentro de esta familia de glicoproteínas se encuentra la proteína específica de preñez B (PSPB) o PAG-1. A partir de la tercera semana de gestación, estas PAG podrán ser detectadas en la circulación sanguínea materna o en la leche y ser utilizadas como biomarcador de la gestación.

1.4.2. Implicaciones de la subnutrición materna durante el primer tercio de gestación sobre el embrión o feto

El periodo de gestación embrionario empieza en el momento que se produce la concepción hasta el final de la fase de diferenciación (aproximadamente el día 42), mientras que el periodo de gestación fetal comprende desde el día 42 hasta el parto (Bech-Sàbat et al., 2009).

Una subnutrición durante la gestación temprana puede afectar al desarrollo y crecimiento del propio embrión o feto, induciendo cambios irreversibles en sus etapas posteriores. Las primeras fases del desarrollo embrionario de los mamíferos son determinantes para su vida adulta, puesto que se producen cambios fundamentales en el ADN que condicionarán la diferenciación celular del embrión (Reik, 2007). La epigenética juega un papel clave en la diferenciación celular. A partir de modificaciones químicas en el ADN y en las proteínas que interaccionan con éste, se regula la expresión de genes a corto, medio y largo plazo sin alterar la secuencia del ADN. Mecanismos como la modificación de las histonas, el remodelado de la cromatina y la metilación del ADN determinan el patrón de expresión génica y, por tanto, la síntesis de proteínas y la diferenciación celular (Meissner et al., 2008). Durante la primera semana de gestación, las células totipotenciales (cada una de ellas capaz de generar un individuo completo y funcional) que conforman la mórula emperezarán a dividirse mitóticamente para formar el blastocisto. Éste estará formado por una capa externa de células denominada trofoectodermo, de la que derivarán las futuras membranas fetales, y una masa de células internas que formarán las tres capas germinales embrionarias: el endodermo, el mesodermo y el ectodermo. A partir de estas tres líneas germinales empezará la organogénesis, en la que se irán diferenciando y especializando grupos celulares para conformar los órganos y sistemas del futuro feto (Hopper, 2015). Durante esta etapa crítica de desarrollo, la estructura, fisiología y metabolismo de los diferentes órganos y sistemas podría verse afectada (Mossa et al.,

2015). La necesidad del embrión de adaptarse a las condiciones uterinas hace que se produzcan cambios moleculares y celulares para adaptar su fisiología al ambiente, modificando la expresión de genes (Fleming et al., 2012; Velazquez, 2015). Este mecanismo de adaptación se denomina programación fetal, y se define como el proceso por el que la nutrición intrauterina u otros estímulos alteran las vías de desarrollo prenatal, induciendo cambios estructurales, metabólicos o fisiológicos postnatales (Nathanielsz et al., 2007). En este aspecto, una nutrición materna deficiente durante las primeras etapas de desarrollo del embrión o feto influirá en la respuesta de sus mecanismos fisiológicos, que se reajustarán para poder hacer frente a esta escasa disponibilidad de nutrientes, estableciendo en el feto el que se denomina como "fenotipo ahorrador" (Hales y Barker, 2012; Priante et al., 2019). A medida que avanza la gestación, se irá reduciendo la gran plasticidad que presentan las primeras células embrionarias. De este modo, si durante el posterior desarrollo fetal o vida postnatal del nuevo individuo cambia la disponibilidad de nutrientes de su ambiente, los mecanismos fisiológicos desarrollados no se ajustarán a la disponibilidad real de nutrientes de su nicho ecológico. Con ello se incrementará el riesgo de padecer enfermedades crónicas a largo plazo como obesidad, enfermedades cardiovasculares o diabetes (Hales y Barker, 2001). Algunos estudios han relacionado una restricción en la dieta materna con una reducción del peso del feto, una alteración en la regulación del transporte de nutrientes (Jansson et al., 2006), una reprogramación fetal del eje IGF (Gallaher et al., 1998) y alteraciones en la vascularización del intestino (Meyer et al., 2010).

1.4.3. Implicaciones de la subnutrición materna durante el primer tercio de gestación sobre la placenta

La placenta juega un papel fundamental en el desarrollo del feto, ya que, además de proporcionar una barrera de protección, permitirá el paso de nutrientes y productos del metabolismo desde la circulación materna a la circulación fetal y viceversa (Casanello et al., 2015). La placenta también tiene una importante función en el reconocimiento materno de la gestación y la secreción de hormonas y factores de crecimiento. Como se ha descrito anteriormente, las células trofoblásticas de la placenta serán las encargadas de secretar el IFN-τ y las PAG. En el último tercio de gestación, la placenta tendrá también un papel fundamental en el mantenimiento de la gestación. Aproximadamente a partir del día 200 de gestación, se produce una reducción en el número de células viables que constituyen el cuerpo lúteo y será la placenta la principal fuente de progesterona extra ovárica (Shemesh, 1990).

En rumiantes la placenta es de tipo cotiledonaria, es decir, las vellosidades coriónicas (la unidad funcional de la placenta fetal) se agrupan en estructuras circulares

denominadas cotiledones, que contactarán con unas estructuras derivadas del endometrio uterino denominadas carúnculas. La íntima unión de estas dos estructuras constituirá un placentoma. En bovinos, entre 100 y 140 placentomas distribuidos por toda la placenta constituirán la superficie por dónde se producirá el intercambio de nutrientes y metabolitos entre la circulación fetal y materna (Haeger et al., 2016). A partir de su formación, la placenta irá sufriendo cambios estructurales para adaptarse a las crecientes necesidades metabólicas del feto. Una reducción en el volumen de tejido conectivo y espesor de la barrera placentaria (compuesta por las diferentes láminas que separan la circulación materna de la fetal), así como un incremento en la superficie de los placentomas permitirán incrementar su eficacia de intercambio a lo largo de la gestación (Estrella et al., 2017). En las primeras etapas de desarrollo de la placenta, el número de placentomas también se irá incrementando, pero permanecerá estable a partir de la segunda mitad de gestación (Laven y Peters, 2001). En este sentido, un periodo de subnutrición durante la gestación temprana podría afectar a la vascularización y funcionalidad de la placenta (Vonnahme et al., 2007), con importantes consecuencias en el desarrollo fetal e incluso en el peso al nacimiento (Robinson, 2017; Sultana et al., 2017). Existen diferentes estudios con resultados contradictorios de cómo un periodo de restricción alimentaria podría afectar al desarrollo de la placenta. Por un lado, algunos autores relacionaron una restricción de nutrientes durante la gestación temprana con una disminución en el peso (Zhu et al., 2007) y superficie (Long et al., 2009) de los placentomas. Otros estudios, en cambio, no observaron diferencias en el peso de los placentomas, pero sí un incremento en su superficie y en el peso de los cotiledones (Taylor, R. K. et al., 2015). Este incremento de peso y superficie podría estar relacionado con mecanismos de adaptación del propio individuo para incrementar la eficacia en la captación de nutrientes en un ambiente restringido (fenotipo ahorrador).

1.4.4. Implicaciones de la subnutrición materna durante el primer tercio de gestación sobre la vida postnatal de la descendencia

Una restricción alimentaria durante el desarrollo fetal puede tener consecuencias directas en el fenotipo de la descendencia e incrementar el riesgo de desarrollar enfermedades metabólicas durante la vida postnatal del individuo.

Varios estudios vinculan la subnutrición peri-implantacional o durante las primeras etapas de la gestación con alteraciones en la fisiología del animal adulto. Long et al. (2010b) describieron una alteración en la regulación de la concentración plasmática de glucosa en terneros procedentes de madres que habían sido subnutridas durante los primeros meses de gestación y posteriormente sobrealimentadas hasta el

parto. Además, estos terneros presentaron un menor peso de sus pulmones y tráquea, y un mayor tamaño de sus fibras musculares a los 16 meses de edad (Long et al., 2010a). En otro experimento, Long et al. (2012) relacionaron la subnutrición materna durante la primera mitad de la gestación con un incremento del tamaño de los adipocitos en terneros sacrificados a los 15 meses de edad. Mossa et al. (2013) describieron una disminución del número folículos antrales en los ovarios, y un incremento del diámetro aórtico y la presión arterial en aquellas terneras cuyas madres habían sido subnutridas durante el primer trimestre de la gestación.

En la especie ovina, Ford et al. (2007) relacionaron la subnutrición durante la primera mitad de gestación con una mayor ingesta de alimento, resistencia a la insulina y obesidad de los corderos. En línea con estos resultados, Gilbert et al. (2005) describieron una predisposición a la hipertensión arterial y una reducción en el número de nefronas en aquellos corderos alimentados *ad libitum* que procedían de madres subnutridas en la primera mitad de la gestación. Contrariamente a lo esperable, Muñoz et al. (2008) reportaron un mayor peso al nacimiento, concentración plasmática de inmunoglobulinas (Ig) G y tasa de supervivencia en corderos procedentes de ovejas restringidas nutricionalmente durante la gestación temprana.

La mayoría de los estudios coinciden en que una restricción de la alimentación materna durante las primeras etapas de gestación no repercutirá en aquellos parámetros relacionados con el desarrollo corporal del animal, como el peso al nacimiento y al destete, o el peso de la canal al sacrificio (Perry et al., 1999; Long et al., 2012) siempre y cuando después del periodo de restricción la madre tenga acceso a una dieta adecuada a sus necesidades durante el resto de la gestación (Taylor, R. K. et al., 2017). Como se ha descrito anteriormente, será durante el último tercio de gestación cuando la alimentación de la madre incidirá más en el crecimiento del feto y del individuo en su periodo postnatal. Además, es importante tener en cuenta que, el estado nutricional de la madre en el momento de la subnutrición y la severidad de ésta, influirán en la magnitud de sus consecuencias.

1.4.5. Implicaciones transgeneracionales de la subnutrición materna durante el primer tercio de gestación

La subnutrición materna durante las primeras etapas de la gestación, no solo podrá afectar al individuo que se está gestando, sino que también podrá tener consecuencias en las siguientes generaciones. La subnutrición materna (F0) durante la gestación puede inducir modificaciones epigenéticas en el feto (F1) para asegurar su supervivencia a ese entorno. Esas adaptaciones podrán implicar una disfunción en sus sistemas endocrinos y metabólicos durante su vida postnatal que afectarán al entorno

uterino durante la gestación de la siguiente generación (F2). Ese ambiente uterino alterado podrá inducir nuevamente modificaciones epigenéticas en el feto, sin que haya existido un periodo de subnutrición materna en este individuo (Matthews y Phillips, 2010). Además, los cambios epigenéticos inducidos por un ambiente adverso pueden ser transmitidos directamente a lo largo de las generaciones (Ford y Long, 2011). Zamenhof et al. (1971) relacionaron una restricción alimentaria durante la gestación (F0) con un reducido peso corporal y cerebral en la descendencia (F1). Cuando estos nuevos individuos que habían sufrido un periodo de subnutrición fetal se reprodujeron, su descendencia (F2) presentó también una reducción en el peso corporal y cerebral, sin que hubiera habido ningún periodo de restricción alimentaria. Otros estudios también describieron un efecto transgeneracional de la subnutrición materna, alterando la funcionalidad del eje hipotalámico-hipofisario-adrenal (Bertram et al., 2008), y la regulación plasmática de glucosa (Zambrano et al., 2005) en, al menos, dos generaciones sucesivas.

Estos resultados evidencian que los efectos de la subnutrición durante la gestación pueden manifestarse en las sucesivas generaciones, quedando latentes modificaciones epigenéticas en el genoma de la descendencia.

2. Objetivos

2. OBJETIVOS

Esta Tesis Doctoral se planteó para evaluar las repercusiones de la subnutrición materna durante el primer tercio de gestación en vacas nodrizas sobre su productividad a corto, medio y largo plazo, evaluando los mecanismos fisiológicos a través de los que la subnutrición peri-implantacional puede afectar al desarrollo embrionario, a su descendencia y a la eficiencia productiva del conjunto vaca-ternero, utilizando las dos razas de vacas autóctonas mayoritarias del Pirineo, la Parda de Montaña y la Pirenaica.

Para abordar este objetivo general, se propusieron los siguientes objetivos parciales:

- Evaluar el efecto de la subnutrición en los rendimientos productivos y reproductivos de la madre durante la gestación y la siguiente lactación, haciendo especial hincapié en el reconocimiento materno y mantenimiento de la gestación, y analizando sus perfiles metabólicos, endocrinos, hematológicos e inmunológicos.

- Evaluar el efecto de la subnutrición materna en los crecimientos de la descendencia durante la lactación, analizando sus perfiles metabólicos, endocrinos, hematológicos e inmunológicos.

- Evaluar el efecto de la subnutrición materna sobre los crecimientos y rendimientos reproductivos de las novillas durante sus fases de recría, primera gestación y lactación, haciendo especial hincapié a la edad a la pubertad y fertilidad, y analizando sus perfiles metabólicos y endocrinos.

3. Diseño experimental

3. DISEÑO EXPERIMENTAL

Se planteó un ensayo con el objetivo de analizar los efectos a corto, medio y largo plazo de una subnutrición durante el primer tercio de gestación de una vaca nodriza adulta criando a su ternero.

El ensayo se dividió en diferentes fases, en función de la etapa fisiológica de los animales, desde el inicio de gestación de la vaca hasta el destete de sus nietos (Figura 2):

1. Fase de GESTACIÓN de las vacas (diciembre 2014 – octubre 2015)
2. Fase de LACTACIÓN de las vacas y sus terneros (octubre 2015 – febrero 2016)
3. Fase de RECRÍA de las novillas (febrero 2016 – enero 2017)
4. Fase de GESTACIÓN de las novillas (enero 2017 – noviembre 2017)
5. Fase de LACTACIÓN de las novillas y su descendencia (noviembre 2017 – febrero 2018)

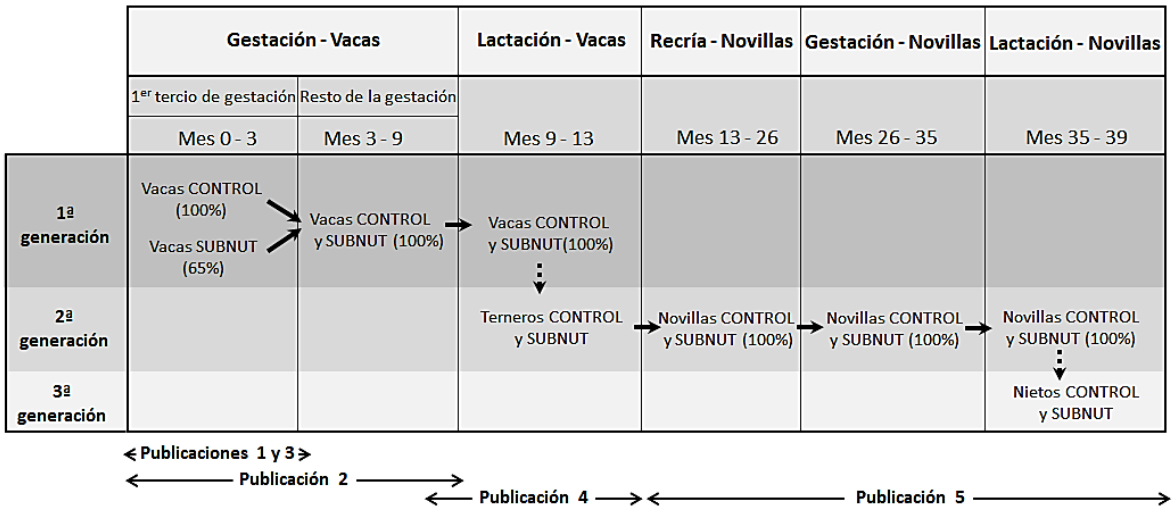

Figura 2. Diseño experimental y manejo alimentario de los animales a lo largo del estudio. Entre paréntesis, porcentaje de los requerimientos nutricionales aportados por la dieta.

Todas las fases de este ensayo, excepto la fase de RECRÍA de las novillas, se llevaron a cabo en la Finca Experimental de la Garcipollera del Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón, situada en Bescós de la Garcipollera (Jaca; 42°37' N, 0°30' O), a 945 m de altitud, con una temperatura media anual de 10,2 ± 0,2 °C y una precipitación anual de 1059 ± 68 mm. La fase de RECRÍA se llevó a cabo en las instalaciones del CITA de Aragón en Zaragoza (41°43' N, 0°48' O), a 225 m de

altitud, con una temperatura media anual de 15,2 ± 0,2 °C y una precipitación anual de 318 ± 63 mm.

Durante la primera fase de GESTACIÓN se utilizaron 75 vacas adultas de raza Parda de Montaña y 40 de raza Pirenaica que estaban criando un ternero. Con el fin de realizar una inseminación artificial a tiempo fijo (IATF) se sincronizaron sus celos a los 65 ± 14 días postparto, mediante un protocolo basado en la aplicación de un dispositivo liberador de progesterona intravaginal (PRID) y 10 µg de hormona liberadora de gonadotropina (GnRH) a día 0. A los 7 días se inyectaron 150 µg de $PGF_{2\alpha}$ y a los 9 días se retiró el dispositivo liberador de progesterona y se inyectaron 500 UI de gonadotropina sérica de yegua preñada (PMSG). A los 11 días se inyectaron 10 µg de GnRH y ocho horas más tarde se inseminaron las vacas con semen procedente de machos Pardos y Pirenaicos de probada fertilidad (Figura 3). Inmediatamente después de la IATF, las vacas se distribuyeron en dos lotes (equilibrados en peso, CC y días postparto) en función de la dieta que recibirían los siguientes 82 días de gestación (1er tercio de gestación). El grupo control recibió una dieta que cubría el 100% de sus necesidades de lactación, gestación y mantenimiento (grupo CONTROL, n = 53, recibieron 10,9 y 10,0 kg materia seca (MS)/animal/día para vacas Pardas y Pirenaicas, respectivamente). Mientras que el grupo subnutrido recibió una dieta que cubría únicamente el 65% de sus necesidades (grupo SUBNUT, n = 62, recibieron 7,0 y 6,4 kg MS/animal/día para vacas Pardas y Pirenaicas, respectivamente). Se utilizó durante esta fase una dieta comercial premezclada (10,96 MJ EM/kg MS y 124 g PB/kg MS, Tabla 1) y se calcularon dichas cantidades utilizando la siguiente fórmula (A.R.C., 1980):

$$\text{Energía necesaria (MJ/día)} = (PV^{0.75} \times 0.5) + (PL \times 5.4)$$

Siendo PV el peso del animal, estipulado a 580 kg para una vaca nodriza adulta, y PL la producción de leche corregida por energía, estipulado a 9 kg en el caso de las Pardas y 8 kg en el caso de las Pirenaicas.

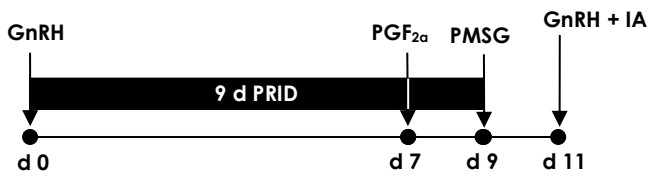

Figura 3. Protocolo utilizado para la sincronización de celos e inseminación artificial a tiempo fijo de las vacas y las novillas.

Tabla 1. Ingredientes y composición química de la dieta comercial premezclada utilizada durante el tratamiento nutritivo.

Ingredientes		**Composición química**	
Heno de alfalfa (%)	25,0	MS (g/kg)	908
Paja de cereal (%)	25,0	Proteína bruta (g/kg MS)	124
Cebada aplastada (%)	25,0	Fibra neutro detergente (g/kg MS)	466
Alfalfa granulada (%)	10,0	Fibra ácido detergente (g/kg MS)	253
Harina de colza (%)	6,5	Lignina ácido detergente (g/kg MS)	40
Pulpa de cítricos (%)	4,5	Cenizas (g/kg MS)	113
Harina de soja (%)	2,5	Energía metabolizable (MJ/kg MS)	11,0
Correctores (%)	1,5		

A los 82 días de gestación se destetaron los terneros. Las vacas que no quedaron gestantes a la IATF se retiraron del ensayo y las restantes se alimentaron con una dieta control que cubría el 100% de sus necesidades durante el resto de la gestación.

Durante la fase de LACTACIÓN, tanto el grupo de vacas CONTROL (n = 37) como el grupo SUBNUT (n = 48) recibieron una dieta para cubrir el 100% de sus requerimientos nutritivos utilizando la misma dieta comercial anteriormente descrita. Los terneros se alimentaron únicamente de leche de sus respectivas madres siguiendo un sistema de doble tetada, que consistía en dos periodos de acceso a la ubre materna de 30 min a las 7:00 y a las 14:30 h. A los 120 días de lactación se destetaron los terneros.

Para la fase de RECRÍA, se seleccionaron únicamente las hembras (a partir de ahora, novillas) y tanto las que procedían de madres CONTROL (n = 17) como las que procedían de madres SUBNUT (n = 19) se alimentaron con 2 kg/animal/día de un pienso comercial (Tabla 2) y heno de pradera y paja *ad libitum*.

Tabla 2. Ingredientes y composición química del pienso utilizado durante la recría de las novillas.

Ingredientes		**Composición química**	
Maíz (%)	47,0	MS (g/kg)	907
Gluten de maíz (%)	15,0	Proteína bruta (g/kg MS)	152
Cebada (%)	15,0	Fibra neutro detergente (g/kg MS)	262
Harina de soja (%)	6,0	Fibra ácido detergente (g/kg MS)	62
Pulpa de remolacha (%)	6,0	Lignina ácido detergente (g/kg MS)	8
Harina de palmiste (%)	4,0	Cenizas (g/kg MS)	60
Aceite de palma (%)	4,0	Energía metabolizable (MJ/kg MS)	14,4
Correctores (%)	3,0		

A los 16 meses de edad las novillas se sincronizaron con un protocolo basado en un dispositivo liberador de progesterona intravaginal y 10 µg de GnRH. A los 7 días se inyectaron 150 µg de $PGF_{2\alpha}$ y a los 9 días se retiró el dispositivo liberador de progesterona y se inyectaron 250 UI de PMSG (la mitad de la dosis administrada a las vacas). A los 11 días se inyectaron 10 µg de GnRH y ocho horas más tarde se inseminaron con semen procedente de un macho Pirenaico de probada fertilidad (Figura 3). Desde la IATF hasta un mes antes del parto, las novillas se alimentaron durante la GESTACIÓN en praderas de fondo de valle siguiendo un sistema de pastoreo tradicional. Esas praderas estaban compuestas básicamente de hierba (*Festuca arundinacea*, *Festuca pratensis* y *Dactilys glomerata*) y leguminosas (*Trifolium repens*) entre otras especies (1191 kg MS/ha) (Rodríguez-Sánchez et al., 2018). Durante el último mes de gestación las novillas recibieron 9 kg/animal/día de heno de pradera y durante la lactación 10 kg/animal/día de la dieta comercial premezclada descrita anteriormente (Tabla 1). Los terneros recién nacidos se alimentaron exclusivamente de leche de sus respectivas madres con acceso ilimitado a la ubre materna. A los 105 días de edad se destetaron los terneros. Durante todo el ensayo todos los animales tuvieron acceso ilimitado a agua y a bloques correctores de vitaminas y minerales.

La metodología de muestreo utilizada, la determinación analítica de los metabolitos y las hormonas, el cálculo de las diferentes variables y el análisis estadístico de los resultados se detalla en cada una de las publicaciones correspondientes.

4. Publicaciones

4.1. A negative energy balance during the peri-implantational period reduces dam IGF-1 but does not alter progesterone or pregnancy-specific protein B (PSPB) or fertility in suckled cows. Noya, A.; Casasús, I.; Rodríguez-Sánchez, J.A.; Ferrer, J.; Sanz, A. Domestic Animal Endocrinology, en prensa. doi: 10.1016/j.domaniend.2019.106418.

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A negative energy balance during the peri-implantational period reduces dam IGF-1 but does not alter progesterone or pregnancy-specific protein B (PSPB) or fertility in suckled cows

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ABSTRACT

The aim of this study was to evaluate the effect of a negative energy balance during the first third of gestation on metabolic, endocrine, and pregnancy recognition parameters in 2 beef cattle breeds adapted to semiextensive conditions. Seventy-five lactating Parda de Montaña and 40 Pirenaica multiparous cows rearing calves were synchronized and timed artificial inseminated (TAI) on day 76 postpartum. Cows were assigned to one of 2 diets (CONTROL or SUBNUT; 100% or 65% of their requirements supplied) until day 82 of gestation. Pregnancy was diagnosed 37 d post-TAI using ultrasound. Blood samples were obtained to determine metabolic (glucose, NEFA, β-hydroxybutyrate, cholesterol, and urea) and endocrine (IGF-1) status throughout the first third of gestation and to determine the concentrations of progesterone and pregnancy-specific protein B (PSPB) in the peri-implantational period. Undernutrition affected both cow and calf performance. The CONTROL cows maintained BCS and BW, whereas SUBNUT cows had negative daily gains. The CONTROL lactating calves had higher BW gains than SUBNUT. These negative effects were more evident in the Pirenaica breed, which was more sensitive to undernutrition. The negative energy balance was reflected in the cows' metabolic profiles, with higher NEFA values and lower IGF-1 concentrations in SUBNUT cows. However, undernutrition did not affect dam pregnancy/TAI or pregnancy recognition and maintenance, confirming that during periods of undernourishment pregnant dams prioritize the allocation of dietary energy toward reproductive functions. Progesterone concentration on day 21 post-TAI (with a 4.8 ng/mL cut-off value) and PSPB on day 26 post-TAI (with a 0.57 ng/mL cut-off value) were determined as the earliest indicators to accurately establish dam pregnancy status, regardless of breed or nutrition treatment. In summary, early undernutrition affected cow performance and metabolic profiles and impaired lactating calf growth, but did not affect progesterone or PSPB concentrations or the pregnancy/TAI rate in suckled cows.

1. Introduction

Beef production systems are adapting to extensive conditions with the aim of reducing feed costs. This means that for long periods, cows will feed only on pastures or low-cost diets, which may compromise their nutritional

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status and reproductive performance. For instance, undernutrition during prepartum and/or postpartum periods negatively impacts pregnancy success and reproductive efficiency [1]. It is well established that when the nutrient requirements for maintenance and lactation exceed intake, fertility, embryo quality, and viability rates are reduced. In fact, many metabolic and endocrine signals involved in reproductive processes are regulated by nutritional status [2]. A negative energy balance can impair the follicular development, the oocyte quality, or the luteinizing hormone secretion, increasing the postpartum anestrus period [3]. Undernourishment following breeding can alter oviductal and uterine support for embryo growth, negatively impacting maternal embryo recognition and pregnancy maintenance. Similarly, alterations in hormone or metabolite concentrations, induced by changes in nutritional inputs, can also affect development of the early embryo and its ability to successfully trigger maternal recognition [4].

Embryo loss is a frequent occurrence that impairs dam efficiency, representing an important source of economic loss for livestock producers [5]. Early and accurate pregnancy detection is key to improve dam reproductive performance, since it allows the reduction of days open and thus the calving interval. Direct techniques such as transrectal palpation or ultrasonography are frequently used, providing an immediate diagnosis as early as day 35 and day 26 after breeding, respectively [6]; however, accuracy requires good technician skills. Indirect techniques, based on the detection of progesterone or pregnancy-specific proteins in cow plasma or milk, or the expression of interferon tau stimulated genes (ISGs) [7], are under development, but their precision and the earliest days when they can be applied remain unclear.

Furthermore, poor nutrition effects may elicit interbreed differences, since genetic background affects metabolic [8] and endocrine status. Parda de Montaña (PA) and Pirenaica (PI) are the 2 main beef cattle breeds adapted to the semiextensive system in the Pyrenees mountain region (Northern Spain). Some interbreed differences have been reported in metabolic and hematologic profiles in response to differing managements, such as reduced granulocyte and mean corpuscular hemoglobin values in feed-restricted PI cows, but not in restricted PA cows [9], or reduced NEFA, total protein, and urea plasma concentrations in PI, but not in PA cows, with restricted nursing periods [10].

We hypothesized that a negative energy balance during the peri-implantational period could be detrimental to dam pregnancy recognition and maintenance, and although interbreed differences have been reported, reproductive functions should not have been affected by the breed, provided they are crucial for the species survival. The aims of this study are to evaluate the effect of an energy-restricted diet during early gestation on performance, metabolic (glucose, NEFA, β-hydroxybutyrate, cholesterol, and urea) and endocrine (IGF-1) status, pregnancy recognition and maintenance, and to establish the earliest day to use the pregnancy-specific protein B (PSPB) concentration as an accurate pregnancy test in PA and PI suckled cows.

2. Material and methods

All procedures were approved by the Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón. The care and use of animals were performed in accordance with the guidelines of the European Union on the protection of animals used for experimental and other scientific purposes [11].

2.1. Animals, management, and diets

This study was conducted at CITA-La Garcipollera Research Station, in the mountain area of the central Pyrenees (Spain, 945 m a.s.l.). Seventy-five PA (560 ± 54.8 kg body weight [BW]; 2.7 ± 0.03 body condition score [BCS] on a 5-point scale) and 40 PI (579 ± 54.9 kg BW; 2.9 ± 0.05 BCS) multiparous cows rearing a single calf were used for the study. The cows were synchronized to estrus at 65 ± 14 d postpartum with a protocol based on a progesterone-releasing intravaginal device (PRID Delta 1.55 g, CEVA, Loudéac, France) and a 10-μg injection of GnRH (Busol, INVESA, Barcelona, Spain), followed 7 d later by a 150-μg injection of prostaglandin $F_{2\alpha}$ (Galapán, INVESA, Barcelona, Spain). After 9 d, the PRID was removed and 500 IU of pregnant mare serum gonadotropin (Serigan, Laboratorios Ovejero, León, Spain) was administered, followed 48 h later by a second injection of GnRH (10 μg). Eight hours after the second GnRH injection, cows were randomly timed artificial inseminated (TAI) with sires of proven fertility (4 PA and 3 PI) by an expert technician. Pregnancy diagnosis to a single AI was performed by ultrasonography using a linear-array 7.5 MHz transducer (Aloka SSD-500V, Aloka, Madrid, Spain) on day 37 ± 2.5 post-TAI.

During the experiment, all cows and calves remained indoors in a loose housing system. After TAI (day 0), cows were group-fed and distributed into 2 maternal nutrition treatments with a total mixed ration (10.96 MJ ME/kg DM and 124 g CP/kg DM) (Table 1) during the first 82 d of pregnancy. The control group (CONTROL, n = 53) was fed a diet that supplied 100% of the estimated energy requirements for cow maintenance, lactation, and gestation (10.9 and 10.0 kg DM/cow/d for PA and PI, respectively); and the nutrient-restricted group (SUBNUT, n = 62) received 65% of their requirements (7.0 and 6.4 kg DM/cow/d for PA and PI, respectively) for a 580-kg beef cow producing 9 kg (PA) or 8 kg (PI) of energy-corrected milk [12]. Groups were randomly balanced according to cow BW (565 ± 60.6 and 568 ± 50.9 kg for CONTROL and SUBNUT, respectively), BCS (2.8 ± 0.27 and 2.8 ± 0.29, respectively), and postpartum period (78 ± 12.0 and 74 ± 14.6 d, respectively) at TAI. Cows were supplied water and vitamin–mineral supplements (lick blocks) ad libitum. During the experiment, suckling calves had a restricted twice-daily nursing system and their diets consisted exclusively of milk.

2.2. Animal weight, BCS assessment, and blood sample collection

Dams were weighed every 2 wk and calves were weighed on day 0, 54, and 82 of the experimental period.

Table 1
Ingredients and chemical composition of feedstuffs used in the experiment (on an as-fed basis).

Ingredients	
Alfalfa hay (%)	25.0
Cereal straw (%)	25.0
Crushed barley (%)	25.0
Dehydrated alfalfa (%)	10.0
Rapeseed meal (%)	6.5
Citrus pulp (%)	4.5
Soybean meal (%)	2.5
Correctors (%) (calcium carbonate, dicalcium phosphate, sodium chloride, vitamins, and trace elements)	1.5
Chemical composition	
DM (g/kg)	908 ± 5.8
CP (g/kg DM)	124 ± 10.2
NDF (g/kg DM)	466 ± 34.8
ADF (g/kg DM)	253 ± 25.1
ADL (g/kg DM)	40 ± 4.7
Ash (g/kg DM)	113 ± 15.3
ME (MJ/kg DM)	11 ± 0.4

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; DM, dry matter; ME, metabolizable energy; NDF, neutral detergent fiber.

The ADG was calculated by linear regression. Dam BCS was registered monthly by 2 expert technicians, based on the estimation of fat covering loin, ribs, and tailhead (using a 1–5 scale). Blood samples were collected every 2 wk for metabolic profiles; monthly for endocrine profiles; on day 14, 18, 21, 28, 42, 56, 69, and 82 post-TAI for plasma progesterone concentration; and on day 25, 26, and 28 post-TAI for PSPB concentration. Blood samples were collected before morning feeding by tail vessel puncture between the sixth and seventh coccygeal vertebrae. Samples to determine glucose, NEFA, β-hydroxybutyrate, cholesterol, and PSPB concentration were collected into 10 mL tubes containing EDTA (BD Vacutainer, Becton-Dickenson and Company, Plymouth, UK). Samples to determine urea, IGF-1, and progesterone concentration were collected into 10 mL heparinized tubes (BD Vacutainer). After bleeding, samples were centrifuged at 1,500 × *g* for 20 min at 4°C and plasma was stored at −20°C until analysis.

2.3. Assays

An automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India) was used to measure blood concentrations of glucose (glucose oxidase/peroxidase method, sensitivity: 0.056 mmol/L); β-hydroxybutyrate (enzymatic colorimetric method, sensitivity: 0.03 mmol/L); cholesterol (enzymatic colorimetric method, sensitivity: 0.256 mmol/L); and urea (kinetic UV test, sensitivity: 0.170 mmol/L). The mean intra- and interassay coefficients of variation for these compounds were <5.4% and <5.8%, respectively. Nonesterified fatty acids (NEFA, enzymatic method, sensitivity: 0.06 mmol/L) were analyzed using a commercial kit (Randox Laboratories Ltd., Crumlin Co., Antrim, UK). The mean intra- and interassay coefficients of variation were 5.1% and 7.4%, respectively. Insulin-like growth factor 1 (IGF-1, enzyme immunoassay, sensitivity: 20 ng/mL) was determined using a solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite, Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). The mean intra- and interassay coefficients of variation were 3.1% and 12.0%, respectively. Plasma progesterone concentration (ELISA test, sensitivity: 0.27 ng/mL) was measured using a specific kit for cattle (Ridgeway Science, Lydney, UK). The mean intra- and interassay coefficients of variation were 8.0% and 10.4%, respectively. Pregnancy-specific protein B (PSPB) (ELISA test, sensitivity: 0.25 ng/mL) was determined using a specific bovine kit (bioPRYN, Bio Tracking Inc., Moscow, Russia). The mean intra- and interassay coefficients of variation were <5%.

2.4. Statistical analysis

All statistics were calculated using SAS statistical package v 9.4 (SAS Institute Inc., Cary, NC, USA). Normality of data was assessed with the Shapiro–Wilk test. Normality could not be confirmed for PSPB concentration, and therefore, it was expressed as a decimal logarithm for further analyses. The ADG of both dams and calves was analyzed using a general linear model (GLM procedure) with the breed (PA vs PI) and nutritional treatment (CONTROL vs SUBNUT) as fixed effects. In the case of cows, BW at TAI was added as a covariate, and in the case of calves, calf gender (male vs female) was added as fixed effect. Pregnancy/TAI and embryo mortality rate were analyzed using a logistic regression model (LOGISTIC procedure) considering breed, nutritional treatment, ADG during the first month of subnutrition (from TAI to ultrasound scanning day), the cow BSC at TAI, and the interval from the last calving to TAI as covariates. Embryo mortality was established in those dams that were diagnosed by ultrasonography as nonpregnant on day 37, but that presented one of these situations: (1) concentrations of progesterone on day 14 and PSPB on day 25 above the cut-off values proposed, (2) concentrations of progesterone on day 14 and PSPB on day 26 above the cut-off values, (3) concentrations of PSPB on day 25 and 28 above the cut-off values, or (4) concentrations of PSPB on day 26 and 28 above the cut-off values. Metabolites (glucose, NEFA, β-hydroxybutyrate, cholesterol, and urea), IGF-1, progesterone, and PSPB concentrations were analyzed using a mixed linear model (MIXED procedure) for repeated measures based on Kenward-Roger's adjusted degrees of freedom solution. The fixed factors were breed and nutritional treatment as the between-subject effects; sampling day as the within-subject effect; animal as the random effect (experimental unit), and the BCS at TAI as a covariate. In case of progesterone and PSPB concentrations, the pregnancy status (pregnant vs nonpregnant) was considered as a fixed effect. Pregnancy/TAI and embryo mortality rate were analyzed using a logistic regression model (LOGISTIC procedure) considering metabolites (on day 0, 14, and 28) and IGF-1 (on day 0 and 28) as covariates. The least square (LS) means of the treatments were estimated per fixed effect, and pairwise comparisons of the means were obtained by the probability of difference (PDIFF) option of the LS means procedure. Estimated cut-off values of progesterone and PSPB for diagnosing a dam as pregnant or nonpregnant were estimated using a linear logistic regression (LOGISTIC procedure), with breed and

nutritional treatment as possible fixed effects. The Youden index was used to determine the sensitivity, specificity, and the cut-off value of the proposed model. Relationships among the studied parameters were determined using Pearson's correlation coefficients. The level of significance for all tests was $P < 0.05$. The results are presented as LS means ± standard error.

3. Results

3.1. Animal performance

No breed effect was found for dam BW during the experiment ($P > 0.05$), but PI dams had higher mean BCS than PA dams (2.7 ± 0.03 vs 2.9 ± 0.04 for PA and PI, respectively, $P < 0.001$). Cow BW and BCS were affected by an interaction between time and nutritional treatment ($P < 0.001$), BW and BCS from the second half of the experimental period being lower in the SUBNUT than in the CONTROL group, as shown in Figure 1. Throughout the experiment, cows in the CONTROL group maintained BW, whereas those in the SUBNUT group experienced a negative ADG (0.11 ± 0.031 vs −0.37 ± 0.026 kg/d, respectively, $P < 0.001$). Regarding calf performance, an interaction effect of breed and nutritional treatment influenced ADG. Calves from PA-CONTROL and PI-CONTROL groups had greater weight gains than their counterparts (0.62 ± 0.020, 0.55 ± 0.020, 0.62 ± 0.034, and 0.44 ± 0.025 kg/d for PA-CONTROL, PA-SUBNUT, PI-CONTROL, and PI-SUBNUT, respectively, $P < 0.05$). However, whereas no differences were found between CONTROL subgroups ($P > 0.05$), ADG was greater in PA-SUBNUT than in PI-SUBNUT calves ($P < 0.001$). No gender effect was found in the calf ADG (0.57 ± 0.017 vs 0.55 ± 0.017 kg/d for male and female, respectively, $P > 0.05$).

3.2. Metabolic and endocrine profiles

Plasma concentrations of glucose, NEFA, β-hydroxybutyrate, cholesterol, urea, and IGF-1, commonly associated with ruminant energy metabolism, were analyzed in order to characterize the nutritional status of suckled cows. Their profiles during the first third of gestation are displayed in Figure 2. Triple interaction effects of breed, nutritional treatment, and sampling day affected both metabolite and IGF-1 concentrations ($P < 0.05$).

Glucose concentrations fluctuated over the course of the experiment. Glucose concentrations in PI-CONTROL cows were equal to or higher than those of their PI-SUBNUT counterparts, unlike PA-CONTROL cows, which had lower values than PA-SUBNUT cows at day 56.

In general, PI had higher NEFA concentration than PA throughout the experiment (0.24 ± 0.017 vs 0.32 ± 0.024 mmol/L for PA and PI, respectively, $P < 0.05$). From the second half of the experiment, PI-SUBNUT had higher NEFA concentrations than PI-CONTROL on day 56 and 82 ($P < 0.05$), and PA-SUNBUT had higher NEFA values than PA-CONTROL from day 56 to the end of the experiment ($P < 0.05$). NEFA levels during the experiment were positively correlated with BCS at TAI, the highest correlations being observed on day 56 ($r = 0.39$, $P < 0.001$).

Few differences were found throughout the experimental period in β-hydroxybutyrate concentrations. PA-CONTROL on day 0 and 82 and PI-CONTROL on day 69 had higher values than their respective SUBNUT counterparts ($P < 0.05$).

Regarding cholesterol concentrations, no differences were found between PA-CONTROL and PA-SUBNUT cows throughout the experiment ($P > 0.05$); however, on day 28, 69, and 82 PI-SUBNUT had lower values than PI-CONTROL ($P < 0.05$). The evolution of cholesterol concentration during the experimental period was similar to that of glucose concentration, with a positive correlation on day 42 ($r = 0.33$, $P < 0.001$).

Similarly, no differences were found in urea concentrations between PA-CONTROL and PA-SUBNUT cows throughout the experimental period ($P > 0.05$), but PI-CONTROL cows had higher values than PI-SUBNUT cows on day 28, 56 ($P < 0.05$), and 69 ($P < 0.001$).

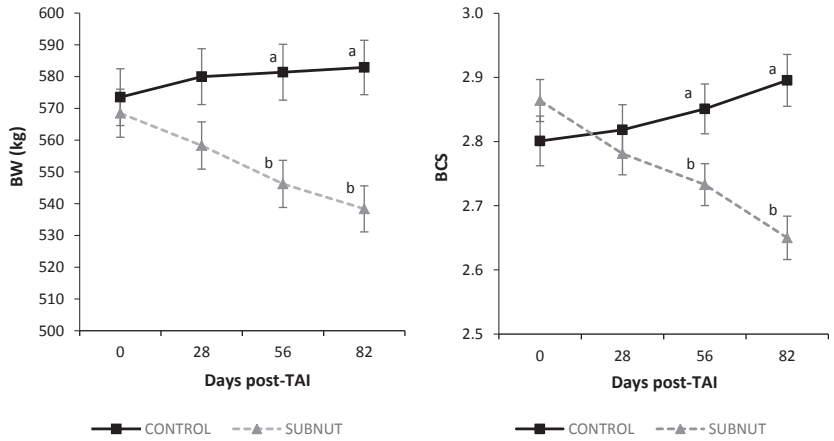

Fig. 1. Body weight (BW) and body condition score (BCS) after TAI of suckled cows according to the nutritional treatment. [a,b]Means at a given time with different superscripts differ significantly ($P < 0.05$); CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy.

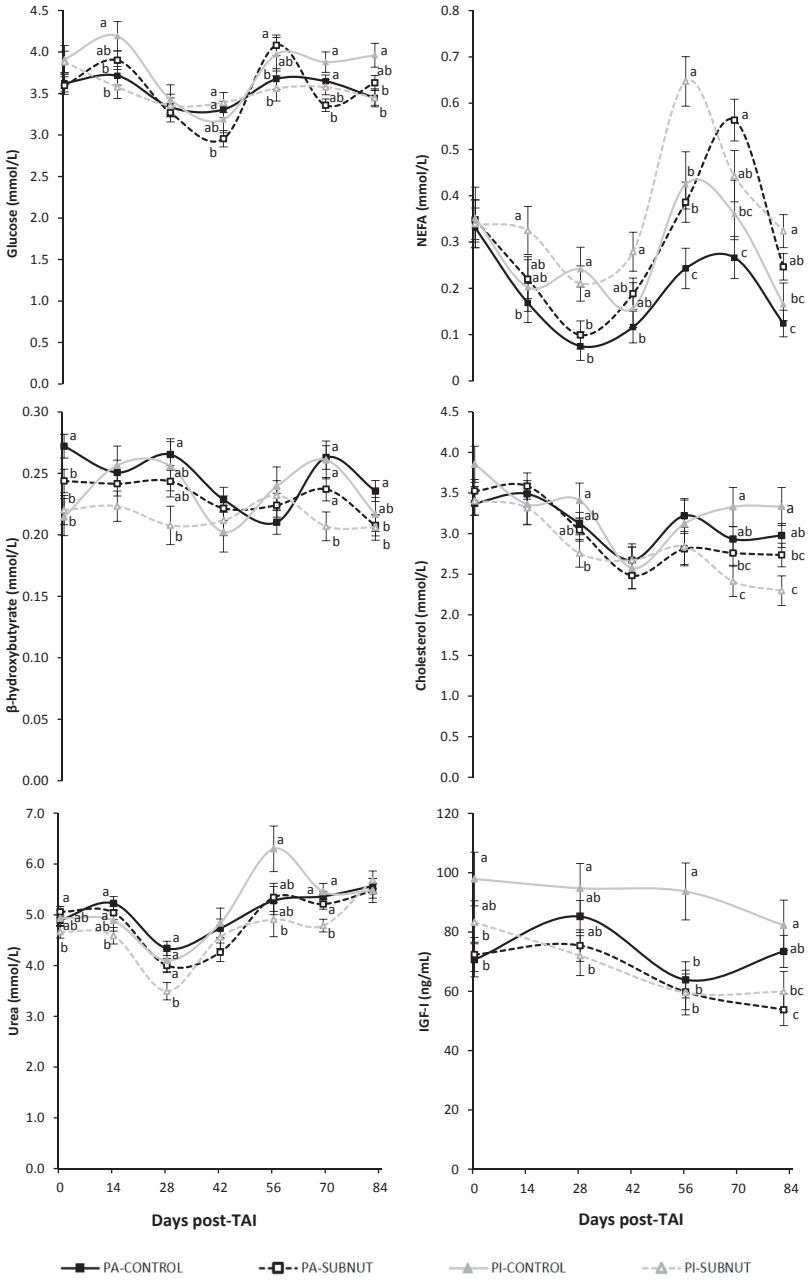

Fig. 2. Plasma concentrations of glucose, NEFA, β-hydroxybutyrate, cholesterol, urea, and IGF-1 after TAI of suckled cows according to the breed and the nutritional treatment. [a-c]Means at a given time with different superscripts differ significantly ($P < 0.05$); PA, Parda de Montaña; PI, Pirenaica; CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy.

In general, CONTROL groups had higher IGF-1 concentrations than SUBNUT groups (82.7 ± 4.65 vs 67.0 ± 3.99 ng/mL for CONTROL and SUBNUT, respectively, $P < 0.05$). Specifically, PA-CONTROL had higher values than PA-SUBNUT on day 82 ($P < 0.01$) and PI-CONTROL higher values than PI-SUBNUT on day 28, 56, and 82 ($P < 0.05$). A negative relationship between IGF-1 and NEFA concentration was found at AI time ($r = -0.26$, $P < 0.01$).

3.3. Progesterone and PSPB concentrations, pregnancy diagnosis, and embryo mortality

Progesterone concentrations were affected by a triple interaction among nutritional treatment, pregnancy status, and sampling day (Fig. 3), but not by breed ($P > 0.05$). No differences were found in progesterone concentration between pregnant-CONTROL and pregnant-SUBNUT dams, or

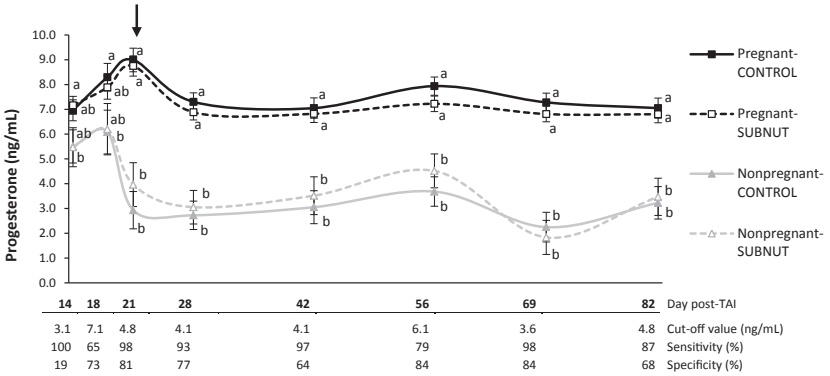

Fig. 3. Progesterone concentrations after TAI of suckled cows according to nutritional treatment and pregnancy status. [a,b]Means at a given time with different superscripts differ significantly ($P < 0.05$); CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; the arrow marks the earliest day for an accurate diagnosis based on progesterone concentration.

between nonpregnant-CONTROL and nonpregnant-SUBNUT throughout the experiment ($P > 0.05$). Pregnancy status affected progesterone concentration, pregnant cows having statistically higher values than their nonpregnant counterparts from day 21 to the end of the assay ($P < 0.001$). The estimated cut-off value of progesterone concentration to determine pregnancy status at each sampling day and the sensitivity and the specificity each model are presented in Figure 3. The earliest accurate cut-off value to diagnose gestation was 4.8 ng/mL on day 21 post-TAI, with an area under the curve (AUC) value of 0.93. On earlier days (14 and 18), the specificity was lower since the difference was not enough to discriminate the progesterone values from a gestational corpus luteum in a pregnant cow from a corpus luteum in the luteal phase in a nonpregnant cow (AUC = 0.66 and 0.77 for day 14 and 18, respectively). On day 28, the accuracy had slightly diminished (AUC = 0.91). Progesterone concentration from pregnant dams was quite constant from day 28 to the end of the experiment regardless of the breed and the nutritional treatment (7.1 ± 2.1 ng/mL).

A triple interaction effect of nutritional treatment, pregnancy status, and sampling day affected the PSPB concentration (Fig. 4). No differences were found between breeds ($P > 0.05$) neither between pregnant-CONTROL and pregnant-SUBNUT dams, nor between nonpregnant-CONTROL and nonpregnant-SUBNUT dams throughout the experiment ($P > 0.05$). Pregnancy status affected PSPB concentration on day 26 and 28, with higher values in pregnant than in nonpregnant dams ($P < 0.001$). No statistical differences were found on day 25 between pregnant-CONTROL and nonpregnant-SUBNUT dams ($P > 0.05$). The estimated cut-off value to diagnose pregnancy status according to PSPB concentration, its sensitivity, and its specificity are displayed in Figure 4. For pregnancy diagnosis at day 25, a 0.76 AUC value was obtained, but no cut-off value was proposed because of the overlap between pregnant and nonpregnant PSPB values. On day 26 and 28, the AUC values were 0.88 and 0.93, respectively, but no significant differences were found between these logistic models ($P > 0.05$). Thus, the first cut-off value obtained to diagnose pregnancy was 0.57 ng/mL on day 26 post-TAI. Concerning only pregnant dams, PSPB concentration increased over time ($P < 0.001$), with no breed or nutritional treatment effect ($P > 0.05$). A negative relationship was found between PSPB and progesterone concentrations in pregnant dams throughout the experiment. Specifically, the PSPB concentrations on day 26 were negatively related to progesterone concentrations on days 14 ($r = -0.41$, $P < 0.01$), 21 ($r = -0.29$, $P < 0.05$), 28 ($r = -0.37$, $P < 0.01$), 56 ($r = -0.45$, $P < 0.001$), and 82 ($r = -0.29$, $P < 0.05$), among others. Concentration of PSPB was also negatively correlated with IGF-1 on day 28 post-TAI ($r = -0.40$, $P < 0.001$).

The pregnancy rate obtained by ultrasonography 37 d post-TAI was 77% (89/115), with no breed (73% vs 85%, for PA and PI) or nutritional treatment (71% vs 82%, for CONTROL and SUBNUT) effect ($P > 0.05$). The ADG during the first month of the experiment, the cow BSC at TAI, the calving to TAI interval, or the metabolite concentrations had not a significant effect on pregnancy rate ($P > 0.05$). Neither IGF-1 on day 0 was related with the fertility rate ($P > 0.05$); however, IGF-1 concentration on day 28 had a negative relationship with fertility rate ($P < 0.01$), the probability to be pregnant decreasing by 2.2% for each extra point of IGF-1.

Embryo mortality rate, diagnosed in 8 dams (8/97 possibly pregnant cows, according to their progesterone and PSPB concentrations), was not related to breed (5/60 PA and 3/37 PI) or nutritional treatment (2/40 CONTROL and 6/57 SUBNUT, $P > 0.05$). The ADG during the first month of the experiment, the cow BSC at TAI, the calving to TAI interval, metabolite, and IGF-1 concentrations had not a significant effect on embryo mortality rate ($P > 0.05$).

4. Discussion

4.1. Animal performance

Nutritional restriction at 65% of cows' requirements over 82 d reduced BCS and BW throughout the study with no difference between breeds, indicating that the estimated requirements, specifically calculated for each breed,

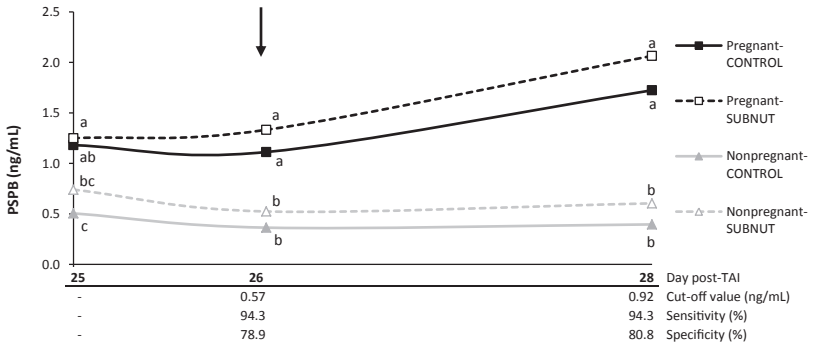

Fig. 4. Pregnancy-specific protein B (PSPB) concentrations after TAI of suckled cows according to nutritional treatment and pregnancy status. [a-c]Means at a given time with different superscripts differ significantly ($P < 0.05$); CONTROL, dams fed 100% of their nutritional requirements from day 0 to day 82 of pregnancy; SUBNUT, dams fed 65% of their nutritional requirements from day 0 to day 82 of pregnancy; the arrow marks the earliest day for an accurate diagnosis based on PSPB concentration.

were well adjusted. The lower calf gains observed in SUBNUT groups resulted from the negative effects of feed restriction on dam milk yield and its protein concentration [13]. However, whereas no differences were found between CONTROL subgroups, PA-SUBNUT calves had higher gains than PI-SUBNUT calves, suggesting that nutritional restriction in PI dams may more severely impair milk yield and/or composition.

4.2. Metabolic and endocrine profiles

Circulating glucose is an indicator of energy balance that shows a strong dependence on the current energy and protein intake at a given time [14]. In our study, CONTROL groups had higher or equal values than SUBNUT groups in most of cases. Similarly, Richards et al [15] found lower glucose concentrations in restricted cows compared to cows fed at maintenance after 30 wk.

A negative energy balance increases plasma NEFA concentration as a consequence of fatty acid release from adipose tissue. In the current study, SUBNUT cows had higher NEFA concentrations than CONTROL cows from the second half of the experiment. Pirenaica cows had higher NEFA concentrations than PA breed, which was related with their higher BCS during the experiment.

Ketogenesis increases blood glucose concentrations when glucose becomes scarce and glycolysis falls to very low levels [16]. In the current study, despite the greater fat tissue mobilization in SUBNUT cows, few differences were found in β-hydroxybutyrate concentration between CONTROL and SUBNUT groups. This implies that β-hydroxybutyrate, which is the predominant circulating ketone body, was not the main energy source used by SUBNUT groups. The mobilization of NEFA from adipose tissue is not associated with concomitant increases in their oxidative metabolite (β-hydroxybutyrate) [10].

Cholesterol is related to glucose concentration [17], with both metabolites indicating a positive energy balance. Accordingly, a positive relationship between them was found in our study. Furthermore, PI-CONTROL had greater cholesterol concentrations than PI-SUBNUT, whereas no differences were found between PA subgroups, highlighting the greater sensitivity to undernutrition of the PI breed.

Blood urea is a good indicator of the protein status of the animal, directly related to degradable protein intake, but also to the catabolism of body protein in periods of energy shortfall [18]. Blood urea concentrations have long been known to reflect inefficient utilization of dietary CP by ruminants [19]; that is, blood urea concentration increases in a cow fed excess dietary protein. In our experiment, CONTROL groups had equal or higher urea concentrations than SUBNUT groups, mostly in PI breed, reflecting their greater CP intake.

In the current study, PI-CONTROL dams had the highest IGF-1 concentrations, whereas PI-SUBNUT concentrations were similar to that obtained in PA groups. The differences between PA-CONTROL and PI-CONTROL cows contrast with other experiments where IGF-1 differences between these breeds were not found [10,18]. Nutrient intake is positively related to IGF-1 concentration [20], and accordingly, IGF-1 concentration was higher in CONTROL than in SUBNUT cows, with negative correlation between NEFA and IGF-1 concentration. At parturition, 6 mo after the nutrient treatment was finished, calves born from CONTROL cows had also higher IGF-1 blood concentration than those from SUBNUT cows [9], highlighting the maternal-embryo cross-talk and its role in embryonic and fetal development.

4.3. Progesterone and PSPB concentrations, pregnancy diagnosis, and embryo mortality

Progesterone plays a central role in the establishment of uterine receptivity to the embryo and drives conceptus elongation through molecular changes induced in the endometrium [21]. A negative energy balance is detrimental for the early growth of ovarian follicles, and after ovulation, progesterone secretion of the corpus luteum can be reduced [22]. In the current study, nutritional treatment did not affect progesterone concentration between pregnant dams, allowing for the maintenance of pregnancy in both CONTROL and SUBNUT cows. On the contrary, other studies have described an inverse relationship between energy intake and systemic progesterone concentration.

High energy intake increases metabolic rate and the blood flow through the liver, resulting in an increased clearance rate of progesterone [23,24]. Accordingly, Nolan et al [25] found 25% lower progesterone concentrations in heifers fed a high versus a low-energy diet.

In our experiment, day 21 was determined to be the earliest accurate day to diagnose pregnancy status based on progesterone concentration, with a 4.8 ng/mL cut-off value and both high sensitivity and specificity values. In agreement with our results, Otavă et al [26] found that in pregnant cows, the progesterone levels increased continuously up to day 21 postfertilization and established the progesterone levels between days 18 and 24 as an indirect method for pregnancy diagnosis. Similarly, Humblot [27] established a combination of $<$3.5 ng/mL on day 0 and $>$5 ng/mL on days 21–24 as criteria to diagnose a dam as pregnant.

Pregnancy-specific protein B, formerly known as pregnancy-associated glycoprotein 1 [28], is a glycoprotein synthesized by the binucleate trophoblastic cells of the bovine placenta [29]. Unlike progesterone, PSPB is a specific pregnancy signal induced as a result of the presence of a conceptus [30]. According to Humblot [27], PSPB concentrations rise from day 15 to 35 to reach 2 to 3 ng/mL at this stage, the critical period for maternal recognition of pregnancy taking place between days 15 and 18 of gestation [28]. The earliest day when the PSPB pregnancy test can yield accurate and consistent results remains unclear, with estimates ranging from day 24 postconception [30], 25 [31], 28 [32], to day 30 [33]. Nevertheless, PSPB clearance from circulation during the postpartum period is extremely slow [34], involving the persistence of high peripheral PSPB concentrations in postpartum cattle. In the current study, the day 25 blood sample was taken on day 100.8 $\pm$ 13.5 after parturition, consistent with the manufacturer's instructions (more than 73 d since last calf). However, residual PSPB concentrations in nonpregnant dams on day 25 did not permit the determination of an accurate cut-off value to diagnose pregnancy. The low metabolic rates of beef compared to dairy cattle might have delayed the clearance of the residual PSPB from the last gestation. The PSPB concentration on day 26 yielded a 0.57 ng/mL cut-off value, with both high sensitivity and specificity and similar accuracy to that from day 28. This suggests that in our conditions, day 26 was the earliest day to diagnose pregnancy, regardless of the nutritional treatment or breed.

Surprisingly, the PSPB concentration was negatively correlated with progesterone values from the critical period of days 15–18 until day 82 post-TAI. We hypothesized that higher PSBP concentration may compensate for lower progesterone production, due to the response by the trophoblastic cells to establish a stronger maternal-embryo cross-talk to permit maternal recognition and ensure the maintenance of gestation. Humblot et al [35] found negative but nonsignificant correlations between circulating progesterone on day 24 and PSPB on day 24, 26, and 30–35 and therefore concluded that there was no relationship between them in pregnant animals. Similarly, López-Gatius et al [36] discounted any potential involvement of progesterone with pregnancy-associated glycoproteins from the placenta or vice versa. However, Ayad et al [37] observed that pregnancy-associated glycoproteins tended to be higher in pregnant females with higher progesterone concentrations. Additional research is needed to determine the role of PSPB in the maternal recognition of a viable conceptus and in pregnancy maintenance, which is not yet fully understood.

In the current study, 77% of cows were pregnant at the TAI, a higher pregnancy rate compared with other studies using similar synchronization protocols [18,38,39], regardless of the breed or the nutritional treatment, probably because of their optimal BCS at TAI. Nutrition determines cow BW and BCS, which underpin fertility rate in postpartum cows [40]. In the current study, despite the SUBNUT group being in a negative energy balance after TAI, these cows' optimal BCS at TAI allowed the conception and maintenance of gestation. Keady et al [41] found similar fertility between a control group fed with ad libitum grass silage as the sole diet and a group supplemented with 5 kg/d of concentrate during late gestation. Contrastingly, Perry et al [42] found that post-AI supplementation improved pregnancy success, and Fontes et al [43] reported an increased pregnancy failure rate associated to a nutrient restriction during early gestation. Metabolite concentration during the first month of the experiment and IGF-1 concentration on day 0 were not related with the pregnancy/TAI rate. Surprisingly, lower IGF-1 concentrations on day 28 were associated with higher pregnancy success. High plasma IGF-1 concentration at TAI has been described as a useful predictor of reproductive success in cattle [23]. Taylor et al [44] reported that cows with plasma IGF-1 values greater than 50 ng/mL at first service exhibited a fivefold increase in likelihood of conception, and Moyes et al [45] found that plasma IGF-1 concentrations in pregnant cows were numerically higher than those of nonpregnant cows after conception; however, these differences were not significant until 15 wk postconception. On the other hand, Falkenberg et al [46] found no significant differences in IGF-1 concentration between cows that conceived at the first AI, in later services, or in cows that did not become pregnant. In the current study, no IGF-1 effect on pregnancy/TAI rate was found at day 0. At that moment, all cows had an optimal BCS and the IGF-1 concentration of all groups was above the threshold before reproduction is adversely affected [47], which implies that IGF-1 concentration did not determine the reproductive performance. From day 0 onward, due to the nutritional treatment, IGF-1 concentration in SUBNUT cows started to decrease, specifically in PI breed. Although the differences in pregnancy/TAI rate between CONTROL and SUBNUT cows were not significant, 57% of pregnant dams belonged to SUBNUT group, while 58% of nonpregnant dams belonged to CONTROL group. That could be the reason why on day 28 pregnant dams (most from SUBNUT group) had lower IGF-1 concentration than nonpregnant dams (most from CONTROL group). Our hypothesis is that despite these lower values at the onset of pregnancy, according to Moyes et al [45], IGF-1 concentration of pregnant dams increases above that of nonpregnant dams as gestation proceeds.

In our study, an 8% embryo mortality rate was reported. Although fertility rates are usually high in beef cattle, pregnancy outcome may decrease due to embryo losses, which can account for up to 29%–39% of pregnancies after

fertilization, most of them between day 8 and 16 after insemination [48]. Nutritional and metabolic status of the cow can affect embryonic development and survival [2]. In beef heifers, Dunne et al [24] found that a short-term (2 wk) reduction in energy intake after AI severely reduced embryo survival rates by 41%, but Doyle et al [23] reported no effect of postinsemination plane of nutrition. In the current study, SUBNUT cows had higher embryo loss rates than their CONTROL counterparts, but the difference was not significant, probably due to the low incidence of embryo losses. Therefore, more studies are needed to assess the impact of the negative energy balance on embryo mortality in adult beef cows.

Therefore, in our study, undernutrition during the first third of pregnancy did not impair the cow reproductive performance and allowed to establish and maintain the gestation. A 65% energy restricted diet was a severe feed restriction, reflected in most of the metabolites and IGF-1 SUBNUT cow profiles. Nevertheless, at the beginning of the experiment, all cows had an optimal BCS to face this nutritional challenge. Animals with lower BCS and a worse metabolic status at the beginning of the study would possibly have obtained a worse reproductive performance. Fernández-Foren et al [49] affirmed that initial body reserves determine the endocrine response to undernutrition. As in our experiment, undernourished animals with optimal initial BCS developed compensatory mechanisms against adverse environmental factors, counteracting the negative effects caused by a food restriction on reproduction. However, it is interesting to highlight that in our study, although the reproductive performance was not initially affected by undernutrition, an altered maternal environment compromised the fetal programming with long-term consequences in the newborns [9].

5. Conclusions

A restrictive diet during the first 82 d after TAI induced a negative energy balance in suckled cows, reflected in higher NEFA and lower IGF-1 concentrations, which affected dam performance and impaired calf growth. These negative effects were more evident in the PI breed, which was more sensitive to feed restriction. Undernutrition did not affect dam pregnancy recognition, maintenance of gestation, or pregnancy/TAI rate, confirming that pregnant dams cope with undernourishment by prioritizing the allocation of dietary energy toward reproductive functions.

CRediT authorship contribution statement

A. Noya: Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft, Writing - review & editing. **I. Casasús:** Methodology, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Writing - original draft, Writing - review & editing. **J.A. Rodríguez-Sánchez:** Methodology, Investigation, Project administration, Writing - review & editing. **J. Ferrer:** Resources, Methodology, Data curation, Funding acquisition, Investigation, Project administration, Writing - review & editing. **A. Sanz:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - original draft, Writing - review & editing.

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Effects of maternal subnutrition during early pregnancy on cow hematological profiles and offspring physiology and vitality in two beef breeds

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Abstract

This experiment evaluated the effects of subnutrition during early gestation on hematology in cows (*Bos Taurus*) and on hematological, metabolic, endocrine, and vitality parameters in their calves. Parda de Montaña and Pirenaica dams were inseminated and assigned to either a control (CONTROL, 100% requirements) or a nutrient-restricted group (SUBNUT, 65%) during the first third of gestation. Dam blood samples were collected on days 20 and 253 of gestation, and calf samples were obtained during the first days of life. Pirenaica dams presented higher red series parameters than Parda de Montaña dams, both in the first and the last months of gestation. During early pregnancy, granulocyte numbers and mean corpuscular hemoglobin were lower in Pirenaica-SUBNUT than in Pirenaica-CONTROL cows. Calves from the SUBNUT cows did not show a physiological reduction in red series values in early life, suggesting later maturation of the hematopoietic system. Poor maternal nutrition affected calf endocrine parameters. Newborns from dystocic parturitions showed lower NEFA concentrations and weaker vitality responses. In conclusion, maternal nutrition had short-term effects on cow hematology, Pirenaica cows showing a higher susceptibility to undernutrition; and a long-term effect on their offspring endocrinology, SUBNUT newborns showing lower levels of IGF-1 and higher levels of cortisol.

KEYWORDS
blood, cortisol, IGF-1, metabolic parameters, peri-implantational period

1 | INTRODUCTION

Beef cattle (*Bos taurus*) production systems have adapted to increasingly extensive management by reducing feed costs. Depending on food availability, cows can suffer periods of undernutrition during some phases of their production cycle, sometimes concomitantly with the rearing of a calf or/and in early pregnancy. Implantation of the embryo and the maternal recognition of pregnancy at approximately day 20 postconception are critical points of gestation (Spencer & Hansen, 2015). Moreover, the peri-implantation period is a crucial time for embryo survival, and it could be a potentially vulnerable period during which adverse programming mediated through poor maternal nutrition might begin. Altered placental angiogenesis, cotyledon weight, and fetal development in beef cattle (Long, Vonnahme, Hess, Nathanielsz, & Ford, 2009), cardiovascular abnormalities in mice (Watkins et al., 2008), altered cardiovascular activity (Torrens et al., 2009), or suppressed behavioral reactions in response to stressful conditions in ewes (Hernandez, Matthews, Oliver, Bloomfield, & Harding, 2010) have all demonstrated the risk of adverse developmental programming and increased chronic

disease incidence attributed to periconceptional undernutrition (Fleming, Velazquez, Eckert, Lucas, & Watkins, 2012).

Hematological parameters have been used routinely to monitor the health and nutritional status of cattle (Strydom et al., 2008), considering that factors such as age, sex, breed, stress, diet, body condition, reproductive status, ambient temperature, or altitude can affect hematological profiles (Krimer, 2011). Any imbalance of hematological parameters will indicate a breakdown in the homeostasis.

Similarly, metabolic parameters are indicators of nutritional status and ruminant energy metabolism. However, these parameters can be permanently altered if some stimuli, such as poor nutrition, are present during fetal programming. During this critical and sensitive fetal stage, the structure, physiology, and metabolism of different organs and systems can be modified, leading to detrimental postnatal metabolic changes (Mossa, Walsh, Ireland, & Evans, 2015). Similarly, undernutrition during fetal life could cause permanent alterations in the endocrine function in the fetus to ensure fetus survival under adverse intrauterine conditions (Kiani et al., 2011; Rhind, 2004). For example, prenatal nutrient availability influences the ability of calves to regulate plasma concentrations of glucose and insulin (Ford & Long, 2011).

Interbreed differences, which may interact with the nutritional level, must be considered, since genetic differences induce changes in hematological or metabolic values (Wuletaw, Wurzinger, Holt, Dessie, & Sölkner, 2011). Parda de Montaña (PA) and Pirenaica (PI) are the two main beef cattle breeds that have adapted to the semi-extensive system of animal husbandry in the Pyrenees mountain region (northern Spain). Differences have been found between these breeds in their neuroendocrine and metabolic adaptation to varied management practices (Blanco, Casasús, & Palacio, 2009; García-Belenguer et al., 1996), which should be considered to choose the genotype better adapted to extensive management characterized by variable food availability to guarantee the maximum herd productivity.

At present, little is known about the physiological mechanism through which maternal subnutrition and breed can alter embryo hematopoiesis, metabolism, or endocrine regulation in cattle. This study's hypothesis was that maternal subnutrition during early pregnancy could trigger effects in dam hematological values and in offspring physiology and vitality, and that the response could differ between genotypes with varying baseline profiles. The aim of this study was to evaluate the effect of undernutrition during the first third of gestation on the hematological parameters in the peri-implantation period (day 20 after artificial insemination, AI) and in the last month of gestation (day 253) in PA and PI beef cows and on hematological, metabolic, and endocrine profiles and vitality of newborn calves.

2 | MATERIALS AND METHODS

All of the procedures were approved by the Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón. The care and use of animals were performed in accordance with the guidelines of the European Union (Directive, 2010E.U.) regarding the protection of animals used for experimental and other scientific purposes (E.U., 2010).

2.1 | Animals and management

This study was conducted at La Garcipollera Research Station in the mountainous area of the central Pyrenees (northeastern Spain, 945 m a.s.l.). Seventy-four PA (560 ± 55 kg live weight (LW); 2.73 ± 0.26 body condition score (BCS) on a 5-point scale) and 40 PI (579 ± 51 kg LW; 2.95 ± 0.28 BCS) multiparous cows rearing calves were synchronized to estrus at 65 ± 14 days postpartum with a protocol based on a progesterone-releasing intravaginal device (PRID Delta 1.55 g; CEVA, Loudéac, France) and a 10 μg injection of GnRH (Busol, INVESA, Barcelona, Spain), followed 7 days later by 150 μg of prostaglandin F2α (Galapán, INVESA). After 9 days, the PRID was removed and 500 IU of pregnant mare serum gonadotropin (Serigan, Laboratorios Ovejero, León, Spain) was administered, followed 48 hr later by a second injection of GnRH (10 μg). Eight hours after the final GnRH injection, the cows were randomly inseminated with proven fertility sires (4 PA and 3 PI) by an expert technician.

On the day of AI, the dams were randomly allocated to two maternal nutrition levels with a total mixed ration (10.96 MJ ME/kg DM and 124 g CP/kg DM) (Table 1) during the first 82 days of pregnancy. The control group (CONTROL, n = 52) was fed a diet that supplied 100% of the estimated energy and protein requirements for cow maintenance, lactation, and gestation (10.9 and 10.0 kg DM/cow/day for the PA and PI, respectively), and the nutrient-restricted group (SUBNUT, n = 62) received 65% of their requirements (7.0 and 6.4 kg DM/cow/day for the PA and PI, respectively). After this treatment phase, all of the dams were fed 100% requirements until parturition. Pregnancy diagnosis was performed by ultrasonography using a linear array 7.5 MHz transducer (Aloka SSD-500V; Aloka, Madrid, Spain) on day 37 post-AI, and the nonpregnant cows were removed from the trial thereafter. During the experiment, all of the cows and calves remained in a loose housing system.

2.2 | Measurements and blood sampling

The cows were weighed and their BCS was registered by two expert technicians based on the estimation of the fat covering the loin, ribs, and tailhead on day 20 post-AI, and again from those who conceived from AI (n = 83) on day 82 post-AI and 1 month before parturition (253 days post-AI). On days 20 and 253 post-AI, blood samples were collected in EDTA tubes (BD Vacutainer Becton-Dickenson and Company, Plymouth, UK) from all of the dams via coccygeal venipuncture. The calves were weighed at birth and their blood sampled once during their first days of life (between days 1 and 11) via jugular venipuncture into EDTA and heparinized tubes. Samples for hematology were refrigerated (4°C) and analyzed within the next 8 hr. Samples for metabolite and hormone concentration were centrifuged at 3,500 rpm for 20 min at 4°C immediately after collection, and the plasma was harvested and frozen at −20°C until analysis.

TABLE 1 Ingredients and chemical composition of feedstuffs used in the experiment (on an as-fed basis)

Ingredients	
Alfalfa hay (%)	25.0
Cereal straw (%)	25.0
Crushed barley (%)	25.0
Dehydrated alfalfa (%)	10.0
Rapeseed meal (%)	6.5
Citrus pulp (%)	4.5
Soybean meal (%)	2.5
Correctors (%) (calcium carbonate, dicalcium phosphate, sodium chloride, vitamins, and trace elements)	1.5
Chemical composition	
DM (g/kg)	907.7 ± 5.8
CP (g/kg DM)	124.1 ± 10.2
NDF (g/kg DM)	466.2 ± 34.8
ADF (g/kg DM)	253.3 ± 25.1
ADL (g/kg DM)	40.3 ± 4.7
Ash (g/kg DM)	113.4 ± 15.3
ME (MJ/kg DM)	11.0 ± 0.4

Note: ADF, acid detergent fiber; ADL, acid-detergent lignin; CP, crude protein; DM, dry matter; NDF, neutral detergent fiber; ME, metabolizable energy.

Concurrently, the cow parturition process was classified into three categories depending on the assistance needed: unassisted, easy-pulled (assisted by hand or with a rope), or hard-pulled (assisted with a fetus extractor). Newborn vitality was evaluated immediately after birth via a modified calf vitality test proposed by Mee (2008a). The parameters were evaluated and their categories were: meconium staining (no staining around the anal area vs. stained), tongue (normal vs. swollen or protruding tongues), calf attitude (attempts to stand vs. no effort to rise), palpebral reflex (actively blinks and closes eyes vs. slow to blink or no reflex), finger suckling reflex (strong vs. weak or absent), and mucous membrane color (bright pink vs. brick red or white/blue).

2.3 | Hematology, hormone, and metabolite assays

Unclotted (EDTA) whole-blood samples from the cows and calves were analyzed using a fluorescent flow cytometry analyzer (Sysmex XT-2000i V; Sysmex Corporation, Kobe, Japan) standardized for the analysis of bovine blood. Hematological analyses included hematocrit (HCT, expressed as a percentage), hemoglobin concentration (HGB, g/dl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin concentration (MCHC, g/dl), red blood cell count (RBC, 10^6 counts/mm^3), red blood cell distribution width (RDW, percentage), white blood cell count (WBC, 10^3 counts per mm^3, including the different leukocyte subtypes: granulocytes (GRAN), lymphocytes (LYM), and monocytes (MON)), number of platelets (PLT, 10^3 counts/mm^3), mean platelet volume (MPV, fl), platelet distribution width (PDW, fl), and plateletcrit (PCT, percentage).

Heparin and EDTA plasma samples (according to the manufacturer's instructions) were used to assess the calves' metabolic and endocrine status. An automatic analyzer (GernonStar; RAL/TRANSASIA, Dabhel, India) was used to measure the blood concentrations of glucose (glucose oxidase/peroxidase method, sensitivity: 0.056 mmol/L) and urea (kinetic UV test, sensitivity: 0.170 mmol/L). The mean intra- and inter-assay coefficients of variation for these molecules were <5.4% and 5.8%, respectively. A commercial kit (Randox Laboratories Ltd., Crumlin Co., Antrim, UK) was used to analyze the concentrations of nonesterified fatty acids (NEFA, enzymatic method, sensitivity: 0.06 mmol/L). The mean intra- and inter-assay coefficients of variation were 5.1% and 7.4%, respectively. A solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite, Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK) was used to determine the plasma cortisol concentration (sensitivity: 5.5 nmol/L) and insulin-like growth factor-I (IGF-1, sensitivity: 20 ng/ml). The mean intra-assay coefficient of variation for cortisol was 7.1% and the mean intra- and inter-assay coefficients of variation for IGF-1 were 3.1% and 12.0%, respectively.

2.4 | Statistical analyses

Data were analyzed with SAS and JMPro statistical software (SAS Institute Inc., Cary, NC). Normality was confirmed by the univariate procedure ($p > 0.05$). The live weights, BCS, and hematological values of the cows in the first and last months of pregnancy were assessed through analysis of variance using a general linear model (the GLM procedure) with dam age (5–10 years old vs. more than 10 years old), breed (PA vs. PI), maternal nutrition (CONTROL vs. SUBNUT), and their interactions as fixed effects. In the samples collected on day 20 post-AI, the pregnancy status (pregnant vs. nonpregnant) was also included as a fixed effect. The live weights, hematological values, and metabolite and hormone concentrations in the calves were assessed through analysis of variance with a mixed linear model (the mixed procedure), including the type of parturition (only for metabolite and hormone concentration analysis), gender (male vs. female), breed, maternal nutrition, and their interaction as fixed effects; the calf age was included as a covariate and the sire used for AI was considered a random effect. The relationship among the calves' hematological values and age, metabolite, and hormone concentrations were determined through Pearson's correlation coefficients. The association between the calves' vitality and breed, maternal nutrition, and type of parturition was assessed using the *F*-test (the FREQ procedure).

All of the statistical analyses were considered significant at $p < 0.05$. Values are expressed as the least square (LS) means. Multiple comparisons among treatments were conducted using Tukey's test.

3 | RESULTS AND DISCUSSION

In the current study, the dam age, the sire used for AI, and the pregnancy status (only in the cow samples collected on day 20 post-AI) had no effect on the parameters measured in the dams or calves.

3.1 | Hematological parameters of dams on day 20 post-AI

The LW, BCS, and hematological values of the dams at the third week post-AI are shown in Table 2. No differences were found in the LW between breeds ($p > 0.05$) on day 20 post-AI, but the BCS was lower in the PA than in the PI cows ($p < 0.001$). Regarding maternal nutrition, the CONTROL and SUBNUT groups had similar LW and BCS ($p > 0.05$), probably because the SUBNUT group had been undernourished during only 20 days.

Cow hematology records were in the normal range for the adult cows (Roland, Drillich, & Iwersen, 2014). The effects of dam breed, nutrition, age, and pregnancy status on these data were analyzed. Breed affected most of the hematological parameters studied on day 20 post-AI. Concerning the white series, the PA cows had higher values of WBC than the PI ($p < 0.05$) due to a higher LYM count ($p < 0.01$). In the red series, the PI had higher values of RBC, HGB, HCT, and MCV than the PA ($p < 0.001$). In platelets, both the PLT and PCT counts were higher in the PA than in the PI cows ($p < 0.001$), while the MPV was higher in the PI cows ($p < 0.001$). This inverse physiological relationship between PLT and MPV was also described in humans with the aim of maintaining a constant PCT value (Lozano et al., 1998).

Maternal nutrition showed a minor effect on cow hematology values on day 20 post-AI, but a significant interaction between breed and maternal nutrition was observed in the granulocyte counts. The values did not differ between maternal nutrition treatments in the

TABLE 2 Hematological parameters of the cows on day 20 post-AI according to the breed and maternal nutrition

	Breed			Maternal nutrition			Significance		
	PARDA	PIRENAICA	S.E.D.	CONTROL	SUBNUT	S.E.D.	Breed	Maternal nutrition	Breed × M. nutrition
n	74	40		52	62				
Live weight (kg)	575	588	10.4	586	577	10.0	ns	ns	ns
Body condition score (1–5)	2.7	2.9	0.05	2.8	2.8	0.05	<0.001	ns	ns
White blood cells (10^3 counts/mm^3)	7.5	6.9	0.29	7.2	7.2	0.30	0.034	ns	ns
Lymphoctyes (10^3 counts/mm^3)	3.6	3.0	0.2	3.2	3.4	0.19	0.003	ns	ns
Monocytes (10^3 counts/mm^3)	0.58	0.55	0.03	0.56	0.57	0.03	ns	ns	ns
Granulocytes (10^3 counts/mm^3)	3.3	3.3	0.18	3.5	3.2	0.19	ns	ns	0.034
Red blood cells (10^6 counts/mm^3)	6.1	6.8	0.11	6.4	6.5	0.12	<0.001	ns	ns
Hemoglobin (g/dL)	10.8	12.6	0.18	11.7	11.8	0.18	<0.001	ns	ns
Hematocrit (%)	32.1	37.2	0.57	34.3	34.9	0.59	<0.001	ns	ns
Mean corpuscular volume (fL)	53.2	55.5	0.18	54.7	54.0	0.18	<0.001	ns	ns
Mean corpuscular hemoglobin (pg)	17.9	18.6	0.17	18.4	18.2	0.17	<0.001	ns	0.008
Mean corp. hemoglobin conc. (g/dL)	33.6	33.7	0.18	33.8	33.5	0.18	ns	ns	ns
Red cell distribution width (%)	17.0	17.0	0.16	17.3	16.7	0.17	ns	<0.001	ns
Platelets (10^3 counts/mm^3)	264.5	198.1	11.9	244.8	217.9	12.3	<0.001	0.029	ns
Mean platelet volume (fL)	5.6	6.0	0.07	5.8	5.8	0.07	<0.001	ns	ns
Platelet distribution width (fL)	16.1	16.2	0.06	16.1	16.1	0.07	ns	ns	ns
Plateletcrit (%)	0.145	0.116	0.01	0.139	0.123	0.01	<0.001	0.025	ns

Note: ns, not significant ($p > 0.05$); *n*, number; CONTROL, 100% fed group; SUBNUT, 65% fed group; S.E.D., standard error of the difference.

PA cows (3.3 vs. 3.4 10^3 GRAN counts/mm^3 for the PA-CONTROL and the PA-SUBNUT, respectively, $p > 0.05$, standard error of the difference (SED) 0.21 10^3), whereas the counts were higher in the PI-CONTROL than in the PI-SUBNUT (3.7 vs. 3.0 10^3 counts/mm^3, respectively, $p < 0.05$, SED 0.31 10^3). Similarly, in an experiment conducted with beef heifers in which a short-term dietary restriction (1.2 vs. 0.4 maintenance energy requirements) was applied for 18 days, Matthews et al. (2015) found no effects on the neutrophil and lymphocyte numbers. Similarly, over a longer period of differential feeding during 10 weeks, Schären et al. (2016) did not observe any biologically relevant effects on white blood cell populations. In the current study, there was an interaction between breed and maternal nutrition in MCH, that is, no differences were found between the PA-CONTROL and the PA-SUBNUT dams (17.8 vs. 18.0 pg, respectively, $p > 0.05$, SED 0.19), but the values were higher in the PI-CONTROL than in the PI-SUBNUT (19.0 vs. 18.3 pg, respectively, $p < 0.01$, SED 0.29). The RDW was conditioned by maternal nutrition, with higher variability in the erythrocyte sizes in the CONTROL group ($p < 0.001$). However, since the RDW values were within the reference range, anisocytosis was discarded. Similar to the current results, Matthews et al. (2015) found that imposing a short-term dietary restriction on beef heifers had no effects on the RBC or HGB concentrations. Meacham, Warnick, Cunha, Hentges, and Shirley (1964) found no differences in the HCT or HGB between bulls receiving diets with 8% versus 15% crude protein over 84 days. In the current study, maternal nutrition resulted in lower PLT counts and PCT in the SUBNUT dams ($p < 0.05$), in contrast to Matthews et al. (2015), who found no effect on the platelet numbers from short-term dietary restrictions. Overall, it is likely that in the current study, undernutrition had only a minor effect on cow hematology on day 20 post-AI because it had been acting for a short time. However, a clear breed-associated susceptibility to undernutrition was observed in the Pirenaica dams in the first month of gestation.

Pregnancy status ($p > 0.05$) did not have any effect, possibly due to the low metabolic and nutrient requirements of the developing fetus during the first month of gravidity (Dänicke et al., 2012). In fact, Mir et al. (2008) described the increase in the HGB, HCT, RBC, MCV, and MCHC values in mid-gestation in crossbreed cows to accommodate the higher need for oxygen consumption in advanced pregnancies. These hematological values returned to lower values in late gestation due to the dilution of blood that occurs as a consequence of increased plasma volume.

3.2 | Hematological parameters of dams in the last month of pregnancy

Similar to the results obtained on day 20 post-AI, the PA and PI presented similar LW ($p > 0.05$) and the PI had higher BCS than the PA on day 253 of pregnancy ($p < 0.05$) (Table 3). No differences were found between the CONTROL and SUBNUT groups in the LW and BCS ($p > 0.05$). The differences between the groups in both parameters registered after 82 days of maternal nutrition treatment (577 vs. 539 kg ($p < 0.01$) and 2.9 vs. 2.6 ($p < 0.001$) for the CONTROL and SUBNUT groups in the LW and BCS, respectively) disappeared 1 month before parturition, probably because the 100% diet received from day 82 post-AI until calving allowed the SUBNUT cows to overcome this difference.

All of the cow hematological parameters registered in the last month of gestation were within the bovine reference range (Roland et al., 2014) except for MPV, which in all of the groups was higher than that referenced due to a physiological increase in the last stage of gestation (Fay, Hughes, & Farron, 1983). The effects of the cow breed, nutrition, and age on these data were analyzed. Neither age nor any interaction among effects influenced the studied parameters. The breed affected most of the red blood cell parameters, since the PA cows had lower values of RBC and HGB ($p < 0.01$) and HCT ($p < 0.05$) than the PI cows, in agreement with previous observations in early pregnancy and with results obtained by García-Belenguer et al. (1996). Parda de Montaña also exhibited lower MCHC ($p < 0.05$) and RDW ($p < 0.01$) than PI dams. No significant differences in the platelet series were observed ($p > 0.05$). These results confirmed that, in physiological conditions, the values of the red series were higher in the PI cows, which provides evidence of interbreed differences that could imply a better adaptation to altitude conditions than the PA dams, in line with a study by Bianca and Näf (1979). Blood parameters are considered important indicators for measuring the adaptation of animals to altitude, which induces hematopoiesis as an adaptive mechanism (Wuletaw et al., 2011).

Maternal subnutrition applied in the early gestation period had no long-term effects on the hematological variables observed one month before calving ($p > 0.05$). This lack of effect suggests that the cows were able to offset the previous differences after they returned to the control diet for 171 days, and therefore their blood profiles were relatively resilient to nutritional stress. It is well known that an adequate nutritional status is essential to restore physiological values of the hematological parameters. In this sense, Meacham et al. (1964) found lower HCT and HGB values when bulls were fed a low-protein diet, but the values were restored after the bulls returned to a control diet for 100 days.

3.3 | Hematological parameters of newborn calves

The calves' LW and blood cell values are displayed in Table 4. The live weights of the newborn calves were higher in the PA breed ($p < 0.01$) than in the PI, in line with previous studies of the same breeds (Álvarez-Rodríguez, Palacio, Casasús, & Sanz, 2010). As expected, the male calves were heavier than the females ($p < 0.01$). However, no differences were found in the calf LW at birth that could be ascribed to maternal nutrition ($p > 0.05$). Accordingly, Mossa et al. (2013) did not find weight differences in calves born to nutrient-restricted and control heifers that were fed at 0.6 and 1.2 of their requirements, respectively, during the 110 first days of gestation.

Regarding offspring hematology, a stress leukogram was observed in 21 calves who were discarded for all subsequent analyses due to extreme granulocytosis (more than 10 10^3 counts/mm^3) or intense lymphopenia (less than 0.2 10^3 counts/mm^3) or both. Neither breed

TABLE 3 Hematological parameters of the cows in the last month of pregnancy according to the breed and maternal nutrition

	Breed			Maternal nutrition			Significance	
	PARDA	PIRENAICA	S.E.D.	CONTROL	SUBNUT	S.E.D.	Breed	Maternal nutrition
n	48	35		30	53			
Live weight (kg)	634	602	11.8	611	626	12.1	ns	ns
Body condition score (1–5)	2.9	3.1	0.06	3.0	2.9	0.06	0.04	ns
White blood cells (10^3 counts/mm^3)	6.6	6.1	0.36	6.2	6.5	0.37	ns	ns
Lymphoctyes (10^3 counts/mm^3)	3.3	3.7	0.24	3.7	3.7	0.24	ns	ns
Monocytes (10^3 counts/mm^3)	0.43	0.48	0.05	0.46	0.46	0.05	ns	ns
Granulocytes (10^3 counts/mm^3)	2.5	2.2	0.31	2.3	2.4	0.32	ns	ns
Red blood cells (10^6 counts/mm^3)	5.7	6.4	0.23	5.9	6.1	0.23	0.006	ns
Hemoglobin (g/dL)	10.3	11.5	0.23	10.7	11.1	0.23	0.002	ns
Hematocrit (%)	30.2	33.2	1.2	31.2	32.2	1.22	0.015	ns
Mean corpuscular volume (fL)	53.2	52.3	1.29	53.1	52.4	1.31	ns	ns
Mean corpuscular hemoglobin (pg)	18.1	18.2	0.39	18.2	18.1	0.4	ns	ns
Mean corp. hemoglobin conc. (g/dL)	34.1	34.8	0.28	34.4	34.5	0.28	0.016	ns
Red cell distribution width (%)	19.0	20.0	0.35	19.2	19.9	0.36	0.008	ns
Platelets (10^3 counts/mm^3)	250.7	256.8	31.1	259.0	248.5	31.6	ns	ns
Mean platelet volume (fL)	7.7	7.9	0.21	7.9	7.7	0.22	ns	ns
Platelet distribution width (fL)	8.6	8.8	0.5	8.7	8.7	0.51	ns	ns
Plateletcrit (%)	0.22	0.21	0.02	0.22	0.21	0.02	ns	ns

Note: ns, not significant ($p > 0.05$); *n*, number; CONTROL, 100% fed group; SUBNUT, 65% fed group; S.E.D., standard error of the difference. No significant interactions among effects were found ($p > 0.05$).

(22.4% of the PA and 32.3% of the PI calves had a stress leukogram) nor maternal nutrition in early pregnancy (28.6% of the CONTROL and 24.0% of the SUBNUT calves, respectively) or their interaction were associated with the leukogram status (*F*-test, $p > 0.05$). This response has been observed regularly in studies on the hematology of newborn calves (Benesi et al., 2012) as a consequence of the high cortisol concentrations produced by a stressful situation, such as the birthing process (Hulbert & Moisá, 2016). Three pairs of twin calves were also removed from the analysis. The effects of breed, maternal nutrition, and gender were therefore analyzed in the data on the remaining 59 calves, which were within the bovine physiological range for calves. No interactions among effects were found.

The breed-associated differences in the red series parameters observed in the dams in the current study did not occur in their newborn calves, which meant that the hematological characteristics inherent to the breed are not congenital but are acquired later during their postnatal life, in accordance with previous studies (Blanco et al., 2009).

Maternal nutrition in early gestation did not influence the calf leukograms, although the fetal immune system develops at the beginning of gestation, including lymphoid thymus and spleen development at approximately days 42 and 55, respectively (Schultz, Dunne, & Heist, 1973). However, maternal nutrition had an effect on the red series parameters, with the calves from the SUBNUT treatment showing lower MCH than their CONTROL counterparts ($p < 0.05$). Given that the MCHC and HGB values were within the reference range, and they did not differ between the nutritional treatments, and hypochromic anemia in the undernourished group was discarded (Almaguer, 2012). Similarly, Dänicke et al. (2012) did not find differences in HCT, WBC, GRAN, LYM, and MON values in newborn calves whose mothers were submitted to a nutritional treatment during the first days of pregnancy. Hematopoiesis is a long process that starts in the blood islands of the embryo yolk sac in the third week of pregnancy and is a continuous process throughout gestation (Tchernia, 1989). Similarly, as the cows recovered most of the values affected by undernutrition during the last 6 months of gestation, the calves could also have restored their blood cell parameters if they were affected.

A gender effect was observed in the platelet series. The female calves exhibited greater values in the MPV and PDW than the male calves ($p < 0.05$). In contrast, Panousis et al. (2018) found that female calves had higher red series values than males, but no differences were observed in the leukocyte and platelet parameters in Holstein calves sampled between 1 and 9 days of life. On the other hand, Tennant, Harrold, Reina-Guerra, Kendrick, and Laben (1974) did not observe any sex-related differences in Jersey and Holstein calves.

The correlations between calf age (days 1 to 11) and the hematological values without and with regard to maternal nutrition

TABLE 4 Hematological parameters of the newborn calves in the first days of life according to the breed, maternal nutrition, and gender

	Breed			Maternal nutrition			Gender			Significance		
	PARDA	PIRENAICA	S.E.D.	CONTROL	SUBNUT	S.E.D.	Female	Male	S.E.D.	Breed	Maternal nutrition	Gender
n	38	21		25	34		32	27				
Live weight (kg)	47.2	39.1	1.63	42.6	43.8	1.32	41.2	45.1	1.38	0.004	ns	0.007
White blood cells (10^3 counts/mm^3)	9.1	7.4	0.7	7.7	8.8	0.67	8.1	8.4	0.71	ns	ns	ns
Lymphoctyes (10^3 counts/mm^3)	3.9	3.0	0.52	3.3	3.7	0.37	3.3	3.6	0.4	ns	ns	ns
Monocytes (10^3 counts/mm^3)	0.10	0.26	0.09	0.24	0.12	0.09	0.17	0.19	0.10	ns	ns	ns
Granulocytes (10^3 counts/mm^3)	5.0	4.3	0.51	4.2	5.1	0.52	4.6	4.7	0.56	ns	ns	ns
Red blood cells (10^6 counts/mm^3)	8.3	8.0	0.49	8.0	8.3	0.33	8.4	7.8	0.35	ns	ns	ns
Hemoglobin (g/dL)	10.8	10.3	0.72	10.5	10.6	0.47	10.9	10.2	0.5	ns	ns	ns
Hematocrit (%)	34.2	31.4	2.61	32.4	33.2	1.48	34.0	31.6	1.59	ns	ns	ns
Mean corpuscular volume (fL)	41.3	39.5	0.94	40.8	39.9	0.88	40.3	40.4	0.60	ns	ns	ns
Mean corpuscular hemoglobin (pg)	13.1	12.9	0.18	13.2	12.8	0.17	12.9	13.1	0.18	ns	0.026	ns
Mean corp. hemoglobin conc. (g/dL)	31.7	32.6	0.44	32.3	32.1	0.22	32.1	32.2	0.23	ns	ns	ns
Red cell distribution width (%)	25.1	24.6	0.57	24.4	25.3	0.54	25.0	24.7	0.57	ns	ns	ns
Platelets (10^3 counts/mm^3)	745.3	738.5	62.0	712.3	771.5	64.0	703.6	780.2	67.0	ns	ns	ns
Mean platelet volume (fL)	6.6	6.6	0.11	6.6	6.6	0.09	6.7	6.5	0.09	ns	ns	0.047
Platelet distribution width (fL)	7.8	7.7	0.22	7.7	7.8	0.2	8.0	7.5	0.22	ns	ns	0.019
Plateletcrit (%)	0.51	0.50	0.04	0.48	0.52	0.04	0.49	0.52	0.05	ns	ns	ns

Note: ns, not significant ($p > 0.05$); *n*, number; CONTROL, 100% fed group; SUBNUT, 65% fed group; S.E.D., standard error of the difference.
No significant interactions among effects were found ($p > 0.05$).

TABLE 5 Correlations between the calf age (days 1–11) and their red and platelet hematological parameters, without and with regard to maternal nutrition

	All calves		CONTROL calves		SUBNUT calves	
	Corr.	Sign.	Corr.	Sign.	Corr.	Sign.
Red cell parameters						
Red blood cells	−0.09	ns	−0.37	ns	0.06	ns
Hemoglobin	−0.16	ns	−0.47	0.017	−0.01	ns
Hematocrit	−0.17	ns	−0.52	0.008	0.01	ns
Mean corpuscular volume	−0.25	ns	−0.59	0.002	−0.13	ns
Mean corpuscular hemoglobin	−0.27	0.041	−0.47	0.019	−0.24	ns
Mean corp. hemoglobin concentration	0.03	ns	0.31	ns	−0.15	ns
Red cell distribution width	0.27	0.043	0.22	ns	0.35	0.043
Platelet parameters						
Platelets	0.62	<0.001	0.69	<0.001	0.61	<0.001
Mean platelet volume	−0.39	0.004	−0.31	ns	−0.48	0.006
Platelet distribution width	−0.16	ns	−0.12	ns	−0.21	ns
Plateletcrit	0.55	<0.001	0.63	0.001	0.54	0.001

Note: ns, not significant ($p > 0.05$); Corr., Pearson's coefficient correlation; Sign., significance; CONTROL, 100% fed group; SUBNUT, 65% fed group.

are displayed in Table 5. No significant correlation between calf age (1–11 days) and any variable of the white series was observed ($p > 0.05$). However, most of the red and platelet hematological parameters were related to calf age. Calf age negatively correlated with the MCH and positively correlated with the RDW. Other studies have shown that the HCT, MCV, and HGB concentrations tend to decrease during the first days of life (Probo et al., 2012). During intrauterine life, the fetus has a relatively hypoxic environment and requires larger erythrocytes to compensate for this situation. During the first days of life, former erythrocytes containing fetal hemoglobin are replaced by new smaller erythrocytes containing hemoglobin A (Brun-Hansen, Kampen, & Lund, 2006). Thus, the decrease in the HGB, HCT, MCV, and MCH values is a physiological process during the first days of life, which was also described by Brun-Hansen et al. (2006). In the platelet series in the current study, both the PLT and PCT increased as the days passed, but the MPV decreased. The correlation between calf age and the PLT agrees with the results of Roland et al. (2014), who described that in correctly developing newborns, the platelet number increases significantly during the first 2 weeks of age and more slowly thereafter over the first 3 months.

Considering maternal nutrition, negative correlations were found between calf age and most of the red cell parameters (HGB, HCT, MCV, and MCH), but only in the CONTROL calves. These results were consistent with the normal physiological development of the calves, which showed a reduction in the red series parameters during the first days of life, suggesting that in the CONTROL newborns, the bone marrow was active and mature enough to start this process immediately after birth. On the contrary, the lack of reduction in the red series in the SUBNUT calves could indicate some delay in the newborn erythropoietic process of replacing fetal erythrocytes. The current study's findings supported the idea that maternal subnutrition during the first third of gestation could trigger a later maturation of the calves' hematopoietic system, although future experiments will be necessary to confirm this.

3.4 | Metabolite and endocrine profiles of newborn calves

The values of the plasma metabolites and hormones of the calves in their first days of life are shown in Table 6 according to breed, maternal nutrition, gender, and type of parturition. No interactions among effects were found. The breed had no significant effects on the calves' metabolite and hormone concentrations, in line with previous studies conducted on cows and calves of the same breeds (Álvarez-Rodríguez & Sanz, 2009; Rodríguez-Sánchez, Sanz, Ferrer, & Casasús, 2018).

Maternal nutrition clearly affected the endocrine profiles, with the CONTROL calves showing higher IGF-1 concentrations ($p < 0.001$) and lower cortisol values ($p < 0.01$) than the SUBNUT calves. Insulin-like growth factor-1 is a hormone involved in muscle growth and is positively related to energy and protein intake (Paradis et al., 2015), which increases its plasma concentration with improved nutritional status (Rodríguez-Sánchez, Sanz, Tamanini, & Casasús, 2015). Similar to previous research, in the current study, the

TABLE 6 Metabolic and endocrine profiles of the newborn calves in their first days of life according to the breed, maternal nutrition, gender, and type of parturition

	Breed			Maternal nutrition			Gender			Parturition				Significance			
	PARDA	PIRENAICA	S.E.D.	CONTROL	SUBNUT	S.E.D.	Female	Male	S.E.D.	UNASSISTED	EASY PULLED	HARD PULLED	S.E.D.	Breed	Maternal nutrition	Gender	Parturition
n	38	21		25	34		32	27		53	3	3					
Glucose (mmol/L)	6.01	5.98	0.268	6.19	5.79	0.260	6.07	5.91	0.295	6.34	5.89	5.73	0.619	ns	ns	ns	ns
Urea (mmol/L)	4.50	3.38	0.463	3.85	4.03	0.415	3.96	3.91	0.475	3.90	4.23	3.68	0.990	ns	ns	ns	ns
NEFA (mmol/L)	0.3	0.3	0.03	0.3	0.3	0.03	0.3	0.3	0.04	0.2^{b}	0.3^{ab}	0.4^{a}	0.08	ns	ns	ns	0.04
IGF-1 (ng/mL)	85.6	82.2	10.90	106.1	61.7	10.40	98.0	69.8	11.81	80.9	58.9	111.9	24.75	ns	0.0001	0.02	ns
Cortisol (nmol/L)	41.9	33.7	12.71	29.0	46.5	5.89	38.7	36.9	6.99	28.1	28.0	57.2	14.13	ns	0.005	ns	0.07

[a-b]Means within a row with different superscripts differ significantly ($p < 0.05$); ns, not significant ($p > 0.05$); n, number; CONTROL, 100% fed group; SUBNUT, 65% fed group; S.E.D., standard error of the difference; UNASSISTED, no assistance in parturition; EASY-PULLED, hand or rope assistance was used in parturition; HARD-PULLED, fetus extractor was used in parturition. No significant interactions among effects were found ($p > 0.05$).

IGF-1 concentration was positively related to the circulating glucose ($r = 0.43$, $p < 0.001$), although no significant differences between the maternal nutrition groups were found in glucose concentrations of the offspring ($p > 0.05$). Hoffman et al. (2016) found no differences in the glucose, triglyceride, and cholesterol concentrations in lambs born to poorly nourished ewes. Conversely, Maresca et al. (2018) described higher glucose concentrations during the first 60 days of life in calves whose mothers had received a low-protein diet from mid-gestation to parturition, supporting the hypothesis of Gardner et al. (2005) that maternal subnutrition during pregnancy could alter the capacity of calves to regulate plasma glucose concentrations during postnatal growth. However, glucose concentrations characterize nutritional status in the short term, and therefore the lack of differences in the current study could indicate that the newborns received a similar diet during their first days of life and their glucose metabolism was not altered. During calf feeding in the first days of life, based only on the maternal colostrum and milk, the CONTROL group could have taken better advantage of the nutritional resources, producing higher IGF-1 concentrations that could have improved their tissue growth and metabolism. Maternal undernutrition may reprogram the fetal IGF-1 system in its ability to respond to acute changes in the substrate supply (Gallaher, Breier, Keven, Harding, & Gluckman, 1998). Similarly, fetal IGF-1 concentration may be altered by maternal nutrition during the earlier stages of development (Rhind, 2004) and the level of protein intake from mid-gestation to parturition can affect calf IGF-1 at birth (Maresca et al., 2018). Accordingly, other authors found a greater reduction in IGF-1 levels in the fetus (Gallaher et al., 1998) and in the lamb (Hoffman, Rokosa, Zinn, Hoagland, & Govoni, 2014) after maternal subnutrition in sheep.

Poor maternal nutrition increased the circulating cortisol levels in the offspring in this study. Cortisol is a hormone synthesized in the adrenal cortex. Its production increases under stress conditions, and consequently it is used as an indicator of stress and animal welfare (Möstl, Maggs, Schrötter, Besenfelder, & Palme, 2002). Cortisol concentrations can also reflect the nutritional state of an animal (Rhind, 2004). In fact, maternal undernutrition can increase the cortisol concentration in the fetus (Binienda et al., 1990) and thus in the newborn calf, in line with the results of the current study. Maternal nutrient restriction can be a cause of prenatal stress, modifying the hypothalamus–pituitary–adrenal function (Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006). Moreover, maternal corticosteroids can induce fetal growth retardation, with lower plasma IGF-1 concentrations. Any delay in fetus development due to maternal undernutrition can lead to a greater fetal cortisol response to undernutrition in late gestation and therefore a greater decrease in IGF-1 (Gallaher et al., 1998). Accordingly, in the current study, a negative correlation between cortisol and IGF-1 ($r = -0.29$, $p < 0.05$) was found in the newborn calves. In fact, the increases in the circulating cortisol level in the SUBNUT calves could have contributed to many metabolic changes and modifications of the immune competency of the newborns.

Regarding the gender effect, surprisingly, the female newborn calves presented higher IGF-1 concentrations than the males

($p < 0.05$), whereas no differences were found in the other metabolic or endocrine parameters according to gender ($p > 0.05$). It is known that in cattle, pre- and post-pubertal plasma IGF-1 concentrations are greater in males than females. Androgens indirectly increase plasma IGF-1 concentrations through increasing plasma growth hormone (GH). However, other authors affirmed that higher IGF-1 concentrations in males are not observed until 3 (Kerr, Manns, Laarveld, & Fehr, 1991) or 4 months of age (Govoni, Hoagland, & Zinn, 2003).

Finally, the type of parturition affected the NEFA concentrations, since the hard-pulled calves presented higher concentrations than the unassisted calves ($p < 0.05$). Furthermore, a tendency was observed in the cortisol concentrations, as the hard-pulled calves showed greater concentrations than the unassisted calves ($p = 0.07$). Negative correlations between the cortisol concentrations and lymphocyte number count ($r = -0.29$, $p < 0.05$) and glucose concentrations ($r = -0.37$, $p < 0.01$) were found. Difficult calving is a stressful situation that increases the plasma cortisol levels in both dams and calves throughout the stimulation of the adrenocorticotropic hormone release (Civelek, Celik, Avci, & Cingi, 2008). As a consequence of the cortisol release, the plasma glucose levels rise due to the increase in liver gluconeogenesis (Drackley, Overton, & Douglas, 2001). In the current study, the calves from dystocic parturitions presented the highest plasma cortisol concentrations, but no statistical differences in the glucose concentrations were found among the groups ($p > 0.05$). Furthermore, a negative correlation was found between cortisol and glucose, suggesting that although the hard-pulled calves should have presented greater glucose concentrations due to their high cortisol concentrations, they did not, most probably due to the low carbohydrate intake of the weakened calves. They needed more time to recover and start ingesting colostrum, and milk later, in the hours after birth. This ingestion delay diminished their glucose and glycogen body reserves. Thus, the calves had to metabolize lipids as an alternative energy source, increasing the NEFA blood concentrations in the calves from dystocic births. Accordingly, a negative correlation was found between the glucose and NEFA concentrations ($r = -0.41$, $p < 0.01$).

3.5 | Vitality test of newborn calves

The relationships between the values of the calf vitality test were assessed immediately after birth, and the breed, maternal nutritional, and type of parturition were analyzed. First, the breed affected the finger suckling reflex, as 95% of the PI calves presented a strong suckling reflex compared to 74% of the PA calves ($p < 0.05$), probably due to the heavier weights at birth registered in the PA breed. This breed effect reflected the higher calf/cow weight ratio at calving (0.08 vs. 0.07 for the PA and PI, respectively, $p < 0.05$). This ratio, used to determine the fetal-maternal disproportion, can compromise the ease of calving (Johanson & Berger, 2003). In the current study, it indicates that the parturition process was less troublesome in the PI than in the PA breed, and thus less traumatic for the newborns. These results were in accordance with those observed in the circulating cortisol concentrations in the newborn calves, although the breed difference was not statistically significant.

Maternal nutrition had no effect on any value of the calf vitality test ($p > 0.05$). High dam BCS at parturition has been described as an important factor that can hinder the parturition process, with negative effects on newborn vitality (Lorenz, Mee, Earley, & More, 2011). Thus, the lack of maternal nutrition effects on the vitality test could be explained because in the current study, no difference in the dam BCS in the last month of gestation was found between the CONTROL and SUBNUT groups.

The type of parturition highly influenced the vitality test results. In general, the parturitions required little assistance, with 53 unassisted, 3 easy-pulled, and 3 hard-pulled parturitions. In the meconium staining test, the unassisted parturitions had the lowest percentage of calves with stained anal areas (2%, 33%, and 33% for unassisted, easy-pulled, and hard-pulled parturitions, respectively, $p < 0.05$). Furthermore, fewer calves with swollen or protruding tongues were in unassisted than in hard-pulled parturitions (2%, 33%, and 100%, for unassisted, easy-pulled, and hard-pulled parturitions, respectively, $p < 0.05$). Most of the calves from the unassisted and easy-pulled parturitions attempted to stand during the calf attitude test (87%, 100%, and 0%, respectively, $p < 0.05$) and had a strong finger suckling reflex (85%, 100%, and 0%, respectively, $p < 0.05$) compared to the calves from the hard-pulled parturitions. Contrarily, the type of parturition did not affect the palpebral test or the mucous membrane color ($p > 0.05$). These results confirmed that after the dystocic births, especially in the hard-pulled parturitions when a fetus extractor was used, the newborns were depressed and had weaker responses to vitality controls, compromising neonatal survival. The premature rupture of the umbilical vessels terminates the oxygen supply from the placenta, first causing respiratory acidosis in the fetus, and if the hypoxia is severe enough, metabolic acidosis later occurs (Murray & Leslie, 2013). Cyanosis of the mucous membranes is a sign of prolonged dystocia, and a weak response or no response to stimulation and poor muscle tone can indicate prolonged and noncompensated acidosis due to fetal hypoxia. Metabolic acidosis is the main cause of suckling reflex loss (Mee, 2008b). Similar to the results of the current study, Schafer and Arbeiter (1995) found that calves with lower vitality test scores had higher plasma cortisol concentrations, with lower levels of lymphocytes and larger neutrophils.

Summarizing the main findings of this study, maternal nutrition in early pregnancy had different breed-related effects on the cow hematological profiles, with the Pirenaica dams showing a higher susceptibility to undernutrition. Furthermore, the results suggest that it could have triggered a later maturation of the fetal hematopoietic system. These cow hematological differences between the maternal nutrition groups, observed in the first third of gestation, disappeared at the end of pregnancy. Few breed differences were found in the neonatal calves, implying that the different hematological profiles observed in the adult cows were not congenital but developed later in life. Dam undernutrition definitely affected the newborn IGF-1 and cortisol concentrations. Furthermore, newborn vitality was highly affected by the parturition type, as the dystocic calves had weaker physiological responses. In conclusion, maternal nutrition had a short-term effect on cow hematology, the PI cows showing

a higher susceptibility to undernutrition; and a long-term effect on offspring endocrinology, SUBNUT newborns showing lower levels of IGF-1 and higher levels of cortisol.

The physiological mechanisms by which maternal subnutrition during the peri-implantation period influenced the hematological, metabolic, and endocrine values of the offspring remain unclear. Further research in this area is necessary to better understand the breed-related adaptive responses coupled with the findings of the current study.

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Maternal nutrient restriction in early pregnancy increases the risk of late embryo loss despite no effects on peri-implantation interferon-stimulated genes in suckler beef cattle

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ABSTRACT

Reducing feeding costs in suckler beef herds to improve economic returns could have detrimental impacts on fertility. This study sought to determine whether maternal nutrient restriction during early pregnancy affects interferon-stimulated gene (ISG) expression in peripheral blood mononuclear cells during the peri-implantation period in two beef cattle breeds. Relationships were also examined between subnutrition and pregnancy failure defined according to ISG fold changes on Days 18 and 21 and to plasma pregnancy specific protein B (PSPB) concentrations on Day 28 post-artificial insemination (AI). Pirenaica or Parda de Montaña dams were assigned to a control (n = 23) or subnutrition (n = 30) group, receiving 100% or 65% of their estimated nutritional requirements from Day 1 to 82 post-AI, respectively. Treatment did not affect ISG expression or fertility. According to ISG fold changes (chi-square P = .023) or PSPB levels (chi-square P = .04) recorded in the subnutrition group, late embryo loss was more likely than in controls. Positive correlation was detected between Day 28 PSPB concentrations and both Day 18 *MX1*, *MX2* and *ISG15* expression, and Day 21 *OAS1* expression. *OAS1* and *MX1* fold changes were found to be the best variables to discriminate pregnancy status. Our findings indicate that maternal nutrient restriction during the first third of pregnancy does not impair embryo signalling yet may increase the risk of pregnancy failure.

1. Introduction

The productivity of livestock enterprises is determined as much by genetic factors as by management factors such as nutrition or environmental conditions (reviewed by Chavatte-Palmer et al., 2018). The economic feasibility of beef cattle herds relies on reduced feeding costs along with the good reproductive performance of dams and high growth rates of their offspring. However, feeding costs minimized through diet restriction or low quality grazing resources could have negative impacts on reproduction (Sanz et al., 2004). A negative energy balance during pregnancy has dramatic effects on postnatal development through its direct influence on foetal growth rate and calf endocrine regulation with detrimental consequences on carcass quality (Long et al., 2009; Funston et al., 2010a; Wang et al., 2015; Lemaster et al., 2017; Taylor et al., 2018; Noya et al., 2019).

During the peri-implantation period, the developing conceptus relies on histotroph secretion into the uterine lumen (Gray et al., 2002; Bazer et al., 2009). Such secretions consist of a complex mixture of nutrients, enzymes, growth factors, hormones, and transport proteins regulated by embryo-maternal crosstalk (Groebner et al., 2011; Bazer et al., 2012; Forde et al., 2014). Pregnancy establishment requires luteal progesterone, which stimulates uterine receptivity for conceptus implantation and development (Spencer et al., 2016), and the pregnancy recognition signal interferon tau (IFN-τ), which orchestrates luteotropic and immune mechanisms for successful embryo implantation (Roberts et al., 1992; Mann and Lamming, 2001). Interferon tau is released by trophoblast cells and induces temporal changes perceptible in local and peripheral tissues (Binelli et al., 2001; Gifford et al., 2007; Pugliesi et al., 2014; Ruhmann et al., 2017). Accordingly, poor embryo signalling during this critical period may lead to pregnancy loss (Garrett

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et al., 1988; Matsuyama et al., 2012; Wijma et al., 2016). During this period of IFN-τ release, the antiviral genes, interferon-stimulated genes (ISGs), undergo short-lived activation (Roberts, 2007). In effect, maternal blood ISG profiles have proved to be an excellent tool to assess conceptus viability during the peri-implantation period in cattle (Green et al., 2010a; Matsuyama et al., 2012; Ahmad Sheikh et al., 2018).

The embryonic period of gestation extends from conception to the end of the differentiation stage (about 45 days) and the foetal period extends from Day 45 to parturition (Committee on Bovine Reproductive Nomenclature, 1972). Pregnancy rates in beef cattle are 50–60%, slightly higher than for dairy cattle (Bridges et al., 2013). However, in both beef and dairy herds, most pregnancy losses occur during the embryonic period (Sreenan and Diskin, 1983). While intrinsic factors in the embryo can reduce fertility (Lonergan et al., 2016), a suboptimal uterine micro-environment has been strongly linked to pregnancy failure (Bazer et al., 2015). Effectively, a large body of literature exists describing the deleterious consequences of maternal undernutrition during the earlier stages of pregnancy on conceptus and placental development (Long et al., 2009, 2010; Wang et al., 2015; Kruse et al., 2017; McLean et al., 2018; Taylor et al., 2018).

The main factors reported to affect the reproductive performance of suckler beef cows are those related to feeding management, calving season, dam breed and calf suckling frequency (Sanz et al., 2004). The present study examines the effects of subnutrition during the first third of pregnancy in two local beef breeds widely distributed in the Spanish Pyrenees, Parda de Montaña (PA) and Pirenaica (PI). The former breed arises from the ancient Brown Swiss cow and its crosses with autochthonous breeds, while PI is a hardy autochthonous breed. To determine the effects of subnutrition we examined: 1) ISG expression in peripheral blood mononuclear cells (PBMCs) 18 and 21 days after artificial insemination (AI) in cows of the two breeds subjected to nutrient restriction from Days 1–82 post-AI; and 2) relationships between subnutrition and pregnancy failure, which was confirmed by ISG fold changes recorded on post-AI Days 18 and 21 and by plasma pregnancy specific protein B (PSPB) concentrations recorded on post-AI Day 28.

2. Materials and methods

2.1. Cattle and herd management

This study was performed at La Garcipollera Research Station in the mountainous area of the central Pyrenees (northeastern Spain, 945 m a.s.l.) from September 2014 to March 2015. Cows were recruited from a large experimental suckler cattle herd including both PA and PI breeds. A full description of the animals included in this study can be found in Noya et al. (2019). The study population was comprised of 53 healthy multiparous cows (7.5 ± 3.5 years) with suckling calves, 34 PA and 19 PI. Exclusion criteria were: mastitis, lameness, digestive disorders and pathological abnormalities of the reproductive tract detectable by ultrasonography.

2.2. Experimental design

All procedures were approved by the Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón. Dams were handled in strict accordance with the guidelines of the European Union (Directive 2010/63/E.U.) on the protection of animals used for experimental and other scientific purposes (E.U., 2010).

Dams were artificially inseminated after a nine-day progesterone (P4)-based synchronization protocol at 65 ± 14 days post-partum. Briefly, cows were treated with a P4 releasing intravaginal device (PRID-Delta, containing 1.55 g of P4; CEVA, Loudéac, France) plus GnRH (10 μg i.m. Busol, INVESA, Barcelona, Spain). Seven days later, animals were also given PGF2α (150 μg Galapán, INVESA, Barcelona, Spain). After a further 48 h, the PRID was removed and animals were injected with equine chorionic gonadotropin (500 IU Serigan, Laboratorios Ovejero, León, Spain). Forty-eight hours after this first injection, the cows received a second GnRH dose. Cows were randomly inseminated by an expert technician eight hours later using semen from corresponding bulls of proven fertility (4 PA and 3 PI).

Table 1
Ingredients of feedstuffs used in the experiment (on an as-fed basis).

Ingredients	% of dietary dry matter
Alfalfa hay	25.0
Cereal straw	25.0
Crushed barley	25.0
Dehydrated alfalfa	10.0
Rapeseed meal	6.5
Citrus pulp	4.5
Soybean meal	2.5
Correctors (calcium carbonate, dicalcium phosphate, sodium chloride, vitamins, and trace elements)	1.5

On the day of AI, dams were randomly allocated to two dietary treatments for the first 82 days of gestation. Dams were fed a diet that met 100% of their energy and protein requirements for maintenance, lactation and gestation (10.9 and 10.0 kg dry matter (DM)/cow/day for PA and PI, respectively) (CONTROL; $n = 23$), or were restricted (SUBNUT; $n = 30$) to 65% of requirements of both protein and energy (7.0 and 6.4 kg DM/cow/day for PA and PI, respectively) (Table 1). Live weight (559 ± 11 kg and 564 ± 12 for SUBNUT and CONTROL, $P = .7912$) and BCS (2.82 ± 0.05 and 2.75 ± 0.06 for SUBNUT and CONTROL, $P = .3529$) were similar in both groups on the day of AI. Feed was provided at 08:00 a.m. and cows were tied up for maximum 2 h until they finished the restricted amount assigned to each one. During the experiment, all cows and calves were loose housed. From Day 83 post-AI until parturition all cows were fed complete rations. Dams were weighed fortnightly. The average daily gain (ADG) was calculated by linear regression. Dam BCS was registered monthly by two expert technicians, based on the estimation of fat covering loin, ribs, and tailhead.

Pregnancy diagnosis was performed by ultrasonography using a linear-array 7.5 MHz transducer (Aloka SSD-500 V, Aloka, Madrid, Spain) on Day 37 post-AI and confirmed at 90 days post-AI. Late embryo mortality was assumed in dams classified as pregnant according to the ISG fold change produced from Day 18 to Day 21 that were diagnosed as non-pregnant by ultrasonography on Day 37 post-AI. This assumption was contrasted with embryo mortality predicted by PSPB concentrations on Day 28. Late embryo/foetal loss was recorded when the 90-day diagnosis proved negative.

2.3. Sample analysis

2.3.1. Blood sample collection

Blood samples were collected from each animal by tail vein puncture into EDTA vacuum tubes (BD Vacutainer™, Becton, Dickinson and Company, Plymouth, UK) on Days 18 and 21 post-AI for PBMC isolation (8 mL) and on Day 28 for plasma PSPB determination (4 mL). Samples were placed immediately on ice, and those for PSPB determination were centrifuged (3500 rpm for 20 min at 4 °C) within 30 min of collection and the plasma stored at −20 °C until analysis.

2.3.2. Pregnancy specific protein B assay

Plasma PSPB concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) kit BioPRYN® (BioTracking Inc., Moscow, Russia) following the manufacturer's instructions. Assay sensitivity was 0.22 ng/mL. Intra- and inter-assay coefficients of variation respectively were: 6.3% and 10.4% for a plasma pool of 2.2 ng/mL, and 5.3% and 6.7% for a plasma pool of 1.3 ng/mL. Baseline levels

calculated in 30 plasma samples from 10 non-pregnant cows were 0.34 ng/mL. Based on optical density (OD) standards for the assay, dams showing plasma PSPB concentrations < 0.6 ng/mL were recorded as non-pregnant, those with concentrations > 1.1 ng/mL as pregnant and those with concentrations between 0.6 and 1.1 ng/mL as being at risk of pregnancy loss (Gábor et al., 2016).

2.3.3. PBMC isolation, RNA extraction and cDNA synthesis

Peripheral blood mononuclear cells were isolated by centrifugation on a Ficoll density gradient (Histopaque, Sigma, St Louis, MO) followed by repeated rinsing in phosphate buffered saline (PBS). Isolated mononuclear cells were lysed in Trizol™ (Invitrogen Corp., Carlsbad, CA, USA) and kept at −80 °C until RNA analysis. Total RNA was extracted according to the method of Chomczynski and Sacchi (1987). RNA concentrations were determined spectophotometrically. Samples were treated with DNAse in the presence of RNAse inhibitors to eliminate contaminating genomic DNA. Complementary DNA (cDNA) was synthesized in a total volume of 20 µL from 1 µg of total RNA in the presence of random primers and reverse transcriptase using the RevertAid H Minus First Strand cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's recommendations.

2.3.4. Quantitative real-time PCR

Messenger RNA expression was determined by quantitative real-time PCR (qPCR) for four target genes—*interferon-stimulated gene 15 (ISG15), 20–50-oligoadenylate synthase 1 (OAS1), myxovirus resistance 1 (MX1)*, and *myxovirus resistance 2 (MX2)*—and two reference genes—*β-actin (ACTB)* and *ribosomal protein L19 (RPL19)* (Table 2). To avoid gene contamination, all primers were selected to span an intron. For each gene, a standard curve was generated by amplifying serial dilutions of a control cDNA sample to check for linearity between initial template concentration and cycle threshold (Ct) values. Amplification was conducted using the SYBR green method of the ABI PRISM™ 7500 sequence detector (Applied Biosystem, Foster City, CA, USA) under the conditions recommended by the manufacturer: an initial activation and denaturation step of 10 min at 95 °C followed by 40 cycles consisting of 10 s at 95 °C and 1 min at 60 °C. PCR reactions were run using 3 µL of 30-fold diluted cDNA as template in a total volume of 8 µL containing 1 × Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Waltham, MA, USA), and 200 nM of forward and reverse primers as reported elsewhere (Serrano-Pérez et al., 2016). Each PCR was run in triplicate and the average used to calculate the relative gene amount. Data were normalized and analysed by the $2^{-\Delta\Delta Ct}$ method using the mean Ct value obtained for the two reference genes and the Ct values for each ISG primer (Schmittgen and Livak, 2008).

2.4. Statistical analysis

The following data were recorded for each animal: parturition and AI date; dam age (two categories: 5–10 years old *vs.* > 10 years old); breed (PA *vs.* PI), treatment (CONTROL *vs.* SUBNUT), *ISG15*, *OAS1*, *MX1* and *MX2* gene expression in PBMCs on Days 18 and 21 post-AI, plasma PSPB concentrations on Day 28 post-AI and pregnancy status (pregnant *vs.* non-pregnant) on Day 90 post-AI.

Data were analysed using the SAS and JMPro package (SAS Institute Inc., Cary, NC). When necessary, data were logit transformed to meet the assumption of normality and homoscedasticity. The normality of data was confirmed by the UNIVARIATE procedure ($P > .05$). Gene expression of ISG on Days 18 and 21 was analysed through analysis of variance using a general linear model (GLM) and dam age, breed, pregnancy status, treatment and their interactions as fixed effects. Multiple comparisons among treatments were performed using Tukey's test.

The Spearman's rho (sr) test was used to identify possible relationships between ISG expression on Days 18 and 21 and plasma PSPB concentrations on Day 28. Decision trees were constructed to explore the importance of the four ISG to explain the pregnancy status using the partition modelling option in JMPro. The partition algorithm searched all possible splits of predictors to best predict the response (pregnancy status). These splits (or partitions) of the data were done recursively to form a tree of decision rules. The variables that explain better the response were selected from the four ISG according to G2 (likelihood-ratio chi-square) test of association and logworth ($-\log(p\text{-value})$) value. The logworth values are the logs of adjusted *p*-values for the chi-square test of independence. Goodness of prediction of the decision three was obtained by a 3-fold cross-validation. Chi-square tests were used to assess associations between pregnancy failure (according to the ISG fold change produced from Day 18 to Day 21 and PSPB concentrations on Day 28) and factors of interest (age, breed and treatment). Data are expressed as the least square (LS) means. Significance was set at $P < .05$.

3. Results

Treatment affected significantly cow average daily gain (ADG; $P < .001$) and body condition score change (BCS; $P < .05$) from AI to Day 82 post-AI. CONTROL cows gained weight and maintained BCS (+0.214 ± 0.10 kg/d and + 0.083 ± 0.073 points BCS/month) whereas SUBNUT cows loss weight and BCS (−0.650 ± 0.09 kg/d and − 0.107 ± 0.062 points BCS/month). The ADG and BCS change were similar in both breeds with no treatment x breed interaction ($P = .6254$ and $P = .9090$ for ADG and BCS respectively).

Table 2
Sequence, NCBI sequence and reference of the primers used for quantitative PCR.

Gene		Sequence (5′ – 3′)	NCBI sequence	Reference
ISG15	Fwd Rev	CGCAGCCAACCAGTGTCT CGTCATGGAGTCCCTCAGA	NM_174366.1	Paradis et al., 2015
OAS1	Fwd Rev	TCATCCGCCTGGTGAAGCACTGG TTGCTCCCAGGCATAGACCGTCAG	NM_001040606.1	Manjari et al., 2016
MX1	Fwd Rev	GTACGAGCCGAGTTCTCCAA ATGTCCACAGCAGGCTCTTC	AF047692	Ribeiro et al., 2014
MX2	Fwd Rev	CTTCAGAGACGCCTCAGTCG TGAAGCAGCCAGGAATAGTG	NM_173941	Ribeiro et al., 2014
ACTB	Fwd Rev	CTGGACTTCGAGCAGGAGAT GATGTCGACGTCACACTTC	AY141970	Monteiro Jr. et al., 2014
RPL19	Fwd Rev	GATCCGGAAGCTGATCAAAG ATTCGAGCATTGGCAGTACC	NM_001040516.1	Serrano-Pérez et al., 2016

Over the study course, 35 of the 53 cows (66%) enrolled became pregnant (n = 14 CONTROL, n = 21 SUBNUT). Of the remaining 18 non-pregnant dams (n = 9 CONTROL, n = 9 SUBNUT), 17 were open at the time of pregnancy diagnosis on Day 37, and one dam was diagnosed as suffering late embryo/foetal loss on Day 90. Mean plasma PSPB concentrations in dams diagnosed as pregnant were 2.35 ± 1 ng/mL. In contrast, PSPB concentrations were under 1.1 ng/mL in 12 of the non-pregnant dams and just over 1.1 ng/mL (1.3 ± 0.45) in the remaining six.

No significant effects were observed for treatment (P = .36), breed (P = .06) or dam age (P = .30) on pregnancy status. Plausible interactions such as breed x treatment or dam age x treatment were not detected.

3.1. Factors affecting ISG mRNA expression

No effects of maternal nutrient restriction or breed on ISG expression were observed. The factors found to significantly affect ISG expression were sampling day, pregnancy status and dam age. Levels of *MX1*, *MX2* and *ISG15* were significantly increased on Day 21 compared to Day 18 (P < .001 for all genes). Higher *MX2* and *ISG15* expression were observed in pregnant than non-pregnant cows (P = .001, P < .001, respectively). Expression levels of *OAS1* were higher and increased between Day 18 and 21 in the pregnant *versus* non-pregnant cows (P < .001) (Fig. 1). Dams aged 5 to 10 years old showed significantly higher expression of *MX1* than dams > 10 years (P < .05) (data not shown).

3.2. Predicting embryo loss

Significant positive correlations were observed between Day 28 PSPB concentrations and Day 18 *MX1*, *MX2* and *ISG15* expression levels (sr: 0.64, sr: 0.45, sr: 0.65, respectively; $P \leq .001$) or Day 21 *OAS1* expression (sr: 0.59; P < .001).

The decision tree developed to predict pregnancy status on Day 37 included the factor fold changes in *MX1, MX2, OAS1* and *ISG15* expression from Days 18 to 21 post-AI. The final decision tree contains 2 nodes (Fig. 2). The combined cutoffs *OAS1* fold change > 3.91 and *MX1* fold change < 0.42 served to define pregnancy status (R^2 = 0.30, Fig. 2). *OAS1* fold change was the first variable able to discriminate between pregnant and non-pregnant dams. Dams exhibiting an increase of *OAS1* fold change higher that 3.91 between Days 18 to 21 were classified as pregnant. In the remaining cows, a node with dams exhibiting a decrease of *MX1* fold change higher than 2.38 (1/0.42) were also classified as pregnant. Out of the 36 dams classified as pregnant, 32 were confirmed as positive on Days 37 and 90. Four dams that fulfilled these thresholds but were diagnosed as non-pregnant on Days 37 and 90 belonged to the SUBNUT group (one PI and three PA dams), and were assumed as suffering pregnancy loss. Two of these dams also showed PSPB concentrations higher than 1.1 ng/mL, and one dam between 0.6 and 1.1 ng/mL. The remaining non-pregnant dams (n = 14) were classified as non-pregnant in the partition analysis, although three of these dams showed PSPB concentrations between 0.6 and 1.1 ng/mL (n = 1) or higher than 1.1 ng/mL (n = 2) on Day 28 (Fig. 2).

A significant association was observed between nutrition level and embryo loss according to ISG fold change (chi-square P = .028) or PSPB concentration (chi-square P = .04). In the group of cows whose nutrition was restricted (SUBNUT), embryo loss was more likely than in the control group (Table 3). Plausible interactions such as breed x embryo loss or dam age x embryo loss were not detected (P > .05).

4. Discussion

This study was designed to examine embryo-maternal crosstalk during the peri-implantation period in beef cows subjected to restricted nutrient intake (from Days 1 to 82 post-AI) by determining the relative expression of ISG in PBMCs on Days 18 and 21 post-AI. Our main findings were that: 1) maternal undernutrition affected neither ISG expression nor fertility, and 2) that, based on ISG fold changes and PSPB concentrations, pregnancy failure was more likely to occur in dams subjected to nutrient restriction.

During early gestation, nutrient demands for embryo growth are lower than in later stages. However, the developing conceptus requires an adequate nutritional uterine microenvironment for normal growth (Groebner et al., 2011; Crouse et al., 2016). The short-term consequence here of 65% nutrient restriction was unimpaired embryo signalling, as neither ISG expression nor fertility were affected. Despite dramatic effects of subnutrition on embryo growth during early stages of pregnancy (Long et al., 2009; Micke et al., 2010; Taylor et al., 2018), our results suggest that most dams were able to develop adaptive responses to guaranty blastocyst formation and pregnancy maintenance (Velazquez, 2015). Similar observations were made in beef heifers subjected to 50 days of 40% nutrient restriction (McLean et al., 2018). However, Kruse et al. (2017) found that 50–80% nutrient restriction for as little as 6 days post-AI in beef heifers gave rise to poorer quality embryos. Similarly, Dunne et al. (1999) detected a significantly reduced embryo survival rate when beef heifers were shifted from high to low herbage allowance for a 10-day period after AI. Older cows seem more capable of supplying nutrients to the conceptus during early pregnancy compared with younger cows (Long et al., 2009; McLean et al., 2018;

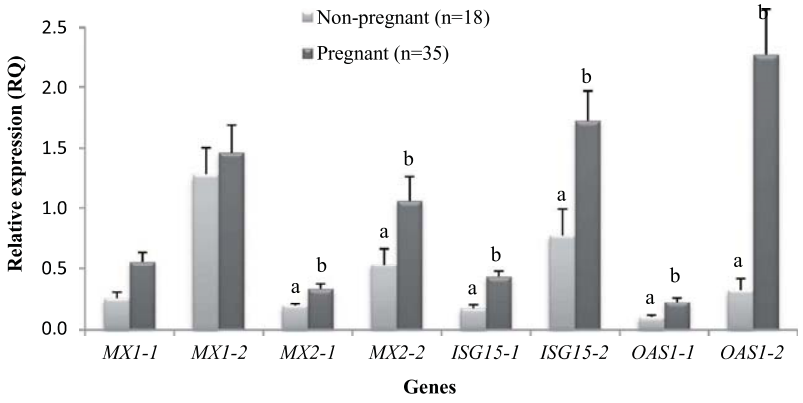

Fig. 1. Relative ISG expression levels recorded in peripheral blood mononuclear cells (PBMC) on Days 18 (1) and 21 (2) post-AI in cows diagnosed as pregnant or non-pregnant on Day 90 post-AI. Bars indicate the mean RQ value ± SEM. [a,b] Statistically significant differences when different letters among groups.

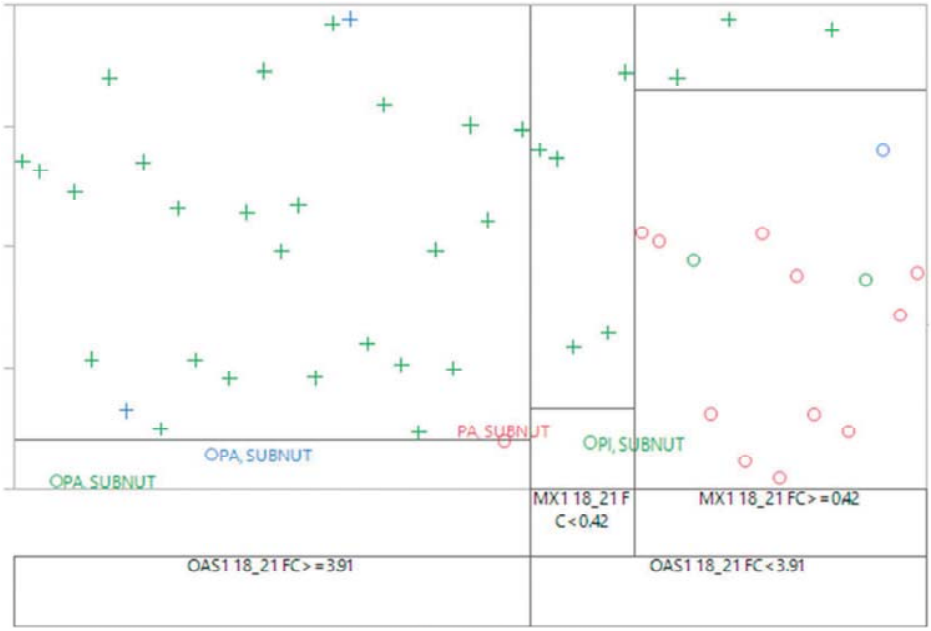

Fig. 2. Binary partition tree. Pregnancy status on Day 90 post-AI represented in the x-direction (+: pregnant, o: non-pregnant) according to partition tree thresholds represented in the y-direction. Colour of point is related to cow's PSPB (ng/mL) on Day 28 post-AI: Red for ≤0.6; Blue for 0.6–1.1; Green for > 1.1. FC: Fold Change, PA: Parda de Montaña, PI: Pirenaica. SUBNUT: diet restricted to 65% of protein and energy requirements during the first 82 days post AI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Proportions of non-pregnant cows and cows suffering embryo loss as defined by fold changes in ISG (A) or concentrations of PSPB (B) according to whether nutrition was restricted (SUBNUT) or complete (CONTROL) within 82 days of AI.

A)	Nutrition	
ISG-defined status	CONTROL	SUBNUT
Non-pregnant	9 (100%)	5 (55.6%)
Embryo loss	0 (0%)	4 (44.4%)

B)	Nutrition	
PSPB-defined status	CONTROL	SUBNUT
Non-pregnant	8 (88.9%)	4 (44.4%)
Embryo loss	1 (11.1%)	5 (55.5%)

Taylor et al., 2018). Some authors argue that a more extended subnutrition period is needed for any perceivable deleterious effects on embryo development (McLean et al., 2018). However, changes in embryo metabolism under these circumstances may result in re-programming of foetal and post-natal development (Funston et al., 2010b). Recently, we observed long-term effects on hormone profiles in calves born from dams that were undernourished in early pregnancy (Noya et al., 2019).

Maternal undernutrition may influence fertility *via* direct effects on the reproductive tract (Hill et al., 1970; Kruse et al., 2017). However, it is unknown to what extent the embryo's metabolism can be down-regulated before it becomes incompatible with life preservation (Leese, 2002). In the present study, as indicated by ISG expression and plasma PSPB levels, an increased risk of pregnancy failure was observed in the subnutrition group especially in PA dams. In dairy cattle, a negative energy balance has been shown to compromise oocyte quality (Snijders et al., 2000). In our experiment, as subnutrition commenced at AI, pregnancy failure in undernourished dams was unlikely due to poor oocyte quality. According to Wiltbank et al. (2016), varying body condition scores in dairy cows from calving to AI play a major role among numerous other factors in increasing the risk of pregnancy loss in the second month of gestation. However, the cows in our trial were fed according to their needs before AI such that no negative energy balance or dramatic change in BCS would be expected (similar mean body condition score changes close to 0 were observed in control and subnutrition group dams; data not shown). Despite the long-term carryover effect of a negative energy balance on reproductive performance (Diskin et al., 2016), post-AI maternal subnutrition seemed here to produce negative effects mainly at later stages of embryo development.

Our findings suggest that PI dams were more able to develop adaptive mechanisms that guaranteed embryo survival during dietary restriction than PA dams. Differences might be attributed to genetic background differences between these two cattle breeds (Villalba et al., 2000; Casasús et al., 2002; Sanz et al., 2003). These differences lead to a higher milk production potential and intake capacity of PA dams during the post-partum period (Casasús et al., 2002), but in consequence, to a higher susceptibility to calf suckling (Sanz et al., 2003; Álvarez-Rodríguez and Sanz, 2009). Interbreed differences have been also observed in the haematological profile response to undernutrition (Noya et al., 2019).

Lastly, as anticipated, the detection of ISGs in maternal blood emerged here as an excellent measure of embryo viability in cattle (Sheikh et al., 2018; Yoshino et al., 2018). Given the trophoblast origin of both IFN-τ and pregnancy associated glycoproteins, positive correlation was observed between PSPB concentrations on Day 28 and both *MX1*, *MX2* and *ISG15* expression on Days 18 and *OAS1* expression on Day 21. Probably, dams classified as non-pregnant with PSPB levels higher than 0.6 ng/mL suffered failure of pregnancy establishment. Decision trees are a reliable and effective decision making technique in medicine (Podgorelec et al., 2002). In our study, dams were classified as

pregnant or non-pregnant according to a partition tree threshold based on ISG fold changes and differentiated according to various decision classes (treatment, breed and PSPB concentration). Since older cows seem to show a lesser response to implantation signals received from the conceptus (Green et al., 2010a), *OAS1* and *MX1* fold changes were found to be the best variables to discriminate pregnancy status in multiparous beef cattle. Previous reports have described the efficiency of OAS1 as biomarker to assess the presence of a viable conceptus in beef (Pugliesi et al., 2014) or dairy cattle (Green et al., 2010b).

5. Conclusions

As an overall conclusion, our findings indicate that maternal nutrient restriction in suckler beef cattle does not influence conceptus signalling during the peri-implantation period. However, pregnancy failure was more likely in dams subjected to nutrient restriction in early pregnancy.

Declaration of Competing Interest

None.

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Long-Term Effects of Maternal Subnutrition in Early Pregnancy on Cow-Calf Performance, Immunological and Physiological Profiles during the Next Lactation

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Simple Summary: Early pregnancy is a crucial stage in the fetus development. In this phase, from undifferentiated cells, equal to each other, tissues and organs start to develop. Nutrition and metabolic status of the cow during pregnancy affect the intrauterine environment and the nutrient source for the fetus. Therefore, cow diet during early pregnancy affects the fetus development and could have long-term consequences on the future calf. In this study, we assessed the effects of a poor maternal diet during the first third of gestation on the performance of cows and calves during the next lactation, as well as the effect on the transfer of immunity from cow to calf. We used Parda de Montaña and Pirenaica cow-calf pairs, two Spanish autochthonous beef breeds. We concluded that maternal undernutrition reduced the body fat reserves of cows at calving, which affected most of the cow productive parameters and the colostrum immunoglobulin concentration. Furthermore, poor maternal diet altered the calf development and metabolic status, with reduced size and weight at weaning, especially in the Pirenaica breed, which prioritized the cow maintenance instead of the calf growth.

Abstract: This study aimed to evaluate the effects of undernutrition during the first third of gestation on cow-calf performance, immunological and physiological profiles during the next lactation in two cattle breeds. Fifty-three Parda de Montaña (PA) and 32 Pirenaica (PI) cows were inseminated, assigned to one of two diets (CONTROL or SUBNUT; 100% or 65% of their requirements) until day 82 of gestation, and fed 100% of the requirements during gestation and next lactation. Cow and calf performance were assessed during lactation. Colostrum and cow-calf plasma samples were analyzed to assess the passive transfer of immunoglobulins and to characterize energy metabolism. At calving, SUBNUT cows had a lower body condition score, which impaired most of the cow-calf parameters. All cows had considerable weight losses during lactation except for SUBNUT-PI cows. Colostrum immunoglobulin G (IgG) concentration was lower in SUBNUT-PI cows, and milk fat content was higher in SUBNUT cows. SUBNUT calves had lower values of body measurements at weaning, and calves born from SUBNUT-PI dams had lower milk intake and the lowest average daily gain (ADG), which was reflected in their lower plasma insulin-like growth factor-1 (IGF-1) concentration. In conclusion, undernutrition in early gestation in suckler cows had long-term effects on offspring postnatal growth, this physiological evidence being more severe in Pirenaica cow-calf pairs.

Keywords: prenatal undernutrition; beef cattle; colostrum; passive transfer immunity; IgG; IgM; IGF-1

1. Introduction

Undernutrition periods are frequent in beef cattle production systems. To reduce feed costs, cattle have had to adapt to low-cost diets or to extensive management, where animal intake depends mostly on food availability. A poor diet because of low quality or low quantity affects the cow production cycle, for example, the peri-implantational period and early gestation. The impact of subnutrition and a poor uterine environment on the embryo/fetus during the first stages of gestation are not fully understood. Long et al. [1] described no differences in calf birth weight in underfed dams during early gestation, whereas Micke et al. [2] reported reduced calf birth weight in nutrient restricted heifers during the first two thirds of gestation. The peri-implantational period is a vulnerable phase, during which adverse programming mediated through poor maternal nutrition might begin [3–5]. Under adverse intrauterine conditions, the fetus could permanently modify some endocrine functions to ensure its survival [6]. The structure, physiology and metabolism of different organs and systems could be modified, leading to detrimental postnatal metabolic changes [7] and predisposing offspring to cardiovascular, metabolic and endocrine diseases in later life [8]. Cow energy status during gestation could also have an effect on the adaptive immune response of their progeny. Different studies evaluated the effect of restricted maternal diet during mid [9] or late [10,11] gestation on passive immunity of beef cattle; however, little is known about the effects of maternal feed restriction in early pregnancy.

Furthermore, these effects could vary depending on the genetic background. Differences in the response to different feeding management have been reported between Parda de Montaña (PA) and Pirenaica (PI) breeds [12,13], which are the two main beef cattle breeds adapted to a semi-extensive system of animal husbandry in the Pyrenees mountain region (northern Spain).

Our hypothesis was that a poor nutritional diet during early pregnancy could adversely impact the passive transfer of immunity from dam to calf, and offspring physiology and growth during its postnatal life, and this effect would be modulated by cow genotype. Therefore, this study aimed to analyze the effects of undernutrition during the first third of gestation on cow-calf performance and their immunological, metabolic and endocrine profiles throughout the following lactation in PA and PI cow-calf pairs.

2. Materials and Methods

All procedures were approved by the Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón. The care and use of animals were performed in accordance with the guidelines of the European Union (Directive 2010/63/E.U.) regarding the protection of animals used for experimental and other scientific purposes [14].

2.1. Animals, Management and Diets

This study was conducted at La Garcipollera Research Station, in the mountain area of the Pyrenees (northeastern Spain, 945 m a.s.l.), from December 2014 to June 2016. Fifty-three PA and 32 PI multiparous cows were synchronized to estrus with a protocol based on a progesterone-releasing intravaginal device (PRID Delta 1.55 g, CEVA, Loudéac, France) and a 10 μg injection of gonadotropin-releasing hormone (GnRH, Busol, INVESA, Barcelona, Spain), followed 7 days later by 150 μg of prostaglandin F2α (Galapán, INVESA, Barcelona, Spain). The PRID was removed after 9 days, and a 500 IU injection of pregnant mare serum gonadotropin (Serigan, Laboratorios Ovejero, León, Spain) was administered. A second 10 μg injection of GnRH was administered 48 h later. Eight hours after the second GnRH injection, cows were randomly inseminated with sires of proven fertility (4 PA males for the PA cows and 3 PI males for the PI cows) by an expert technician. After fixed-time artificial insemination (AI), cows were distributed into 2 nutritional treatments and fed individually with a total mixed ration (alfalfa hay, 25.0%; cereal straw, 25.0%; crushed barley, 25.0%; dehydrated alfalfa, 10.0%; rapeseed meal, 6.5%; citrus pulp, 4.5%; soybean meal, 2.5%; and vitamin-mineral complex, 1.5%; Table 1) during the first 82 days of pregnancy. The control group (CONTROL, n = 37, 574 ± 8.9 kg live weight (LW); 2.80 ± 0.038

body condition score (BCS) on a 5-point scale) fed a diet that supplied 100% of the estimated energy requirements for cow maintenance, lactation and gestation (10.9 and 10.0 kg DM/cow/d for PA and PI, respectively); and the nutrient-restricted group (SUBNUT, n = 48, 568 ± 7.6 kg LW; 2.86 ± 0.032 BCS) received 65% of their requirements (7.0 and 6.4 kg DM/cow/d for PA and PI, respectively), calculated for a 580 kg beef cow producing 9 kg (PA) or 8 kg (PI) of energy-corrected milk [15]. After this treatment phase, the CONTROL group maintained its LW and BCS (583 ± 8.6 kg LW; 2.90 ± 0.040 BCS), whereas they decreased in SUBNUT animals (538 ± 7.2 kg LW; 2.65 ± 0.033 BCS, $p < 0.001$) [16]. After the first third of gestation, all dams were fed 100% of the requirements during the remainder of gestation and the next lactation, using the same total mixed ration described above. Feed was provided at 8:00 and cows were tied up for maximum 2 h until they finished the restricted amount assigned to each one. During lactation, suckling offspring had a restricted twice-daily nursing system, comprising two 30 min periods at 7:00 and 14:30. Their diets only consisted of colostrum and milk from their respective mothers. Calves were weaned at the age of 120 days.

Table 1. Chemical composition of feedstuffs used in the experiment (on an as-fed basis).

Chemical Composition	
DM (g/kg)	908
CP (g/kg DM)	124
NDF (g/kg DM)	466
ADF (g/kg DM)	253
ADL (g/kg DM)	40
Ash (g/kg DM)	113
ME (MJ/kg DM)	10.96

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid-detergent lignin; ME, metabolizable energy.

2.2. Cow and Calf Performance

Cows and calves were weighed fortnightly to calculate by linear regression the average daily gain (ADG) during lactation. The cow BCS was assessed at calving, in the middle and at the end of lactation by 2 expert technicians, based on the estimation of fat covering the loin, ribs and tailhead [17]. Morphometric measurements of calves were assessed on d 7 and 120 after birth. The variables were height at withers (distance from the floor to the highest point of the withers), height at rump (distance from the floor to the highest point of the internal tuberosity of ilium), rump width (maximum distance between iliac tuberosities), rump length (distance from the ischial tuberosity to the external iliac tuberosity), body length (distance from the cranial side of the shoulder blades to the caudal side of the ischial tuberosity), and heart girth (circumference immediately behind the shoulder blades in a plane perpendicular to the body axis). Cow samples for progesterone plasma analyses were collected into heparinized tubes (BD Vacutainer Becton-Dickenson and Company, Plymouth, UK) twice per week during lactation. Blood samples were centrifuged at 3500 rpm for 20 min at 4 °C immediately after collection. Plasma samples were harvested and frozen at −20 °C until analysis. Plasma progesterone concentration (ELISA test, sensitivity: 0.27 ng/mL) was measured using a specific kit for cattle (Ridgeway Science, Lydney, UK). The mean intra-assay and inter-assay coefficients of variation were 8.0% and 10.4%, respectively. The onset of luteal activity in cows after calving was considered when progesterone concentration was >1 ng/mL. If cows had not ovulated prior to the end of lactation (d 120), the interval to first ovulation after calving was regarded as this date [18].

2.3. Colostrum and Milk Composition, Milk Yield and Calf Milk Intake

Colostrum samples were manually collected twice: in Period 1 (from 0 to 12 h postpartum) and in Period 2 (from 12 to 24 h postpartum). Samples from each udder quarter were pooled. On d 23 postpartum, milk yield was recorded by the oxytocin and machine milking method 6 h after calf removal [19]. Fat, protein, lactose and somatic cell count of colostrum and milk were analyzed with an

infrared scan (Milkoscan 4000; Foss Electric Ltd., Hillerød, Denmark). Milk data for fat and protein content were used to calculate energy-corrected milk (ECM) yield (adjusted to 3.5% fat and 3.2% protein [20]).

On d 25 and 120 postpartum, calf milk intake was estimated by the weigh–suckle–weigh method. Calves were weighed before and after one 30 min suckling period each in the morning (7:00) and afternoon (14:30). The calf daily milk intake was the sum of the weight differences of the 2 suckling periods (adapted from Rodrigues et al. [21] and Shee et al. [22]).

2.4. Immunoglobulin Concentration in Colostrum and Cow-Calf Plasma

To determine plasma immunoglobulin (Ig) G and IgM concentration in cows, blood samples were collected into EDTA tubes (BD Vacutainer Becton-Dickenson and Company, Plymouth, UK) at d 253 post AI (approximately 1 month before calving) and at calving. Colostrum samples were collected at Period 1 (0–12 h postpartum) and Period 2 (12–24 h postpartum), and calf blood samples were taken 48 h after birth (adapted from McGee et al. [11]). Blood samples were centrifuged at 3500 rpm for 20 min at 4 °C and plasma and colostrum samples were frozen at −20 °C until analysis. Concentration of IgG and IgM (ELISA bovine test, sensitivity: 4.8 ng/mL for IgG and 4.5 ng/mL for IgM) were determined using a specific bovine kit (Bovine IgG ELISA Quantitation Set, Cat.No. E10-118; and Bovine IgM ELISA Quantitation Set, Cat.No. E10-101; Bethyl, Montgomery, TX, USA). Plasma samples were diluted at 1:300,000 and 1:20,000 for the IgG and IgM analysis, respectively, and colostrum samples were diluted at 1:500,000 and 1:50,000 for the IgG and IgM analysis, respectively. ELISA tests were carried out according to the manufacturer's guidelines. To reduce nonspecific binding, 1:9 diluted gelatin from coldwater fish skin (No. G7765, Sigma-Aldrich, St Louis, MO, USA) was added to the set blocking solution. Low binding tubes (Protein LoBind tube 2.0 ml, Eppendorf, Hamburg, Germany) were used to minimize protein sample loss. The mean intra- assay and inter-assay coefficients of variation were 3.2% and 5.5% for IgG, and 2.5% and 2.7% for IgM, respectively.

2.5. Metabolic and Endocrine Profiles of Cows and Calves

Blood samples were collected monthly into EDTA or heparinized tubes by coccygeal (in cows) or by jugular (in calves) venipuncture. Furthermore, in the case of IGF-1, blood samples were previously taken every month from cows during the first third of gestation (when the maternal nutritional treatment was applied). Blood samples were centrifuged at 3500 rpm at 4 °C, and plasma samples were taken and frozen at −20 °C until analysis. An automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India) was used to measure blood concentration of glucose (glucose oxidase/peroxidase method, sensitivity: 0.056 mmol/L) and urea (kinetic UV test, sensitivity: 0.170 mmol/L). The mean intra-assay and inter-assay coefficients of variation for these molecules were <5.4% and <5.8%, respectively. Non-esterified fatty acids (NEFA, enzymatic method, sensitivity: 0.06 mmol/L) were analyzed using a commercial kit (Randox Laboratories Ltd., Crumlin Co., Antrim, UK). The mean intra- and inter-assay coefficients of variation were 5.1% and 7.4%, respectively. Insulin-like growth factor 1 (IGF-1, enzyme immunoassay, sensitivity: 20 ng/mL) was determined using a solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite, Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). The mean intra-assay and inter-assay coefficients of variation were 3.1% and 12.0%, respectively.

2.6. Statistical Analyses

All statistics were calculated using the SAS statistical package v 9.4 (SAS Institute Inc., Cary, NC, USA). The normal distribution of data was assessed with the Shapiro-Wilk test ($p > 0.05$). Normality could not be confirmed for somatic cell count and postpartum anoestrus length; therefore, their values were expressed as a decimal logarithm for further analyses. The ADG of dams and calves, milk chemical composition, postpartum anoestrus length and Ig plasma concentration in calves were analyzed with a generalized linear model (GLM procedure) with the nutritional treatment (CONTROL vs. SUBNUT), breed (PA vs. PI) and their interaction as fixed effects. The cow BCS at calving was included as

a covariate. The cow BCS, cow and calf LW, colostrum chemical composition, cow plasma and colostrum Ig concentration, morphometric measurements in calves, nutritional metabolites (glucose, NEFA and urea) and hormone (IGF-1 and progesterone) concentration were analyzed using a mixed linear model (MIXED procedure) for repeated measures based on Kenward–Roger's adjusted degrees of freedom solution. The fixed factors were nutritional treatment, breed, and their interactions as the between-subject effects, sampling day as the within-subject effect, animal as the random effect (experimental unit), and the cow BCS at calving was included as a covariate. The least square (LS) means of the treatments were estimated per fixed effect, and pair-wise comparisons of the means were obtained by the probability of difference (PDIFF) option of the LS means procedure. Association between nutritional treatment or breed with the cow luteal activity was assessed using the F-test (FREQ procedure). The relationship between metabolite and hormone concentration was determined through Pearson's correlation coefficients. The level of significance for all tests was $p < 0.05$. Results are presented as LS means ± standard error.

3. Results

3.1. Cow and Calf Performance

Nutritional treatment in early gestation affected cow BCS at calving (Table 2), and CONTROL cows had higher BCS than SUBNUT cows ($p = 0.032$); however, these differences disappeared at mid-lactation and at weaning ($p > 0.05$). Regarding the breed, PI cows had higher BCS than PA cows ($p < 0.001$) during all lactation.

Table 2. Live weight (LW) and body condition score (BCS) of cows throughout lactation, according to the nutritional treatment and the breed.

Traits	Nutritional Treatment		Breed		RSD	Significance	
	CONTROL	SUBNUT	PA	PI		Nut. Treat.	Breed
Cow LW (kg)							
At calving	605	592	598	599	54.4	n.s.	n.s.
At weaning	586	576	575	588	53.5	n.s.	n.s.
BCS (1–5							
At calving	2.8 ^{a}	2.7 ^{b}	2.7 ^{b}	2.9 ^{a}	0.22	0.032	<0.001
At mid lactation	2.9	2.8	2.7 ^{b}	3.0 ^{a}	0.26	n.s.	<0.001
At weaning	3.0	3.0	2.8 ^{b}	3.1 ^{a}	0.28	n.s.	<0.001

^{a,b} Means within a row with different superscripts differ significantly ($p < 0.05$); n.s., not significant ($p > 0.05$); CONTROL, 100% fed group; SUBNUT, 65% fed group; PA, Parda de Montaña; PI, Pirenaica; RSD, residual standard deviation.

Cow LW was not influenced by the nutritional treatment, breed or time ($p > 0.05$, Table 2). The calf LW was affected by a triple interaction among the nutritional treatment, breed and time ($p = 0.006$, Table 3). The PA calves (CONTROL and SUBNUT) were heavier at birth than their PI counterparts. At weaning, no differences were found among PA and CONTROL-PI calves, and SUBNUT-PI calves were the lightest ($p < 0.001$), namely, 29 kg less than their CONTROL-PI counterparts.

Concerning ADG, an interaction between nutritional treatment and breed was observed in cows and calves (Table 3). During lactation, all groups had LW losses, except for those of SUBNUT-PI cows, which were negligible. Contrarily, SUBNUT-PI calves had the lowest ADG ($p = 0.042$). A negative correlation was found between ADG of cows and calves ($r = -0.37$, $p < 0.001$). The cow BCS at calving positively influenced the calf ADG during lactation ($r = 0.30$, $p = 0.006$), with 0.25 kg/d extra for each extra BCS point.

Table 3. Calf live weight (LW) and average daily gain (ADG) of both cows and calves throughout lactation, according to the interaction between nutritional treatment and breed.

Traits	Nutritional Treatment × Breed				RSD	Significance
	CONTROL-PA	SUBNUT-PA	CONTROL-PI	SUBNUT-PI		
Calf LW (kg)						
At birth	45 [a]	46 [a]	39 [b]	40 [b]	5.9	0.002
At weaning	149 [a]	146 [a]	155 [a]	126 [b]	19.9	<0.001
ADG (kg/d)						
Cows	−0.151 [b]	−0.188 [b]	−0.179 [b]	−0.004 [a]	0.1742	0.014
Calves	0.807 [a]	0.792 [a]	0.860 [a]	0.672 [b]	0.1582	0.042

[a,b] Means within a row with different superscripts differ significantly ($p < 0.05$); CONTROL, 100% fed group; SUBNUT, 65% fed group; PA, Parda de Montaña; PI, Pirenaica; RSD, residual standard deviation.

Nutritional treatment in early gestation had no effects on any morphometric parameters on d 7 of life (Table 4). On d 120, height at withers ($p = 0.046$), rump and body length ($p = 0.020$ and $p = 0.022$, respectively) and heart girth ($p = 0.004$) were higher in the CONTROL than in SUBNUT calves. The breed affected most of the morphometric traits because PA calves had higher values than PI calves on d 7 and 120 in height at withers ($p = 0.001$ and $p < 0.001$, respectively) and heart girth ($p < 0.001$ and $p = 0.024$, respectively), on d 7 in rump width ($p = 0.045$) and on d 120 in height at rump ($p = 0.002$). The heart girth was positively influenced by the cow BCS at calving ($p < 0.001$), with 8.2 cm extra for each extra BCS point.

Table 4. Calf morphometric measurements throughout lactation, according to the nutritional treatment and the breed (cow BCS at calving included as a covariate).

Traits	Nutritional Treatment		Breed		RSD	Significance		
	CONTROL	SUBNUT	PA	PI		Nut. Treat.	Breed	BCSc
Height at withers (cm)								
d 7	74	73	75 [a]	72 [b]	3.4	n.s.	0.001	n.s.
d 120	94 [a]	93 [b]	95 [a]	92 [b]	3.2	0.046	<0.001	n.s.
Height at rump (cm)								
d 7	78	78	79	77	3.7	n.s.	n.s.	n.s.
d 120	101	99	101 [a]	98 [b]	4.0	n.s.	0.002	n.s.
Rump width (cm)								
d 7	18	18	19 [a]	18 [b]	1.9	n.s.	0.045	n.s.
d 120	26	26	26	26	2.4	n.s.	n.s.	n.s.
Rump length (cm)								
d 7	21	21	22	21	1.8	n.s.	n.s.	n.s.
d 120	35 [a]	34 [b]	34	35	2.8	0.020	n.s.	n.s.
Body length (cm)								
d 7	67	66	67	66	3.9	n.s.	n.s.	n.s.
d 120	97 [a]	95 [b]	96	96	4.9	0.022	n.s.	n.s.
Heart girth (cm)								
d 7	86	84	88 [a]	83 [b]	5.1	n.s.	<0.001	<0.001
d 120	119 [a]	115 [b]	118 [a]	115 [b]	5.5	0.004	0.024	<0.001

[a,b] Means within a row with different superscripts differ significantly ($p < 0.05$); n.s., not significant ($p > 0.05$); CONTROL, 100% fed group; SUBNUT, 65% fed group; PA, Parda de Montaña; PI, Pirenaica; RSD, residual standard deviation; BCSc, cow body condition score at calving.

Four months after calving, 12 cows did not have any luteal activity, with no nutritional treatment (8% vs. 19% for CONTROL and SUBNUT, respectively, $p > 0.05$) or breed effect (19% vs. 7% for PA and PI, respectively, $p > 0.05$). No differences were found in postpartum anoestrus length between CONTROL and SUBNUT cows (40 vs. 46 days, respectively, $p > 0.05$). Pirenaica cows needed fewer days to recover the luteal activity than PA (38 vs. 49 days, respectively, $p = 0.035$). The BCS at calving was correlated with the postpartum anoestrus length ($r = -0.47$, $p < 0.001$).

3.2. Colostrum and Milk Composition, Milk Yield and Calf Milk Intake

Colostrum chemical composition was not influenced by the nutritional treatment ($p > 0.05$, Table 5). The breed had a significant effect because colostrum lactose content was higher ($p = 0.015$) and somatic cell count was lower ($p = 0.043$) in PI than in PA cows in those samples collected in Period 1 (0–12 h postpartum). Regarding the time when the colostrum sample was taken, the protein concentration decreased ($p < 0.001$) and the lactose concentration increased ($p < 0.001$) from Period 1 to Period 2 (12–24 h postpartum).

Table 5. Colostrum composition, according to the nutritional treatment and the breed (cow BCS at calving included as a covariate).

Traits	Nutritional Treatment		Breed		RSD	Significance		
	CONTROL	SUBNUT	PA	PI		Nut. Treat.	Breed	BCSc
Fat (%)								
Period 1	3.5	3.2	3.5	3.2	1.83	n.s.	n.s.	n.s.
Period 2	3.3	3.9	3.3	4.0	2.34	n.s.	n.s.	n.s.
Protein (%)								
Period 1	17.5 ^{y}	18.3 ^{y}	18.1 ^{y}	17.7 ^{y}	2.85	n.s.	n.s.	n.s.
Period 2	11.6 ^{z}	11.3 ^{z}	11.0 ^{z}	12.0 ^{z}	3.07	n.s.	n.s.	n.s.
Lactose (%)								
Period 1	3.2 ^{z}	3.1 ^{z}	3.0 ^{b,z}	3.3 ^{a,z}	0.49	n.s.	0.015	n.s.
Period 2	3.5 ^{y}	3.5 ^{y}	3.4 ^{y}	3.6 ^{y}	0.55	n.s.	n.s.	n.s.
Somatic cell count (n × 10^3/mL)								
Period 1	1276	1043	1526 ^{a}	872 ^{b}	-	n.s.	0.029	<0.001
Period 2	1464	1510	1890	1170	-	n.s.	n.s.	<0.001

^{a,b} Means within a row with different superscripts differ significantly ($p < 0.05$); ^{y,z} Means within a column and trait with different superscripts differ significantly by period ($p < 0.05$); n.s., not significant ($p > 0.05$); CONTROL, 100% fed group; SUBNUT, 65% fed group; PA, Parda de Montaña; PI, Pirenaica; Period 1, from 0 to 12 h postpartum; Period 2, from 12 and 24 h postpartum; RSD, residual standard deviation; BCSc, cow body condition score at calving.

Nutritional treatment did not affect milk yield on d 23 but influenced milk chemical composition (Table 6). SUBNUT dams had higher milk fat concentration than CONTROL dams ($p = 0.010$). The breed affected most milk traits, with higher milk yield and somatic cell counts ($p = 0.008$ and $p = 0.002$, respectively), and lower fat and lactose concentration ($p < 0.001$) in PA than in PI cows. No interaction between nutritional treatment and breed was found in milk traits, and therefore, milk yield on d 23 did not differ within breed between the two nutritional treatments (CONTROL-PA, 10.6 kg/d; SUBNUT-PA, 9.2 kg/d; CONTROL-PI, 8.4 kg/d; SUBNUT-PI, 8.2 kg/d; standard error of the mean, 0.23 kg/d). The cow BCS at calving was correlated with milk protein concentration ($r = 0.29$, $p = 0.008$) and ECM yield ($r = 0.24$, $p = 0.030$).

The fat and lactose concentration from colostrum to milk on d 23 increased approximately 35.3% and 52.6%, respectively, and the protein concentration and somatic cell counts decreased approximately 79.6% and 83.2%, respectively.

Table 6. Cow milk yield and composition, and calf milk intake, according to the nutritional treatment, the breed or their interaction (cow BCS at calving included as a covariate).

Traits	Nutritional Treatment		Breed		RSD	Significance		
	CONTROL	SUBNUT	PA	PI		Nut. Treat.	Breed	BCSc
Milk yield d 23 (kg/d)	9.5	8.7	9.9 [a]	8.3 [b]	2.11	n.s.	0.008	0.049
Energy-corrected milk yield (kg/d)	10.2	9.6	10.0	9.7	2.20	n.s.	n.s.	0.049
Fat (%)	4.4 [b]	4.8 [a]	4.2 [b]	4.9 [a]	0.60	0.010	<0.001	0.016
Protein (%)	3.6	3.7	3.6	3.7	0.30	n.s.	n.s.	0.030
Lactose (%)	4.7	4.7	4.6 [b]	4.9 [a]	0.29	n.s.	<0.001	n.s.
Somatic cell count ($n \times 10^3$/mL)	184	174	314.4 [a]	102.1 [b]	-	n.s.	0.002	n.s.

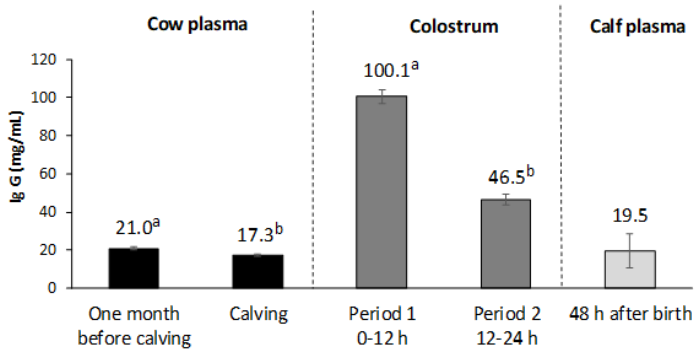
Traits	Nutritional treatment × Breed				RSD	Significance	
	CONTROL-PA	SUBNUT-PA	CONTROL-PI	SUBNUT-PI		N.T. × Breed	BCSc
Calf milk intake (kg/d)							
d 25	9.4 [a]	9.0 [a]	8.2 [a]	6.7 [b]	1.53	<0.001	0.030
d 120	7.5 [a]	7.5 [a]	5.6 [b]	5.1 [b]	1.93	<0.001	0.030

[a,b] Means within a row with different superscripts differ significantly ($p < 0.05$); n.s., not significant ($p > 0.05$); CONTROL, 100% fed group; SUBNUT, 65% fed group; PA, Parda de Montaña; PI, Pirenaica; RSD, residual standard deviation; N.T., nutritional treatment; BCSc, cow body condition score at calving.

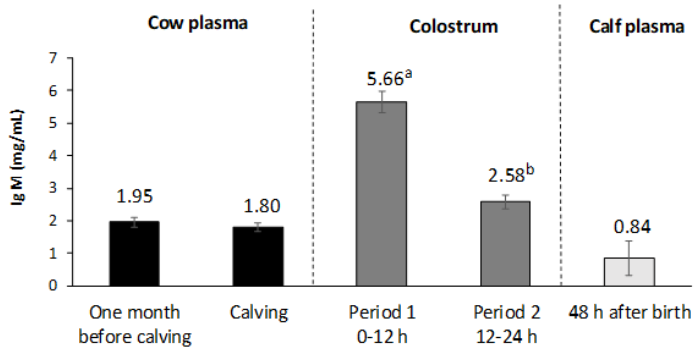
Regarding the calf milk intake, an interaction was found between nutritional treatment and breed. SUBNUT-PI calves had the lowest milk intake on d 25 ($p < 0.001$), whereas no differences were found among CONTROL-PI and PA calves ($p > 0.05$). On d 120, differences between PI groups disappeared, and PA calves had higher intake than PI calves ($p < 0.001$). Calf milk intake values decreased from the beginning to the end of lactation ($p < 0.001$). Calf milk intake on d 25 was correlated with cow milk yield on d 23 ($r = 0.47$, $p < 0.001$), with cow and calf ADG during lactation ($r = -0.53$, $r = 0.63$, respectively, $p < 0.001$) and with calf LW at weaning ($r = 0.74$, $p < 0.001$). The BCS at calving influenced the calf milk intake on d 25 and 120 ($p = 0.030$), with 1.6 kg/d extra for each extra BCS point.

3.3. Immunoglobulin Concentration in Colostrum and Cow-Calf Plasma

The cow Ig concentration was not related to the nutritional treatment during early gestation or breed ($p > 0.05$). Plasma IgG concentration significantly decreased from 1 month before calving to calving by 17.7% ($p < 0.001$, Figure 1). The IgG concentration of colostrum during the first 12 h postpartum was six-fold higher than that from cow plasma at calving. From Period 1 to Period 2, colostrum IgG concentration decreased approximately 54% ($p < 0.001$). The colostrum IgG concentration in Period 1 was correlated with the calf ADG throughout lactation ($r = 0.34$, $p = 0.002$). An interaction was found between nutritional treatment and breed in average colostrum IgG concentration (between Period 1 and 2). No differences were found between CONTROL-PA and CONTROL-PI colostrum IgG concentration (74.1 ± 4.57 ng/mL vs. 76.0 ± 7.29 ng/mL, respectively, $p > 0.05$), but SUBNUT-PA had higher IgG values than SUBNUT-PI cows (82.16 ± 4.48 ng/mL vs. 62.1 ± 4.97 ng/mL, respectively, $p < 0.004$). In calf plasma IgG concentration, no nutritional treatment or breed effect was observed ($p > 0.05$). The calf plasma IgG concentration was higher (12.9%) than that obtained in cow plasma at calving and was correlated with calf ADG throughout lactation ($r = 0.32$, $p = 0.022$). Regarding IgM, cow plasma Ig concentration also decreased at the end of gestation but not significantly ($p > 0.05$). Colostrum IgM concentrations in Period 1 were three-fold higher than those obtained in cow plasma at calving, and from Period 1 to Period 2, its concentration halved ($p < 0.001$). Calf IgM concentration was not affected by maternal nutrition or breed ($p > 0.05$), and its value was lower (−53.3%) compared with dam plasma concentration.



(**a**)



(**b**)

Figure 1. (**a**) Mean IgG concentrations in dam plasma, colostrum and calf plasma in the peripartum. (**b**) Mean IgM concentrations in dam plasma, colostrum and calf plasma in the peripartum. [a,b] Means with different superscripts between cow plasma or between colostrum samples differ significantly ($p < 0.05$).

3.4. Metabolic and Endocrine Profiles of Cows and Calves

Metabolic and endocrine profiles are presented in Figure 2 (cows) and Figure 3 (calves). In cows, CONTROL-PA had the lowest plasma glucose concentrations throughout lactation, with statistical differences in SUBNUT-PA in month 2 ($p < 0.05$). Pirenaica cows had higher average glucose concentration than PA (3.2 ± 0.06 vs. 3.0 ± 0.04 mmol/L, respectively, $p = 0.005$).

Regarding NEFA values, PI dams had higher average concentration than PA (0.36 ± 0.021 vs. 0.31 ± 0.014 mmol/L, respectively, $p = 0.033$). At calving, CONTROL-PI cows had the highest NEFA concentrations ($p < 0.05$).

In calves, glucose concentration was higher in CONTROL-PA than in SUBNUT-PA throughout lactation, with statistical differences in month 3 ($p < 0.05$). In general, CONTROL-PI had higher NEFA concentration than SUBNUT-PI, with statistical differences in month 2 ($p < 0.05$). Urea concentration was higher in PA than in PI calves at the beginning of the lactation, but from month 2, all animals had similar values. Regarding IGF-1 concentration, an interaction between nutritional treatment and breed was observed, because no statistical differences were found between CONTROL-PA and SUBNUT-PA average values during lactation (105.3 ± 5.83 ng/mL vs. 91.8 ± 5.54 ng/mL, respectively, $p > 0.05$), but CONTROL-PI had higher concentrations than SUBNUT-PI (122.3 ± 9.72 ng/mL vs. 77.8 ± 6.43 ng/mL, respectively, $p < 0.001$). Specifically, CONTROL-PI calves had higher values of IGF-1 than the other groups in month 2 and month 3 ($p < 0.05$). A correlation was found between the cow IGF-1 concentration in early gestation (d 28) and calf IGF-1 concentration at birth ($r = 0.33$, $p = 0.003$). Furthermore, calf IGF-1 concentration during lactation was also positively related to its ADG ($r = 0.63$, $p < 0.001$) and negatively with the cow ADG ($r = -0.28$, $p = 0.011$).

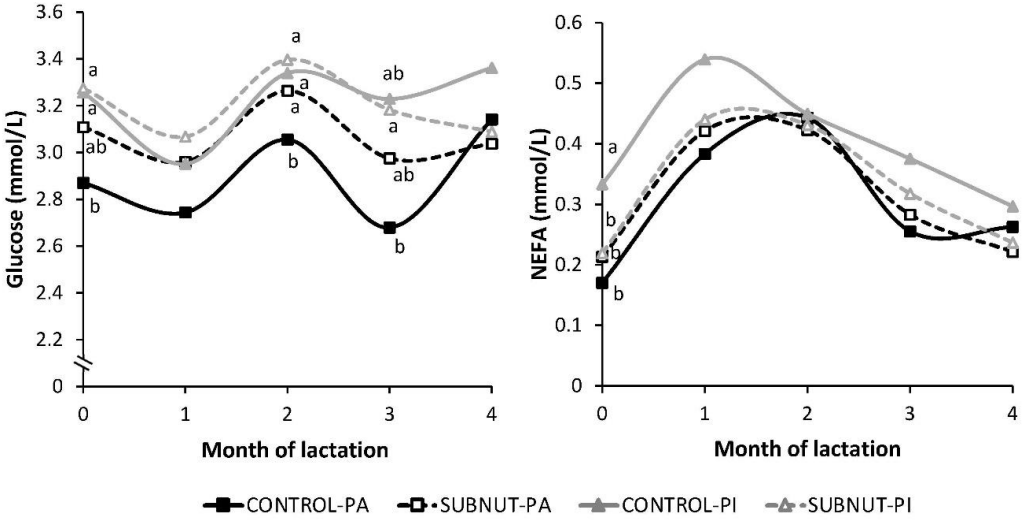


Figure 2. Cow metabolic profiles throughout lactation, according to the nutritional treatment and the breed. [a,b] Means within a month with different superscripts differ significantly ($p < 0.05$); CONTROL, 100% fed group; SUBNUT, 65% fed group; PA, Parda de Montaña; PI, Pirenaica.

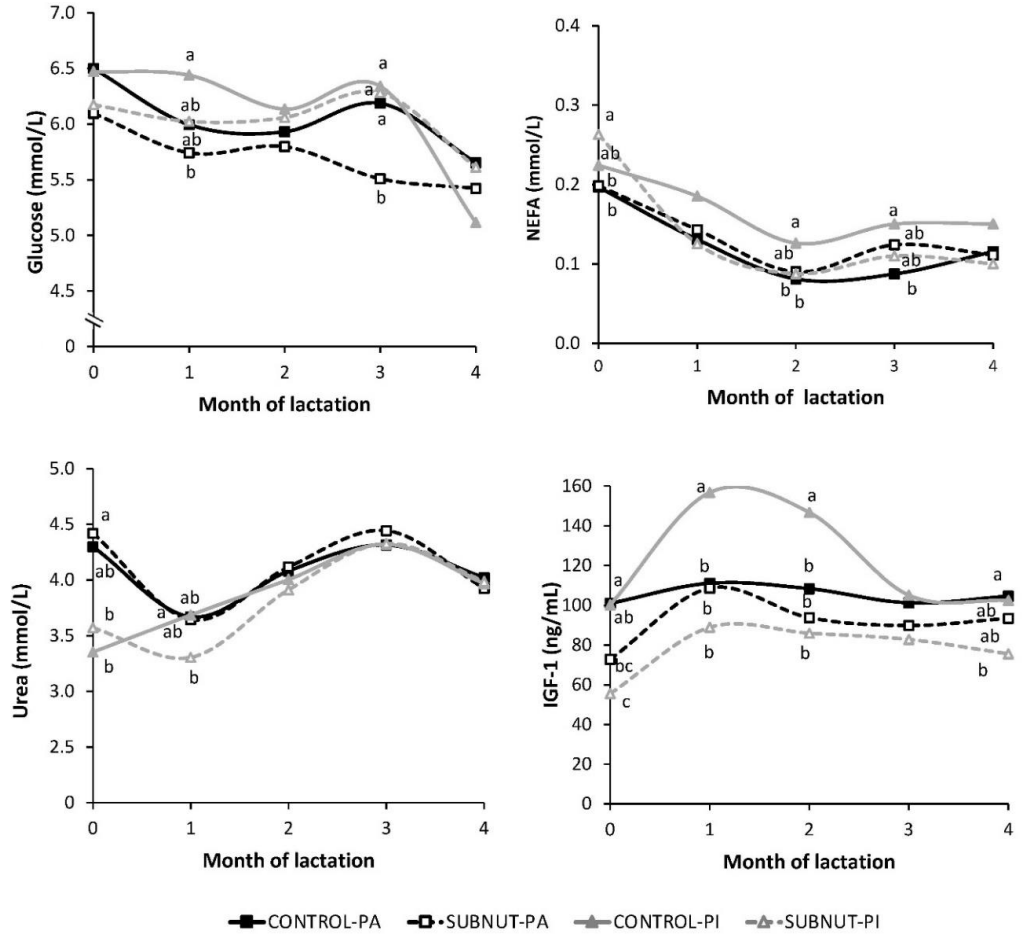


Figure 3. Calf metabolic and endocrine profiles throughout lactation, according to the nutritional treatment and the breed. [a,b,c] Means within a month with different superscripts differ significantly ($p < 0.05$); CONTROL, 100% fed group; SUBNUT, 65% fed group; PA, Parda de Montaña; PI, Pirenaica.

4. Discussion

4.1. Cow and Calf Performance

Poor nutrition during the first third of gestation had long-term effects on cow-calf performance. Although LW at calving was similar in CONTROL and SUBNUT cows, the BCS was lower in the SUBNUT group. This finding suggests that SUBNUT cows were not able to recover their body fat reserves due to the high energy demand in the last stages of gestation [23]. The discordance between BCS and LW shows that despite that these two variables have a strong relationship, the BCS reflects the body fat reserves of the animal, linked to its metabolic status, whereas LW is mainly linked to the animal dimensions, with a high hereditary component [24]. Furthermore, discordances between LW and BCS are common after calving due to changes in the carcass, digestive tract or visceral adipose tissue weight [25]. In our experiment, at the beginning of gestation, when the nutritional treatment started, the CONTROL and SUBNUT groups were BCS and LW balanced [13]. The BCS differences found at calving disappeared afterward, suggesting that when the high energy requirements from the end of gestation and the beginning of lactation decreased, the cows increased their BCS throughout lactation. By contrast, the cow ADG had negative values, with discordance between LW and BCS again. The BCS difference at calving between CONTROL and SUBNUT cows also affected calf performance, with large differences between CONTROL-PI and SUBNUT-PI calf LW at weaning but not at birth. To reduce bovine fetal growth, a severe nutritional restriction is required during at least the last half or third of the pregnancy [5,26,27]. The energy dietary restriction in early pregnancy could not influence calf birth weight, especially when cows received feed supplementation in the second half of gestation to ensure normal birth weight [28]. Although fetal size should not be affected by nutrition received during early and mid-pregnancy, placental characteristics may be altered [29,30]. In the current study, no differences were found in birth LW but were found at weaning; thus, fetal growth was adequate, but possibly due to some alterations in the fetal programming together with a low milk intake capacity, the correct postnatal calf development was altered [3]. Nutrition during early pregnancy could have more subtle effects on organ and tissue development, with potential long-term consequences, whereas nutrition during later pregnancy impacts on fetal and carcass growth most [26]. In the first third of gestation, SUBNUT cows had a negative energy balance, which means that the fetus had to adapt its metabolism to a poor uterine environment. In most of the cases, these fetal adaptive mechanisms were irreversible with consequences in postnatal life [31], highlighting the crucial role of maternal nutrition during the first stages of gestation.

Regarding the breed, PI had higher BCS during lactation and lower calf LW at birth than PA, in concordance with the results reported by Sanz et al. [32] and Rodríguez-Sánchez et al. [12]. The differences found in ADG between CONTROL-PI and SUBNUT-PI calves indicate that PI breed is more sensitive to undernourishment than PA, in line with the results of Noya et al. [13], with greater long-term detriment on its offspring postnatal development.

The morphometric measurements indicated a faster body growth of CONTROL calves throughout lactation. No differences were found in the first week of life; however, CONTROL calves were larger than SUBNUT calves at weaning in most of the parameters registered. The fetus intrauterine growth in the SUBNUT group seemed to be adequate, with similar LW at birth between groups, but during lactation, the CONTROL group grew faster than the SUBNUT group. The breed influenced some body measurements in the first week of life, at weaning, or both, proving the inter-breed morphology and development differences.

The BCS at calving had a substantial influence on the resumption of cow ovarian cyclicity. The CONTROL group required fewer days to recover its ovarian activity, although the difference was not significant. Regarding the breed, the PI cows had a shorter postpartum anoestrus length, related to their higher BCS at calving [33]. Sanz et al. [34] reported no differences in the postpartum anoestrus length between PA and PI cows, confirming our hypothesis that the breed difference found in our study was attributed to a BCS effect. The degree of the negative energy balance that the cows undergo

in early lactation modifies the time needed for the first ovulation. During a negative energy balance, luteinizing hormone pulses are suppressed and dominant follicles that develop have a lower chance of producing a sufficient amount of estradiol to induce a pre-ovulatory gonadotrophin surge [35].

4.2. Colostrum and Milk Composition, Milk Yield and Calf Milk Intake

The nutritional treatment applied during early gestation did not affect the colostrum chemical composition. Similarly, Quigley and Drewry [23] found that the manipulation of cow diet to alter the fat content of colostrum did not result in increased colostrum energy content. Neither the breed nor BCS at calving had any effect on its quality, in concordance with Morrill et al. [36]. Colostrum has an extremely high protein concentration; however, the protein content declines sharply a few hours after calving because of the Ig transfer ceases from the dam's circulation to the mammary secretions [37]. Thus, in this study, the protein content decreased significantly from Period 1 to Period 2, and the lactose content increased.

After calving, the duration of the transition from colostrum to milk can last between two and seven days [38,39], during which the lactose content increases and the fat, protein and somatic cell count values decrease [40]. However, other authors have reported that the fat content in mature milk can also increase [41], as observed in our results. In our experiment, in the transition from colostrum to milk, the somatic cell counts decreased drastically. The high colostrum somatic cell counts were not related to a mastitic infection but a high temporary permeability of tight junctions between the mammary epithelial cells [42].

The milk fat content on d 23 was higher in SUBNUT than in CONTROL cows. This may be associated with the higher milk yield of CONTROL than SUBNUT cows, despite the fact that it was not significant. Milk yield and fat percentage are negatively correlated [43], because an increase in milk yield implies a dilution effect in fat milk constituents [44]. Inter-breed differences were supported by our results because PA cows had higher milk yield with lower fat and lactose content than PI, in accordance with the results reported by Álvarez-Rodríguez et al. [18]. Both in colostrum and milk, PA cows had higher somatic cell counts than PI, which can be associated with their higher milk yield [45].

An interaction was observed here between breed and maternal nutritional treatment in calf milk intake on d 25, which was lower in SUBNUT-PI calves than in the others, although the milk yield of SUBNUT-PI cows on d 23 was similar to that of their CONTROL-PI counterparts. These differences may be due to a lesser digestive tract development of SUBNUT-PI calves, either during fetal life or immediately after birth, which impaired their milk intake capacity. The calf milk intake at the beginning of lactation could be a good indicator of the calf LW at weaning, but a moderately accurate method to estimate the cow milk yield in suckler cows. Calf milk intake on d 25 had a positive relationship with calf ADG during lactation and a negative relationship with cow ADG. Animals with higher milk aptitude, such as PA, prioritize the allocation of dietary energy to the milk yield, increasing calf ADG values, instead of their own weight gains. By contrast, lactating PI dams prioritized their own maintenance over milk production and the development of their offspring, accordingly with the results obtained by Sanz et al. [32].

4.3. Immunoglobulin Concentration in Colostrum and Cow-Calf Plasma

Plasma concentrations of IgG and IgM in cows were not influenced by the nutritional treatment during early gestation or the breed. They decreased in the last month of gestation, although not significantly in the case of IgM. The reduction of plasma Ig in the third trimester of gestation is a physiological phenomenon: Ig are transferred from a dam's circulation into the udder tissue [46]. Plasma IgG drop is described from the eighth week before calving, whereas IgM concentration starts to decrease from the fourth week before calving [47].

Colostrogenesis occurs as early as five weeks before calving [48], suggesting that the undernutrition applied during the first third of gestation should not affect the colostrum Ig concentration [49]. However, the lower BCS at calving of SUBNUT cows could indirectly affect the colostrum Ig

concentration. In our study, colostrum IgG concentration was higher in SUBNUT-PA than in SUBNUT-PI dams, supporting the hypothesis that PI could be more sensitive to negative energy balance.

In this study, IgG and IgM concentration in colostrum decreased dramatically from Period one to Period two because of the cessation of the Ig transfer immediately before calving, in accordance with the results reported by Barrington and Parish [37]. Notably, colostrum Ig concentration was negatively associated with the interval from calving to colostrum collection [50].

Our findings did not support the hypothesis that maternal undernutrition could adversely impact the passive transfer of immunity from dam to calf. Despite the lower colostrum IgG concentration in SUBNUT-PI cows, no differences between nutritional treatments or breeds were observed in IgG and M plasma concentrations in calves, in accordance with studies that have described no adverse impact of maternal dietary restriction on calf passive immunity [10,49]. By contrast, Burton et al. [51] found that the absorption of IgG1, IgG2, IgM, and IgA were reduced in calves born from nutrient-restricted dams, but the colostrum Ig concentration was not affected. Hammer et al. [52] described higher IgG plasmatic values in lambs whose mothers were nutrient restricted during pregnancy, suggesting that the fetal gastrointestinal system may be programmed to be more efficient in extracting nutrients, namely, large molecules such as Ig. In the current study, IgG concentration in colostrum and calf plasma was correlated with calf ADG. Wittum and Perino [53] described an indirect effect of passive transfer status on calf ADG and weaning weight because of its effect on calf morbidity. By contrast, Cummins et al. [54] reported no differences in daily weight gains during lactation in calves with different plasma IgG concentrations 24 h after birth.

4.4. Metabolic and Endocrine Profiles of Cows and Calves

The cow glucose profiles were related to inter-breed differences, with higher concentrations in PI cows, and to the current diet received during lactation, instead of the nutritional treatment applied during early pregnancy. Glucose profiles are strongly linked with the short-term effect of the current energy and/or protein intake [12].

The BCS difference at calving due to undernutrition in early pregnancy was reflected in NEFA profiles, specifically in PI cows. Non-esterified fatty acids concentrations were 50% higher in CONTROL-PI than in SUBNUT-PI cows. During early lactation, an imbalance is observed between the high energy requirements and the reduced intake capacity [35], and cows mobilize body fat reserves as an energy source, increasing the plasma NEFA concentrations [55] and leading to LW losses. In this study, CONTROL-PI cows mobilized more lipid stores, increasing their plasma NEFA concentration. Non-esterified fatty acids concentration in all groups was higher between month one and month two of lactation, by the time the peak milk yield is attained, with the greatest nutritional demands [18].

Regarding calf metabolic profiles, glucose concentration was higher than that obtained in cows, accordingly with the calf diet [56]. Glucose is sourced from milk and, furthermore, during the first month of life, glucose intake is insufficient to maintain the normal plasma glucose concentration, and liver gluconeogenesis is activated [57]. In this study, early maternal nutrition had little impact on calf glucose metabolism. Similarly, other studies have described no effect of nutrient restriction during early gestation on calf glucose basal concentration [58,59].

Non-esterified fatty acids concentration in calves decreased at the beginning of lactation with a nadir in month 2, probably due to the peak milk yield of cows. After calving, calves moved from an intrauterine diet comprising primarily glucose and amino acids to a diet higher in fat [26], with increased energy content that reduced the high plasmatic NEFA concentration observed in the first days of life. From month 2, CONTROL-PI calves had higher values than their counterparts, which could reflect a higher amount and turnover of adipose tissue.

Urea values were not related to maternal nutrition in early pregnancy but to calf nutrition, reflecting its dependence on current energy and protein intake [60]. Blood urea concentration is influenced by the degree of protein catabolism and the ratio of energy to protein in the diet. Some

authors have also described a plasma urea increase in newborns after a high amount of colostrum intake due to its high protein concentration [57].

Maternal nutrition affected calf plasma IGF-1 concentration. In general, CONTROL calves had higher IGF-1 concentrations than SUBNUT calves. These differences between nutritional treatment groups were higher in PI breed, supporting that the long-term effects of maternal subnutrition are greater in PI than in the PA breed, in accordance with results reported by Noya et al. [13]. The IGF-1 concentration was correlated with calf ADG and reflected in the lower LW of SUBNUT-PI calves at weaning. The IGF-1 is critical in the control of animal growth, and greater serum IGF-1 concentration is associated with faster growth rates [61]. In newborns, the growth hormone-IGF-1 axis is already functional, and the still low plasma IGF-1 concentration increases approximately 2.5-fold up to nine months of age [61]. In this study, poor maternal nutrition during the first third of pregnancy could have reprogrammed the fetal IGF-1 system in its ability to respond to acute changes in the substrate supply [62], resulting in evidence of different calf IGF-1 concentrations at birth, when the cow performance would still have little impact on calf metabolic and endocrine profiles. Our results imply a transgenerational relationship between IGF-1 concentrations of cows during early gestation, which were conditioned by the cow feeding level, with the IGF-1 values of their progeny at birth. Similarly, Maresca et al. [63] described lower calf IGF-1 concentration at birth after maternal protein intake restriction from mid-gestation to calving. In our study, although these IGF-1 differences decreased at the end of lactation, SUBNUT calves had reduced LW and body measurements at weaning, reflecting a residual effect of maternal undernutrition on offspring postnatal growth.

5. Conclusions

In this study, the undernutrition applied during the first third of gestation in suckler cows had long-term effects on the productive efficiency of the cow-calf pair in the following lactation. Undernourished cows had a lower BCS at calving, which impaired most of the studied cow-calf traits. Additionally, feed restriction could have modified the calf fetal programming, which was reflected in their lower performance and IGF-1 concentration after birth. These long-term effects, associated with a reduced calf milk intake, impaired body growth and LW at weaning of SUBNUT calves, these effects being more severe in Pirenaica cow-calf pairs. Further research should investigate the impact of early maternal subnutrition on the post-weaning efficiency of beef heifers and young bulls.

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Effects of Developmental Programming Caused by Maternal Nutrient Intake on Postnatal Performance of Beef Heifers and Their Calves

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Simple Summary: The nutrient intake of a cow during the early stages of pregnancy can have detrimental effects on the developing fetus. The adaptive response of the fetus to the restricted intrauterine environment can modify organ development. In this study, we assessed the consequences of maternal undernutrition in the first third of gestation on female offspring during its rearing, gestation, and lactation periods. We confirmed that maternal nutrient restriction decreased the heifer live weight at weaning (four months old); this difference disappeared at puberty, the end of rearing, and during the following gestation and lactation periods. Consequently, heifers from nutrient-restricted dams had impaired metabolic status around the onset of puberty. Maternal undernutrition reduced the number of antral follicles at breeding (16 months old); however, it did not affect the pregnancy rate after a single artificial insemination nor the calf weight gains during the first lactation period. In conclusion, early maternal subnutrition had long-term effects on heifer postnatal performance during the first four months of life which compromised heifer metabolic status during rearing but did not affect the reproductive performance during its first gestation and lactation periods.

Abstract: In this study, we evaluated the effects of maternal subnutrition in early pregnancy on the growth and reproductive performance of female offspring during their rearing, first gestation, and lactation. We inseminated 21 Parda and 15 Pirenaica multiparous cows and assigned them to a CONTROL (100% of nutrition requirements) or SUBNUT (65%) diet until day 82 of gestation. Cows were fed 100% requirements afterward. During the rearing of female offspring, growth, physiological profiles and ovarian follicular dynamic were studied. At 16 months old, heifers were inseminated. After first calving, dam–calf weights were recorded during lactation. Heifers born from CONTROL cows were heavier at weaning (four months old) than heifers born from SUBNUT cows, but this difference disappeared at the end of rearing and during the first gestation and lactation periods. All heifers reached puberty at a similar age and live weight. During rearing, SUBNUT heifers had higher concentrations of non-esterified fatty acids, urea, and cholesterol and a lower antral follicle count than CONTROL, but no difference was found in their fertility rate. After heifer first calving, dam–calf weights were similar among groups. In conclusion, maternal undernutrition reduced offspring postnatal gains at weaning, compromising metabolic status and follicle population during rearing but did not impair performance in the first gestation and lactation periods of beef heifers.

Keywords: puberty; fertility; metabolic and endocrine profile; early pregnancy; malnutrition; long-term effects

1. Introduction

Beef cattle production systems have increasingly adapted to extensive conditions to reduce feed costs. Animals are managed during long periods on grazing systems, where food availability depends exclusively on pastures. Many factors can influence the quality and quantity of this nutrient source during the cow–calf production cycle. Restricted nutrition can occur during early gestation when critical processes such as embryonic cell lineage allocation and maternal recognition of pregnancy take place. The nutritional microenvironment in ovaries and reproductive tract induces cellular and molecular alterations in the peri-implantation embryo to adapt its physiology to a poor environment, modifying the establishment of founder cell lineages or gene activation [1,2]. This developmental strategy can produce adverse embryo and/or fetal programming, resulting in offspring with a higher risk of developing deleterious phenotypes in adulthood [1,3]. Through epigenetic mechanisms, maternal nutrition is linked to offspring phenotype as a consequence of the organism memory of past metabolic and environmental events [4,5].

In general, most studies established that the consequences of feed restriction during early pregnancy on progeny growth can be alleviated by adequate nutrition in late pregnancy with few consequences on postnatal growth [6–8]. However, the impact of gestational undernutrition, either in early, mid or late gestation, on the reproductive performance of the progeny is not fully understood. Several studies reported negative effects on reproductive traits of the female offspring, with lower antral follicle counts [9], impaired ovulation rate [10] or decreased fertility [10,11]. In contrast, other studies described equal [12] or higher [13] numbers of antral follicles and higher ovulation rate [13] in females from nutrient-restricted dams. To explain these contradictory results, Smith et al. [13] suggested that the effects of maternal nutrient restriction depends on the degree and timing of this restriction and the post-restriction diet. In cattle, few studies have evaluated the consequences of embryo and/or fetal programming in a poor uterine environment on heifer reproductive performance and whether these potential effects can be devolved to heifer offspring which is important to consider when designing feeding practices in the beef cattle husbandry. Micke et al. [14] indicated that a refining nutritional program during early gestation may optimize production objectives in the progeny.

These effects could vary depending on the breed and genetic background. Metabolic and endocrine responses to management can differ in the function of the breed [15–17]. In this study, Parda de Montaña (PA) and Pirenaica (PI) animals were used, because they are the two main beef cattle breeds adapted to a semi-extensive system of animal husbandry in the Pyrenees mountain region in Northern Spain.

Our previous results highlighted that dam nutrition in early pregnancy had long-term effects on offspring growth during the first four months of life, with this physiological evidence being more pronounced in PI cow–calf pairs [18]. In the current study, we hypothesized that poor maternal nutrition could have a residual impact on postnatal performance of beef heifers during rearing, first gestation, and lactation periods. We aimed to evaluate the effects of adverse prenatal environment caused by maternal energy intake on postnatal growth, metabolism, and reproduction of PA and PI heifers and on their offspring weights during their first lactation.

2. Materials and Methods

All the procedures were approved by the Animal Ethics Committee of the Centro de Investigación y Tecnología Agroalimentaria (CITA) of Aragón, Spain. The care and use of animals were performed in accordance with the guidelines of the European Union (Directive 2010/63/EU) regarding the protection of animals used for experimental and other scientific purposes [19]. Gestation and lactation phases of this study were conducted at CITA-La Garcipollera Research Station (the mountain area of the central Pyrenees, Huesca, Spain, 945 m above sea level (a.s.l.)), and the rearing phase at CITA-Montañana Research Station (Zaragoza, Spain, 225 m a.s.l.). The experimental design of this study is shown in Figure 1.

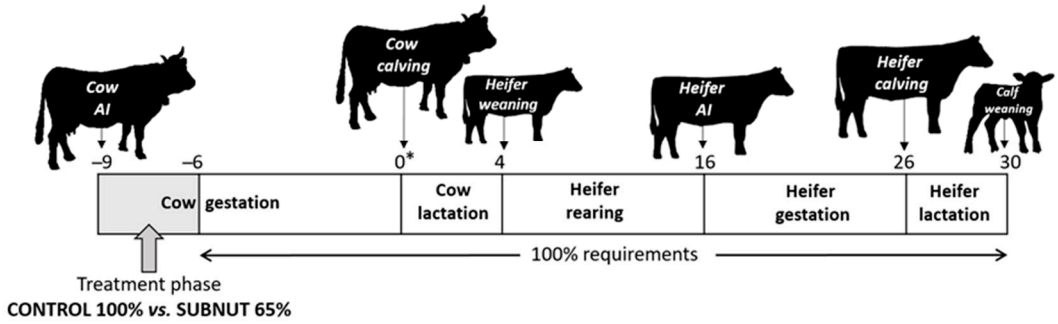

Figure 1. Experimental design. * month; CONTROL, cows fed 100% of their nutritional requirements during the first third of pregnancy; SUBNUT, cows fed 65% of their nutritional requirements during the first third of pregnancy; AI, artificial insemination.

2.1. Management during Cow Gestation (Maternal Nutrition Treatment) and Lactation

We synchronized 53 PA and 32 PI multiparous cows which were then artificially inseminated (AI) and distributed into two nutrition treatments from the day of AI, both fed a total mixed ration (alfalfa hay 25.0%, cereal straw 25.0%, crushed barley 25.0%, dehydrated alfalfa 10%, rapeseed meal 6.5%, citrus pulp 4.5%, soybean meal 2.5%, and vitamin–mineral complex 1.5%; Table 1) during the first third of gestation as described in Noya et al. [15]. Briefly, the control group (CONTROL, 574 ± 8.9 kg live weight (LW); 2.80 ± 0.038 body condition score (BCS) on a 5 point scale [20]) was fed a diet that supplied 100% of the estimated energy requirements for cow maintenance, lactation, and gestation; and the nutrient-restricted group (SUBNUT, 568 ± 7.6 kg LW; 2.86 ± 0.032 BCS) received 65% of their requirements. After this treatment phase, the CONTROL group maintained its LW and BCS (583 ± 8.6 kg LW; 2.90 ± 0.040 BCS), whereas they decreased in SUBNUT animals (538 ± 7.2 kg LW; 2.65 ± 0.033 BCS, $p < 0.001$), reported by Noya et al. [21]. All dams were fed 100% of the requirements during the remainder of gestation and the next lactation, using the same total mixed ration described above. Feed was provided at 08:00 and cows were tied up for a maximum of 2 h until they finished the restricted amount assigned to each. Calves' diets consisted exclusively of maternal milk in a restricted twice-daily nursing system. Calves were weaned at the age of 120 days.

Table 1. Chemical composition of feedstuffs used in the experiment (on an as-fed basis).

Chemical Composition	Total Mixed Ration †	Concentrate ‡	Meadow Hay §	Barley Straw Φ
DM (g/kg)	908	907	886	902
CP (g/kg DM)	124	152	154	40
NDF (g/kg DM)	466	262	569	796
ADF (g/kg DM)	253	62	320	456
ADL (g/kg DM)	40	8	58	58
Ash (g/kg DM)	113	60	98	65
ME (MJ/kg DM)	11.0	14.4	9.8	7.5

† Diet used during cow gestation (maternal nutrition treatment) and lactation and heifer lactation; ‡, §, Φ diet used during heifer rearing; § diet used during the last month of heifer pregnancy; DM, dry matter; CP, crude protein; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; ADL, acid-detergent lignin; ME, metabolizable energy.

2.2. Heifer Management during Rearing

After calf weaning, only female calves were selected for this study. We distributed 21 PA and 15 PI heifers into two groups, according to their maternal nutrition: heifers from CONTROL cows (CONTROL heifers, $n = 17$ (15 PA + 2 PI)) and heifers from SUBNUT cows (SUBNUT heifers, $n = 19$ (6 PA + 13 PI)). Four-month-old heifers were housed in a pen and fed 2 kg/head/day of a commercial concentrate (corn 47%, corn gluten feed 15%, barley 15%, soya flour 6%, sunflower pulp 6%, carob flour 4%, palm oil 4%, and vitamin–mineral complex 3.2%) plus ad libitum meadow hay and barley

straw (Table 1) during rearing. The meadow hay intake was recorded (offer minus refusal) per group (CONTROL versus SUBNUT), and their values are expressed as mean values.

Sixteen-month-old heifers were synchronized to estrus with a protocol based on a progesterone-releasing intravaginal device (PRID Delta 1.55 g, CEVA, Libourne, France) and a 100 µg injection of gonadotropin-releasing hormone (GnRH, Cystoreline, CEVA, Libourne, France), followed 7 days later by a 25 mg injection of prostaglandin F2α (Enzaprost T, CEVA, Libourne, France). After 9 days, the PRID was removed and 250 IU of pregnant mare serum gonadotropin (Foligon, Intervet International B.V., Boxmeer, The Netherlands) was administered, followed 48 h later by a second injection of GnRH (100 µg). Eight hours after the second GnRH injection, heifers were inseminated with a PI sire of proven fertility by an expert technician.

2.2.1. Heifer Growth during Rearing

Heifers were weighed monthly during rearing (from 4 to 16 months of age) to calculate the average daily gain (ADG) by linear regression. The body development of the heifers was studied by recording their size measures at 4 (weaning), 12, and 16 months of life (AI). The variables assessed were height at withers (distance from the floor to the highest point of the withers), hearth girth (circumference immediately behind the shoulder blades in a plane perpendicular to the body axis), rump width (maximum distance between iliac tuberosities), and rump length (distance from the ischial tuberosity to the external iliac tuberosity). The external pelvic area was estimated as the product of rump width and rump length [22].

2.2.2. Heifer Reproductive Performance during Rearing

To determine the onset of puberty, progesterone concentration was assessed from 8 to 16 months of age (AI). Samples were collected by coccygeal venipuncture into heparinized tubes (BD Vacutainer Becton-Dickenson and Company, Plymouth, UK) on days 1 and 10 every 28 day period [23]. Samples were centrifuged at 1500× *g* for 20 min at 4 °C immediately after collection, and the plasma was harvested and frozen at −20 °C until analysis. Plasma progesterone concentration (ELISA test, sensitivity: 0.27 ng/mL) was measured using a specific kit for cattle (Ridgeway Science, Lydney, UK). The mean intra-assay and inter-assay coefficients of variation were 8.0% and 10.4%, respectively. The age at puberty was defined as the date of collection of the first sample that contained ≥1 ng/mL of plasma progesterone. The mature LW of the heifers was established at 580 kg for both breeds [24] to calculate the percentage of mature LW at which heifers reached puberty. Follicle population, corpus luteum, and ovary size were recorded at 9.5, 13.0, and 15.5 months of life by ultrasonography (Aloka SSD-500V, Aloka, Madrid, Spain) using a linear-array 7.5 MHz transducer. The total number of follicles per pair of ovaries was recorded in three categories: small (<5 mm in diameter), medium (between 5 and 10 mm in diameter), and large follicles (>10 mm in diameter) [25]. The diameters of the dominant follicles, corpus luteum, and the ovaries were assessed by considering the average between measurements of their two perpendicular axes [26]. Pregnancy diagnosis was performed by ultrasonography on day 37 post-AI to assess the pregnancy rate to single AI.

2.2.3. Heifer Metabolic and Endocrine Profiles during Rearing

To assess the metabolic and endocrine status of heifers during rearing, blood samples were collected every three months into EDTA or heparinized tubes to determine glucose, non-esterified fatty acids (NEFAs), urea, cholesterol, and insulin-like growth factor 1 (IGF-1) concentrations. Furthermore, in the case of IGF-1, blood samples were previously taken every month from their mothers during the first third of gestation (cow gestation, when the maternal nutritional treatment was applied) and from heifers during their suckling period (cow lactation [18]). Blood samples were centrifuged at 1500× *g* for 20 min at 4 °C, and plasma samples were taken and frozen at −20 °C until analysis. An automatic analyzer (GernonStar, RAL/TRANSASIA, Dabhel, India) was used to measure blood concentrations of glucose (glucose oxidase/peroxidase method, sensitivity: 0.056 mmol/L), urea (kinetic

UV test, sensitivity: 0.170 mmol/L), and cholesterol (enzymatic colorimetric method, sensitivity: 0.026 mmol/L). The mean intra-assay and inter-assay coefficients of variation for these molecules were <5.4% and <5.8%, respectively. Non-esterified fatty acids (NEFAs, enzymatic method, sensitivity: 0.06 mmol/L) were analyzed using a commercial kit (Randox Laboratories Ltd., Crumlin Co., Antrim, UK). The mean intra-assay and inter-assay coefficients of variation were 5.1% and 7.4%, respectively. Insulin-like growth factor 1 (enzyme immunoassay, sensitivity: 20 ng/mL) was determined using a solid-phase enzyme-labeled chemiluminescent immunometric assay (Immulite, Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd, UK). The mean intra-assay and inter-assay coefficients of variation were 3.1% and 12.0%, respectively.

2.3. Heifer Management during Gestation and First Lactation

From AI to one month before the expected calving date, pregnant heifers grazed on mountain meadows (4 heifers/ha) following the traditional management system [24]. These pastures were primarily composed of grasses (*Festuca arundinacea, Festuca pratensis*, and *Dactylis glomerata*), legumes (*Trifolium repens*), and other species (1191 kg dry matter/ha). During this period, two heifers experienced pregnancy losses. From the last month of gestation, heifers were housed and fed 9 kg/head/day of meadow hay (Table 1). After the first calving, heifers received 10 kg/head/day during lactation of the same dry total mixed ration described above (Table 1). Heifers reared their calves until weaning at day 105. Calves had free access to suckle their dams and received no other feed during lactation period. Water and vitamin–mineral supplements (lick block) were supplied in all phases throughout the experiment.

Heifers and their calves were weighed at calving and weaning to calculate their average daily gain (ADG). The heifer body condition score (BCS) was assessed at calving by two expert technicians, based on the estimation of fat covering loin, ribs, and tail head (0 to 5 scale [20]), and calving ease was classified into two categories: assisted or unassisted. Assisted calving included all types of assistance, from manual pull to caesarean section.

2.4. Statistical Analyses

All statistics were calculated using SAS statistical package v.9.4 (SAS Institute Inc., Cary, NC, USA). The normal distribution of data was assessed with the Shapiro–Wilk test ($p > 0.05$). Heifer BCS at calving, ADG of heifers and calves (heifer progeny), and the age and percentage of mature LW when heifers reached puberty were analyzed with a generalized linear model (GLM procedure) with the nutritional treatment (CONTROL versus SUBNUT), breed (PA versus PI), and their interaction as fixed effects. In the case of ADG of calves (heifer progeny), sex was considered as a fixed effect. The LW of heifers and their calves (heifer progeny), body size measures, follicle population, corpus luteum and ovary size, and metabolite (glucose, NEFAs, urea, and cholesterol) and hormone (IGF-1) concentration were analyzed using a mixed linear model (MIXED procedure) for repeated measures based on Kenward–Roger's adjusted degrees of freedom solution. The fixed factors were nutritional treatment, breed, and their interactions as the between-subject effects, sampling day as the within-subject effect, and animal as the random effect (experimental unit). In the case of LW of calves (heifer progeny), sex was considered as a fixed effect. The least squares (LS) means of the treatments were estimated per fixed effect, and pair-wise comparisons of the means were obtained by the probability of difference (PDIFF) option in the LS means procedure. Fertility rate, percentage of pubertal heifers at 12 and 16 months of age, calving assistance, and male/female calf ratio were assessed using the F-test (FREQ procedure). Relationships among the studied parameters were determined using Pearson's correlation coefficients. The level of significance for all tests was $p < 0.05$. The results are presented as LS means ± standard error (SE) in the text or with the residual standard deviation (RSD) in the tables.

3. Results

No significant interactions between maternal nutrition and breed were found in our results throughout the study; thus, the results were examined separately.

3.1. Heifer Growth during Rearing

The CONTROL heifers were heavier than the SUBNUT heifers at weaning ($p = 0.020$). These differences disappeared thereafter, and both groups had similar LW at AI (Table 2). During rearing, SUBNUT heifers had higher ADG than CONTROL heifers, but this difference was not significant ($p > 0.05$). Regarding the breed, despite no differences being found among breeds at weaning ($p > 0.05$), PA had higher LW at AI than PI heifers ($p = 0.036$) which reflected the higher weight gains during rearing in PA than in PI heifers ($p = 0.002$).

Table 2. Heifer productive performance and body size measures during rearing according to maternal nutrition and breed.

Item	Maternal Nutrition		Breed		RSD	*p*-Value	
	CONTROL	SUBNUT	Parda	Pirenaica		Maternal Nutrition	Breed
Heifer performance							
LW at weaning (kg)	152 [a]	133 [b]	147	138	10.3	0.020	0.270
LW at AI (kg)	415	400	420 [a]	395 [b]	20.5	0.199	0.036
ADG during rearing (kg/d)	0.741	0.792	0.823 [a]	0.710 [b]	0.0757	0.148	0.002
Age at AI (months)	16.0	15.7	15.6	16.1	0.42	0.248	0.056
Height at withers							
At 4 months (weaning, cm)	95	92	95	92	2.9	0.156	0.126
At 12 months (cm)	115	113	117 [a]	112 [b]	3.3	0.208	0.010
At 16 months (cm)	121	120	124 [a]	118 [b]	2.8	0.435	0.001
Heart girth							
At 4 months (weaning, cm)	119	115	118	115	5.3	0.060	0.223
At 12 months (cm)	162	158	163 [a]	157 [b]	5.5	0.110	0.027
At 16 months (cm)	175	173	178 [a]	170 [b]	5.9	0.416	0.006
External pelvic area							
At 4 months (weaning, dm^2)	9.6	8.7	8.8	9.5	1.07	0.146	0.242
At 12 months (dm^2)	18.3	17.5	18.7 [a]	17.1 [b]	1.42	0.343	0.043
At 16 months (dm^2)	21.9	21.0	22.6 [a]	20.3 [b]	1.31	0.208	0.002

CONTROL, heifers from cows fed 100% of their requirements in early pregnancy; SUBNUT, heifers from cows fed 65% of their requirements in early pregnancy; RSD, residual standard deviation; LW, live weight; AI, artificial insemination; ADG, average daily gain. [a,b] Means within a row with different superscripts differ significantly ($p < 0.05$).

Regarding heifer growth, body size was not influenced by maternal nutrition (Table 2). No differences were found in any size trait during rearing between CONTROL and SUBNUT heifers ($p > 0.05$). Inter-breed differences were evidenced from 12 months of age onward, since PA heifers had higher values than PI in terms of height at withers, heart girth, and external pelvic area, both at 12 and 16 months of age ($p < 0.05$). The heart girth during rearing was highly correlated with the heifer LW ($r = 0.99$, $p < 0.001$), with a good predictive capacity ($y = 4.67x - 401.23$, $r^2 = 0.98$, where y = LW and x = heart girth).

During rearing, the average hay intake was 5.36 kg DM/day for CONTROL and 4.57 kg DM/day for SUBNUT heifers.

3.2. Heifer Reproductive Performance during Rearing

All heifers reached puberty at a similar age (12.0 ± 1.6 months) and LW (340 ± 30.3 kg, which was 59% of expected mature LW), regardless of the maternal nutrition or breed ($p > 0.05$, Table 3). Of the heifers, 56% were pubertal at 12 months (average age at puberty) and 91% had reached puberty at 16 months. Heifers were inseminated at 16 months of age (average LW 408 kg, which was 70%

of expected mature LW). Heifer age at puberty was negatively correlated with its LW at weaning 8 months before ($r = -0.44$, $p = 0.001$).

Table 3. Reproductive performance of heifers during rearing according to maternal nutrition and breed.

Items	Maternal Nutrition		Breed		RSD	*p*-Value	
	CONTROL	SUBNUT	Parda	Pirenaica		Maternal Nutrition	Breed
Age at puberty (months)	12.0	12.1	11.6	12.6	1.58	0.905	0.169
LW at puberty (kg)	341	336	350	327	23.8	0.659	0.076
Mature LW at puberty (%) †	59	58	61	56	4.8	0.723	0.055
Puberty reached by 12 months (%) ‡	63	50	63	60	-	0.210	0.272
Puberty reached by 16 months (%)	94	89	95	87	-	0.409	0.333
Fertility to a single AI (%)	78.6	81.3	82.4	76.9	-	0.343	0.328

† 580 kg of expected mature LW for both breeds; ‡ % of animals that reached puberty before the mean age at puberty reported in each group; CONTROL, heifers from cows fed 100% of their requirements in early pregnancy; SUBNUT, heifers from cows fed 65% of their requirements in early pregnancy; RSD, residual standard deviation; AI, artificial insemination.

Regarding ovarian follicle population (Table 4), at 9.5 months of age, SUBNUT heifers had more medium follicles than CONTROL heifers ($p = 0.019$). At 13 months of age, CONTROL heifers had more large follicles than SUBNUT heifers ($p = 0.041$), and at 15.5 months of age, CONTROL heifers had more small follicles ($p = 0.011$) and less large follicles ($p = 0.032$) than SUBNUT heifers. No differences were found in the presence or absence of a corpus luteum, in its diameter, or in the ovary size between CONTROL and SUBNUT heifers at either age ($p > 0.05$). Regarding breed, PA had more large follicles ($p = 0.044$) and larger ovary diameter ($p = 0.009$) than PI heifers at 9.5 months of age. At 15.5 months of age, the diameter of the dominant follicle ($p = 0.017$) and the ovary diameter ($p = 0.003$) were higher in PA than in PI heifers. The ovary diameter at 15.5 months was positively correlated with the heart girth of heifers at weaning 11.5 months before ($r = 0.64$, $p < 0.001$).

Table 4. Follicle population, corpus luteum, and ovary size of heifers during rearing according to maternal nutrition and breed.

Items	Maternal Nutrition		Breed		RSD	*p*-Value	
	CONTROL	SUBNUT	Parda	Pirenaica		Maternal Nutrition	Breed
Small follicles (<5 mm)							
At 9.5 months (*n*)	8	9	10	7	4.4	0.365	0.217
At 13 months (*n*)	10	10	9	11	4.1	0.964	0.432
At 15.5 months (*n*)	16 [a]	11 [b]	13	14	4.5	0.011	0.418
Medium follicles (5 < x < 10 mm)							
At 9.5 months (*n*)	0.8 [b]	2.5 [a]	1.8	1.4	1.45	0.019	0.524
At 13 months (*n*)	0.9	1.9	2.1	0.7	1.79	0.234	0.100
At 15.5 months (*n*)	1.4	0.8	0.9	1.3	1.40	0.364	0.637
Large follicles (>10 mm)							
At 9.5 months (*n*)	0.8	0.4	0.8 [a]	0.4 [b]	0.49	0.108	0.044
At 13 months (*n*)	0.9 [a]	0.4 [b]	0.5	0.8	0.57	0.041	0.367
At 15.5 months (*n*)	0.4 [b]	0.9 [a]	0.9	0.4	0.51	0.032	0.056
Dominant follicle diameter							
At 9.5 months (mm)	11.2	9.5	10.9	9.8	1.69	0.054	0.227
At 13 months (mm)	11.1	10.2	10.9	10.5	3.19	0.544	0.807
At 15.5 months (mm)	10.5	11.4	12.4 [a]	9.5 [b]	2.31	0.451	0.017

Table 4. *Cont.*

Items	Maternal Nutrition		Breed		RSD	*p*-Value	
	CONTROL	SUBNUT	Parda	Pirenaica		Maternal Nutrition	Breed
Corpus luteum							
Heifers with CL at 13 months (%)	88	72	84	73	-	0.191	0.246
CL diameter at 13 months (mm)	19.2	17.9	18.6	18.5	4.25	0.601	0.968
Heifers with CL at 15.5 months (%)	94	83	95	80	-	0.282	0.186
CL diameter at 15.5 months (mm)	13.2	17.2	16.6	13.8	4.13	0.119	0.265
Ovary diameter							
At 9.5 months (mm)	14.0	14.4	15.5 ^{a}	12.9 ^{b}	1.41	0.639	0.009
At 13 months (mm)	18.6	17.5	18.3	17.7	2.02	0.325	0.608
At 15.5 months (mm)	17.8	18.8	19.9 ^{a}	16.7 ^{b}	1.73	0.292	0.003

CONTROL, heifers from cows fed 100% of their requirements in early pregnancy; SUBNUT, heifers from cows fed 65% of their requirements in early pregnancy; RSD, residual standard deviation; CL, corpus luteum; ^{a,b} means within a row with different superscripts differ significantly ($p < 0.05$).

The mean fertility rate to a single AI was 80%, regardless of maternal nutrition or breed ($p > 0.05$, Table 3).

3.3. Heifer Metabolic and Endocrine Profiles during Rearing

Metabolic and endocrine profiles of the heifers are presented in Figure 2.

Glucose concentration did not differ between CONTROL and SUBNUT heifers during rearing ($p > 0.05$), and PA had higher concentration than PI at AI ($p = 0.036$).

The SUBNUT heifers had higher NEFA concentration than CONTROL heifers at 13 months of age ($p = 0.004$), whereas no differences were found among breeds ($p > 0.05$).

Urea concentration in the SUBNUT heifers tended to be higher than in CONTROL heifers throughout rearing, specifically at 13 months ($p = 0.053$). Regarding the breed, PA heifers had higher urea concentration than PI heifers with statistical differences at month 13 ($p = 0.027$). Urea concentration was correlated with the heifer ADG during rearing ($r = 0.65$, $p < 0.01$).

The SUBNUT heifers had higher cholesterol concentrations than CONTROL heifers during rearing, with statistical differences at month 13 ($p = 0.043$). No differences were found among breeds ($p > 0.05$). A strong relationship was found between cholesterol and urea concentrations at 13 ($r = 0.70$, $p < 0.001$) and 16 months ($r = 0.52$, $p = 0.002$).

No differences were found in IGF-1 concentration between CONTROL and SUBNUT heifers, or between PA and PI heifers throughout rearing ($p > 0.05$). The mean IGF-1 concentration of heifers during rearing was correlated with their age at puberty ($r = -0.71$, $p < 0.001$) and their ADG during rearing ($r = 0.44$, $p = 0.009$). The mean IGF-1 concentration of heifers during rearing was positively correlated with the IGF-1 concentration during their lactation period (0–4 months old; $r = 0.42$, $p = 0.013$), which, in turn, was correlated with the IGF-1 concentration of their mothers during the first third of pregnancy (maternal nutrient treatment, $r = 0.35$, $p = 0.035$).

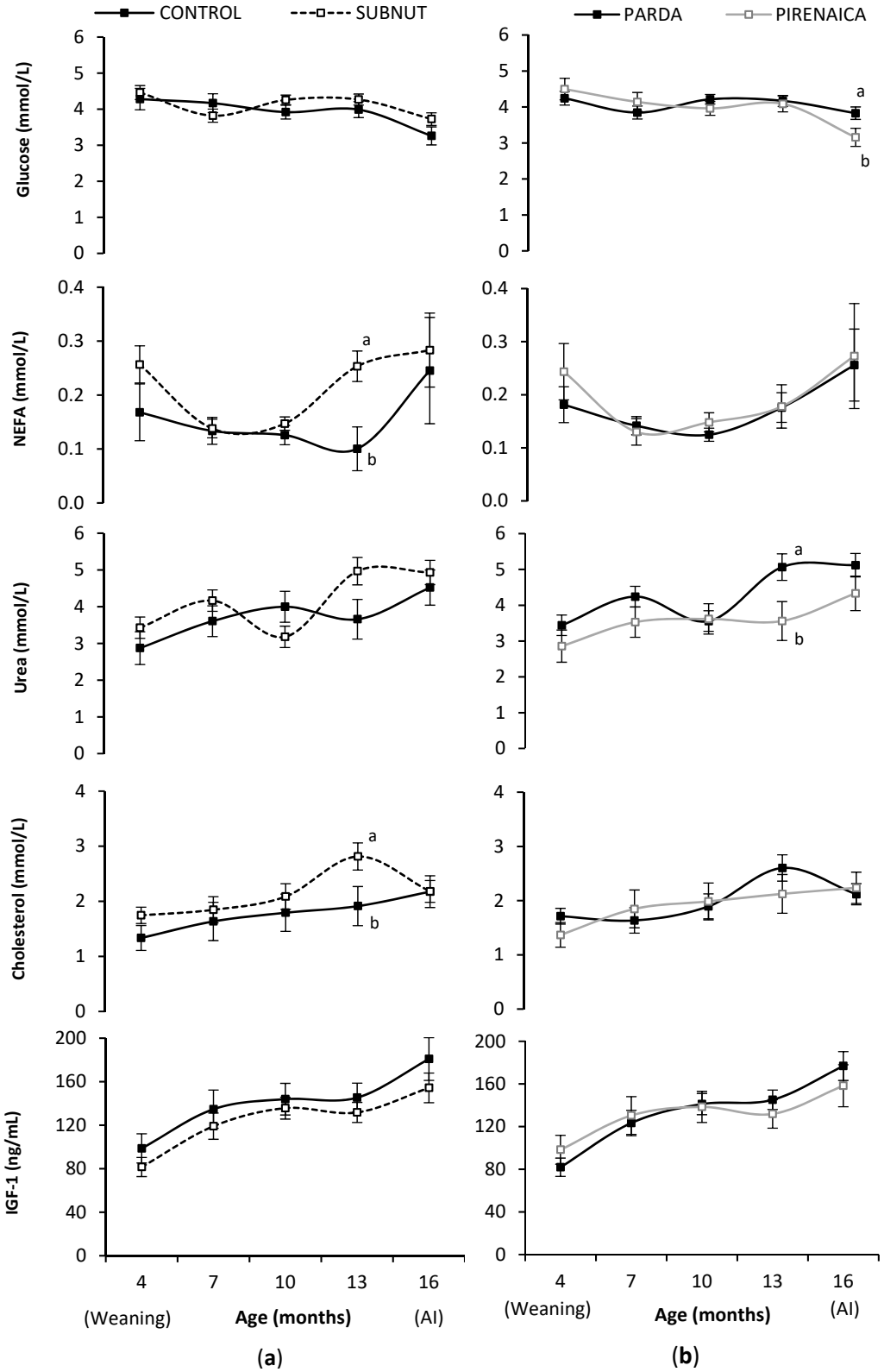

Figure 2. Heifer metabolic and endocrine profiles during rearing according to (**a**) maternal nutrition and (**b**) breed. CONTROL, heifers from cows fed 100% of their requirements in early pregnancy; SUBNUT, heifers from cows fed 65% of their requirements in early pregnancy; AI, artificial insemination. [a, b] Means at given age with different letters differ significantly ($p < 0.05$).

3.4. Heifer Performance during Gestation and First Lactation

The CONTROL and SUBNUT heifers had similar LWs and ADGs during gestation and the following lactation period ($p > 0.05$, Table 5). Similarly, no differences were found between PA and PI LWs and ADGs during gestation and the following lactation ($p > 0.05$), but PA had lower BCS at calving than PI ($p = 0.001$). No differences in the calving assistance were found between the CONTROL and SUBNUT or between PA and PI heifers ($p > 0.05$).

Table 5. Heifer performance during gestation and first lactation according to maternal nutrition and breed.

Items	Maternal Nutrition		Breed		RSD	*p*-Value	
	CONTROL	SUBNUT	Parda	Pirenaica		Maternal Nutrition	Breed
Heifer performance							
Gestation ADG (kg/day)	0.334	0.283	0.298	0.319	0.0969	0.275	0.645
LW at calving (kg)	520	491	516	494	33.0	0.103	0.204
BCS at calving	3.0	3.0	2.8 ^{b}	3.2 ^{a}	0.16	0.425	0.001
Age at calving (months)	26.4	26.3	26.1	26.6	1.52	0.844	0.584
Calving assistance (%)	26.7	16.7	25.0	18.2	-	0.304	0.338
LW at weaning (kg)	469	452	478	443	42.0	0.445	0.124
Lactation ADG (kg/day)	−0.519	−0.349	−0.373	−0.494	0.2318	0.168	0.323
Calf performance							
Male/female calf ratio	8/7	3/9	8/8	3/8	-	0.109	0.163
LW at birth (kg)	35	34	36	33	3.7	0.321	0.134
LW at weaning (kg)	111	105	122 ^{a}	94 ^{b}	19.4	0.505	0.012
Lactation ADG (kg/day)	0.720	0.680	0.814 ^{a}	0.587 ^{b}	0.1918	0.684	0.031

CONTROL, heifers from cows fed 100% of their requirements in early pregnancy; SUBNUT, heifers from cows fed 65% of their requirements in early pregnancy; RSD, residual standard deviation; ADG, average daily gain; AI, artificial insemination; BCS, body condition score; calving assistance, assisted (from manual pull to caesarean section) or unassisted calving; LW, live weight; ^{a,b} means within a row with different superscripts differ significantly ($p < 0.05$).

Regarding the calves born from heifers, the male/female calf ratio was lower in SUBNUT and in PI heifers than in CONTROL and PA heifers but not significantly ($p > 0.05$). Maternal nutrition had no effects on calf LW at birth or ADG during lactation ($p > 0.05$). Regarding breed, PA and PI calves had similar LWs at birth ($p > 0.05$), but PA calves had higher ADG than PI calves during lactation ($p = 0.031$) which implied higher LW at weaning ($p = 0.012$). Calf sex had no effect on LW at birth (36 ± 1.4 versus 33 ± 1.4 kg for male and female calves, respectively, $p > 0.05$), at weaning (103 ± 7.4 versus 111 ± 5.9 kg for male and female calves, respectively, $p > 0.05$), or on ADG during lactation (0.636 ± 0.0872 versus 0.775 ± 0.0872 for male and female calves, respectively, $p > 0.05$).

The calf ADG during lactation was correlated with its dam LW at calving ($r = 0.65$, $p < 0.001$). Calf LW at weaning was highly correlated with the LW of heifer when it was weaned ($r = 0.61$, $p = 0.001$).

4. Discussion

4.1. Growth of Heifers and Their Calves

Maternal nutrition in early pregnancy impaired the heifer LW at weaning (at the end of its suckling period); however, this difference disappeared during subsequent heifer rearing, gestation, and lactation. This result suggests that SUBNUT heifers exhibited compensatory growth to mitigate the growth delay recorded at weaning. The ADG of SUBNUT heifers during rearing was higher than that of CONTROL heifers, although not significantly. Maternal nutrition in early pregnancy most impacts on organ and tissue development, with potential long-term consequences during any postnatal age, rather than on fetal growth, which is associated with maternal nutrition during later pregnancy [27]. These long-term consequences were evidenced in the offspring endocrine profiles at birth [15], in their performance during lactation [18], and at weaning, with retarded growth in SUBNUT heifers in the current study.

The similar LW during rearing, gestation, and first lactation of heifers suggested that the growth delay observed at weaning was not a permanent stunting and an adequate nutrition could overcome these LW differences, agreeing with the results reported by Freetly et al. [28]. In our study, after the first calving, the calf LW at birth and growth rate were not affected by the early nutritional treatment, suggesting that maternal nutrition did not affect the performance of this second generation, at least during lactation. However, since females of these breeds reach maturity when they are 4 to 5 years old [29], further studies are needed to determine if the heifer growth delay at weaning would impact the attainment of adult weight and mature size. Regarding breed, PA heifers had higher growth rates during rearing, BCS at calving, and calf LW at weaning than PI heifers, in accordance with previous studies [30], which evidenced similar interbreed differences.

To make management decisions considering these breed differences, heart at girth was confirmed to be a useful, objective, and easily obtainable parameter to estimate heifer LW, especially in those livestock production systems where the ability to use a scale is limited.

4.2. Heifer Reproductive Performance

In the current study, all heifers reached puberty at a similar age and LW, regardless of the maternal nutrition or the breed. Despite the growth delay observed at weaning in the SUBNUT group, adequate nutrition during rearing could prevent the potential consequences. Puberty is reached at a critical LW of around 55% of the mature LW, irrespective of the growth patterns [31]. Aligned with our results, Corah et al. [32] reported that age at puberty was not affected in female progeny of heifers fed a diet that met 65% of their recommended energy intake, although in that case, the energy restriction was performed during the last third of gestation. Similarly, Smith et al. [13] described no differences in age at puberty between control and maternal nutrient-restricted sheep during the first 55 days of gestation. Regarding breed, due to the higher ADG during rearing, PA heifers reached puberty 1 month earlier and 23 kg heavier than PI heifers, although these differences were not significant. In a previous study, PA and PI heifers reached puberty at a similar LW, but two months earlier than in our study due to the fact of their higher growth rates after weaning [30].

In our study, the follicular population was affected by maternal nutrition. Differences in medium and large follicle counts and the diameter of the dominant follicle were found; however, these results need to be interpreted as they are dependent on the estrus cycle phase of each animal at the scanning day. Otherwise, the number of small follicles is related to the number of follicles present in the ovaries, reflecting the number of remaining primordial follicles and, thus, the ovarian reserve [33]. The lack of differences in numbers of large follicles at 9.5 months of age, before the mean age at puberty (12 months of age), agrees with our finding that CONTROL and SUBNUT heifers reached puberty at a similar age. At 15.5 months of age, the number of small follicles was higher in CONTROL than in SUBNUT heifers. The differentiation of primordial follicles in the ovaries of the female fetus occurs between days 90 and 140 of gestation [9]. A restricted maternal environment in early pregnancy may affect this process, impairing the ovarian reserve [9,34,35]. Mossa et al. [36] described lower antral follicle count in calves born to 60% energy-restricted cows before conception to the end of the first trimester. Maternal nutrient restriction during early gestation reduces the proliferation of ovarian germ-cells and alters the expression of genes that regulate apoptosis in the follicle and granulosa cells, both mechanisms contributing to reduce the number of ovarian primordial follicles [37]. Additionally, we hypothesize that the differences found in IGF-1 concentrations between CONTROL and SUBNUT cows during early pregnancy, which we observed in an earlier phase of this study [21], could have resulted in subsequent changes in fetal hormone levels and in its metabolic pathways, which in turn may have altered gene expression in fetal gonads [38]. Several studies associated a high antral follicle count with improved reproductive efficiency [9,25,35,39], whereas a lower follicle count was related to a reduced lifetime reproductive capacity [40]. In our study, maternal subnutrition could have triggered a disruption in normal gonadal development, reducing the antral follicle count; however, it did not impair the pregnancy success. The ovary diameter was not affected by maternal nutrition in our study.

In contrast, Wilkins et al. [41] described lower ovarian weights in heifers from undernourished dams from 80 days of pregnancy to parturition. Regarding the breed, PA heifers had higher numbers of large follicles and bigger ovaries than PI at 9.5 months of age, which agrees with our results that PA heifers reached puberty one month earlier than PI, although this difference was not significant. Parda de Montaña heifers had also larger ovary size than PI at 15.5 months of age, in line with the larger body size measures in PA than in PI heifers.

In the current study, 80% of heifers were pregnant with a single AI, which is a higher pregnancy rate than reported other studies using a similar synchronization protocol [30] or cosynch protocols [42]. This high pregnancy rate could be associated with the optimal body development of heifers at AI (approximately 70% of the mature LW). Gasser [43] recommended breeding for the first time when heifers have reached the 65% of their expected mature LW. Maternal undernutrition did not impair the fertility rate to a single AI. To the best of our knowledge, few studies have reported the effect of the plane of maternal nutrition in early gestation on the fertility rate of the progeny. Martin et al. [23] and Cushman et al. [12] reported that increasing maternal nutrient intake during late gestation improved the pregnancy rate of their daughters. Based on our results, we partially reject our initial hypothesis because no immediate effects were observed on heifer reproductive performance. This could be because the maternal nutrient restriction applied in our study during the first third of pregnancy was not sufficient to induce changes in heifer fertility at first breeding; however, more studies are needed to assess the long-term consequences of a reduced antral follicle count on their lifetime reproductive performance. Regarding the breed, no differences in fertility rate were found between PA and PI heifers in our study, in agreement with previous studies conducted with the same breeds [30].

Surprisingly, in the current study, the ratio of male to female calves was not balanced in SUBNUT and PI heifers, with three times more female than male calves being born, although the differences were not significant. Some authors agree with the Trivers and Willard [44] sex ratio theory, which states that mothers that experienced severe environmental shocks, such us undernutrition, give birth to female calves. This mechanism could protect those fetuses having greater chances of being reproductively successful [45–47]. At the beginning of this study, SUBNUT dams had a negative energy balance during the first third of the heifer gestation and PI breed was more sensitive to undernutrition than PA [15]. However, studies involving more animals are needed to demonstrate the sex ratio theory in the progeny.

4.3. Heifer Metabolic and Endocrine Profiles

The heifer metabolic and endocrine profiles during rearing exhibited long-term consequences of maternal nutrition, which modified the physiological response of the offspring. Interbreed differences between PA and PI were evidenced.

Glucose profiles are strongly linked with the short-term effect of the current energy and/or protein intake [30]. Furthermore, prenatal nutrient availability may influence the ability of calves to regulate glucose blood concentration during postnatal growing [48]. In our experiment, the lack of differences in glucose concentrations throughout rearing between CONTROL and SUBNUT heifers indicated that glucose metabolism was not affected in their postnatal lives. Similarly, other studies have described no effect of nutritional restriction during early pregnancy on calf basal glucose concentration [11]. Regarding the breed, the higher glucose concentration at AI in PA than in PI could suggest that PA could have consumed more hay and straw (which were offered ad libitum, unlike the concentrate) than PI. This would be consistent with the higher LW and body size measures evidenced at AI.

The NEFA concentrations of SUBNUT heifers were higher than those of the CONTROL group, especially at 13 months of age, around puberty onset. We hypothesize that the higher NEFA concentration in SUBNUT heifers may be due to the extra metabolic effort that allowed SUBNUT heifers to reach a similar LW to that of CONTROL heifers at puberty and breeding. These differences at 13 months of age could also be related to the higher number of large follicles found in CONTROL heifers at this time. According to previous studies [49–51], an increase in NEFA concentration may inhibit the

granulosa cell survival and proliferation, inducing a reduction in the growth rate of dominant follicles and in the estradiol production. Breed did not affect NEFA metabolism, since concentrations were similar throughout rearing.

Urea concentration tended to be higher in SUBNUT than in CONTROL heifers, especially around puberty onset. Plasma urea concentration is positively correlated with dietary protein intake [52,53], but also with the catabolism of body protein in periods of energy shortfall [30]. The higher requirements in SUBNUT than CONTROL heifers could force heifers to catabolize amino acids from tissue proteins, increasing the urea concentration [54]. In the current study, urea concentration was strongly related to the ADG. Likewise, PA had higher urea concentration than PI which agrees with their higher growth rates.

Cholesterol concentration was higher in SUBNUT than in CONTROL heifers around puberty onset. Cholesterol concentration is positively related with nutrient intake [55]. In our study, cholesterol levels may be associated to the higher liver metabolic rates of SUBNUT heifers, increasing the secretion of very-low-density lipoproteins to blood which increased plasma cholesterol concentrations [56]. Urea and cholesterol concentrations were positively related in the current study. Cholesterol is a precursor for steroid hormone synthesis [57]; therefore, Anderson et al. [58] suggested that an increase in serum cholesterol may influence the reproductive development. However, in our study, no differences in age or LW at puberty onset were found.

Nutritional restriction during the fetal development might affect the IGF axis [48]. In a previous phase of this study, heifers from nutrient-restricted cows had lower IGF-1 concentrations than heifers from control cows at birth [15]. A transgenerational relationship was described between IGF-1 concentrations of cows during early gestation and those from heifers during their suckling period [18]. However, no differences were found between CONTROL and SUBNUT heifers during rearing. The optimal feeding level and growth rate of SUBNUT heifers during rearing may have been reflected in increased IGF-1 concentrations, balancing the IGF-1 values between CONTROL and SUBNUT heifers. Similarly, Maresca et al. [48] described lower IGF-1 concentrations at birth in calves whose mothers had received a low-protein diet from mid-gestation to parturition, with these differences disappearing during calf postnatal growth. Insulin-like growth factor 1 is related to skeletal and muscle development in growing cattle [59] and higher serum IGF-1 concentration is associated with faster growth rates [60]. In our study, IGF-1 concentration was highly correlated with ADG and age at puberty ($r = -0.7$), confirming that IGF-1 is an important mediator involved in the onset of puberty in cattle [61] and could be a good indicator to estimate the age at puberty. The mean IGF-1 concentration of heifers in earlier ages (0–4 months) was moderately correlated with the IGF-1 concentration of heifers during rearing (4–16 months), and to the IGF-1 concentration of their mothers during the first third of gestation (maternal nutritional treatment). No differences were found in IGF-1 concentration between breeds, which agrees with the results reported by Álvarez-Rodríguez et al. [16] and Rodríguez-Sánchez et al. [30].

5. Conclusions

Maternal undernutrition in early gestation had some long-term effects on female offspring postnatal performance. Heifers born from underfed cows were lighter at the beginning of the rearing period with this difference disappearing at the end of rearing and during the following gestation and lactation periods. Consequently, heifers from underfed cows increased their nutrient requirements for growth and their metabolic status was impaired during the rearing period. Maternal undernutrition influenced the heifer ovarian development with reduced antral follicle count at breeding but did not affect the age and weight at the onset of puberty nor pregnancy success at single artificial insemination (16 months old) or their calves' weights during the first lactation. As heifers grow until they are five years old, further research is needed to determine if the growth delay at weaning, the impaired metabolism observed during rearing, and the reduced antral follicle count will impact the attainment of

adult weight, mature size, and reproductive lifespan in heifers having suffered maternal undernutrition during their early fetal development.

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5. Consideraciones finales

5. CONSIDERACIONES FINALES

El sistema de producción en la vaca nodriza se desarrolla en condiciones cada vez más extensivas para reducir costes en la alimentación, con lo que es habitual que los animales sufran periodos de subnutrición durante su ciclo de producción. Esta Tesis Doctoral se planteó para analizar los efectos a corto, medio y largo plazo que tiene la subnutrición durante el primer tercio de gestación, tanto en la vaca y su ternero lactante, como en la vida postnatal del ternero que se estaba gestando, y en el caso de las terneras, durante la posterior recría, gestación y lactación. Para ello se utilizaron las dos razas autóctonas más representativas del Pirineo aragonés, la Parda de Montaña y la Pirenaica.

Los principales resultados y consideraciones que se obtuvieron a lo largo de este estudio fueron:

5.1. En las vacas y sus terneros durante la fase de GESTACIÓN

Durante el período en el que se aplicó la subnutrición (primeros 82 días de gestación), las vacas disminuyeron su **peso** y **condición corporal**. Esta restricción nutritiva se vio reflejada en los **perfiles metabólicos, endocrinos** y **hematológicos** de estos animales. Se observó en las vacas subnutridas una mayor concentración de AGNE a causa de una mayor movilización de las reservas lipídicas, una menor concentración plasmática de IGF-1 y una disminución del recuento de granulocitos y plaquetas. Estos efectos fueron más severos en la raza Pirenaica que en la Parda de Montaña, lo que indicó que esta raza presentaba mayor sensibilidad a una restricción alimentaria. Con estos resultados se pone de manifiesto la importancia que tiene la base genética de un animal para afrontar un nuevo estímulo o situación ambiental. El genotipo modulará su respuesta adaptativa, obteniéndose diferentes valores en sus parámetros fisiológicos en función de la raza. Estudios anteriores también han descrito diferencias en las respuestas fisiológicas de estas dos razas ante un determinado manejo (Garcia-Belenguer et al., 1996; Álvarez-Rodríguez et al., 2010). Estas consideraciones raciales deberían tenerse en cuenta en una explotación ganadera a la hora de planificar su ciclo de producción y manejo alimentario.

La subnutrición también disminuyó el **crecimiento de los terneros** que las vacas estaban criando durante esta fase. De acuerdo con los resultados de Walker et al. (2004), la situación de balance energético negativo habría disminuido la producción de leche de las madres, repercutiendo negativamente en el crecimiento de los terneros.

En cuanto a los parámetros reproductivos de las vacas, la restricción alimentaria no influyó en la **tasa de fertilidad**. Mediante un protocolo de IATF se obtuvo una tasa de fertilidad media del 77%. Esta alta tasa de fertilidad, sin diferencias entre vacas subnutridas o del grupo control, se atribuyó a la óptima CC de las vacas en el momento de la IATF (2,8 sobre 5). Si las vacas hubieran tenido un menor estado de engrasamiento en el momento de la IATF, la restricción alimentaria habría comprometido más su estado metabólico, pudiéndose reflejar en una disminución de sus rendimientos reproductivos (D'Occhio et al., 2019). Un protocolo de sincronización adecuado, junto con una buena técnica de inseminación y un semen de calidad serán tres elementos indispensables para el éxito reproductivo, pero si el animal no tiene un estado nutricional mínimo para poder activar y mantener los mecanismos fisiológicos de la reproducción, su eficacia reproductiva disminuirá, poniendo en duda la inversión económica y de tiempo para su sincronización e inseminación.

La subnutrición temprana no afectó al reconocimiento y mantenimiento de la gestación. La dieta no influyó en la **expresión de genes estimulados por el IFN-τ** los días 18 y 21 post-IATF, ni se observaron diferencias significativas en el número de **pérdidas embrionarias** entre el grupo control y el subnutrido, aunque la subnutrición podría haber incrementado el riesgo de reabsorción embrionaria. Los cambios en la expresión de los genes OAS1 y MX1 entre el día 18 y 21 post-IATF fueron un buen indicador para diagnosticar el estado de preñez de una vaca. Los niveles de **progesterona** y **PSPB** en vacas gestantes y no gestantes no se vieron afectados por su nivel de alimentación. Como hemos destacado anteriormente, el buen estado nutricional de los animales permitió en el grupo de vacas subnutridas activar diferentes mecanismos compensatorios para hacer frente a una situación ambiental adversa y poder mantener la gestación. En las vacas gestantes, la concentración plasmática de progesterona fue incrementando hasta el día 21 de gestación, y posteriormente se mantuvo constante hasta el día 82 (final del muestreo). En vacas no gestantes, la concentración disminuyó a partir del día 18 post-IATF, de manera que en nuestras condiciones de trabajo se estableció el día 21 post-IATF como el día más temprano y preciso (mejor sensibilidad y especificidad) para diagnosticar el estado de preñez de una vaca en función de sus niveles plasmáticos de progesterona. No obstante, algunos animales pueden tener un ciclo estral con más de dos o tres oleadas foliculares, de manera que se alarga la fase luteínica y, por tanto, la vida del cuerpo lúteo. Al ser la progesterona una hormona no específica de la preñez ligada a la funcionalidad del cuerpo lúteo, como herramienta *per se* para el diagnóstico de gestación se recomendaría repetir su análisis aproximadamente 5 días más tarde, o asociarla a un método de diagnóstico de gestación complementario. En las vacas gestantes, los niveles plasmáticos de PSPB

aumentaron desde el día 25 al día 28 post-IATF, independientemente de la dieta del animal, mientras que en vacas no gestantes los niveles de PSPB disminuyeron ligeramente. A pesar de las recomendaciones del fabricante del kit comercial de detección y cuantificación de la PSPB, que recomendaba su uso como herramienta para el diagnóstico de gestación a partir del día 28 post-IATF, en nuestras condiciones de trabajo este método arrojó unos buenos resultados de sensibilidad y especificidad a partir del día 26 post-IATF. En cualquier caso, fue necesario respetar un periodo mínimo de 73 días desde el último parto para que las concentraciones residuales de PSPB de la última gestación no incrementaran el número de falsos positivos. A diferencia de la progesterona, al ser la PSPB una molécula específica de la preñez, su cuantificación cómo herramienta *per se* para el diagnóstico de gestación sería un método eficaz, rápido y económico. En nuestro ensayo, la concentración de PSPB se determinó en sangre periférica, sería interesante estudiar si la cuantificación de PSPB en leche presentaría los mismos resultados de sensibilidad y especificidad a día 26, ya que en una explotación de vacas lecheras facilitaría en gran medida la obtención de las muestras biológicas.

A partir del primer tercio de gestación, los terneros se destetaron y finalizó el tratamiento nutritivo diferenciado. La alimentación que recibieron los animales durante los últimos 6 meses de gestación, adaptada a sus necesidades nutricionales, permitió que las vacas subnutridas durante el primer tercio de gestación restablecieran sus parámetros hematológicos, con valores similares a los del grupo control en el último mes de gestación. No se observó tampoco ningún efecto de la dieta recibida ni de la raza sobre la concentración plasmática de **inmunoglobulinas G** y **M** de las vacas un mes antes de la fecha prevista de parto.

5.2. En las vacas durante la fase de LACTACIÓN

Las vacas que fueron subnutridas presentaron un **peso** al parto similar al de las vacas del grupo control, ya que durante los dos últimos tercios de la gestación compensaron la diferencia establecida por el tratamiento nutritivo. No obstante, su **CC** al parto fue inferior a la de las vacas del grupo control, hecho que influyó en la mayoría de los parámetros productivos de vacas y terneros durante la lactación. Una vez más, destaca el importante papel que juega el estado nutricional de un animal dentro de su ciclo de producción, siendo la alimentación un pilar básico en el buen funcionamiento de una explotación ganadera.

La dieta recibida durante la gestación temprana no afectó a la concentración plasmática de **Ig G** y **M** de las vacas en el momento del parto, pero se observó un

descenso en su concentración desde el último mes de gestación (aunque en el caso de las Ig M este descenso no fue significativo). Unas semanas antes del parto, la vaca empieza a producir calostro y las Ig son transferidas desde el plasma sanguíneo a la glándula mamaria, produciéndose una reducción fisiológica en su concentración plasmática (Franklin et al., 2005; Herr et al., 2011).

La alimentación de la vaca al principio de gestación no afectó a la **composición química del calostro**. La formación del calostro es un proceso fisiológico que empieza aproximadamente 5 semanas antes del parto, de manera que la subnutrición aplicada durante el primer tercio de gestación no tendría que tener un efecto directo en su composición química. Sin embargo, el calostro de las vacas Pirenaicas subnutridas tuvo una menor concentración de Ig G que sus homólogas Pardas, mientras que no hubo diferencias entre razas en los grupos control. Esta disminución de la concentración de Ig G en el calostro no tuvo consecuencias en la posterior concentración plasmática de los terneros, pero evidenció nuevamente una mayor sensibilidad a la restricción alimentaria en las vacas Pirenaicas. Se observó, independientemente de la dieta durante la gestación temprana y de la raza, que durante las primeras horas postparto la concentración de Ig en el calostro disminuía alrededor de un 50% debido a que, inmediatamente después del parto, se detiene la transferencia de Ig desde la sangre materna al calostro (Barrington y Parish, 2001). Este hecho pone de manifiesto la importancia de asegurar en el recién nacido la ingesta de calostro dentro de las primeras 4 horas de vida para la correcta transferencia de inmunidad pasiva.

En cuanto a la **producción de leche** tres semanas después del parto, la subnutrición durante el primer tercio de gestación disminuyó el porcentaje de grasa de la leche. Esta disminución podría estar asociada a la menor producción de leche (aunque no de forma significativa) de las vacas subnutridas, produciéndose un efecto de concentración en sus constituyentes (Jenkins y McGuire, 2006).

La restricción alimentaria a principios de la gestación no afectó a la duración del **anestro postparto** de las vacas, sin embargo, se observó una relación inversa entre la CC al parto de la vaca y la duración del anestro postparto. Una situación de balance energético negativo suprime la secreción pulsátil de LH, de manera que el folículo dominante que se está desarrollando tendrá menor capacidad de producir una cantidad suficiente de estradiol para inducir la secreción preovulatoria de GnRH. En este sentido, las vacas Pirenaicas, con mayor CC al parto que las Pardas, necesitaron menos días para reiniciar su actividad ovárica. Estos resultados ponen de manifiesto que, de acuerdo con otros trabajos previos de nuestro grupo (Sanz et al., 2004), una correcta alimentación durante el último tercio de gestación que garantice una CC adecuada

en el momento del parto, juega un papel esencial para reducir el intervalo entre partos y mejorar la eficiencia productiva del rebaño.

A lo largo de la lactación (120 días de duración), todas las vacas perdieron peso excepto el grupo de vacas Pirenaicas subnutridas, evidenciando las diferentes respuestas adaptativas de un animal en función de la raza y su manejo alimentario previo. Después del parto, son frecuentes las variaciones de peso debido a cambios en el volumen de tejido adiposo visceral, modificaciones en el tamaño del sistema digestivo, o a causa de la involución uterina (Baldwin et al., 2004). Contrariamente a la evolución del peso de las vacas durante la lactación, la CC fue aumentando. Una vez disminuyeron las grandes necesidades nutricionales derivadas de las últimas etapas de gestación y del inicio de la lactación, las vacas empezaron a reponer sus reservas de tejido graso. En las primeras etapas postparto son frecuentes estas discordancias entre la evolución del peso vivo y la CC.

En nuestro ensayo, el aumento de la CC estuvo reflejado en los **perfiles metabólicos** de las vacas, ya que al incrementar las reservas corporales la concentración plasmática de AGNE fue disminuyendo, coincidiendo con la disminución de la producción de leche una vez pasado el pico de producción.

5.3. En los terneros durante la fase de LACTACIÓN

La subnutrición materna durante el primer tercio de gestación no afectó al **peso** al nacimiento de los terneros. De acuerdo con los resultados descritos por Greenwood y Cafe (2007), una restricción materna durante las primeras etapas de la gestación está asociada a una alteración en el desarrollo de órganos y tejidos, con consecuencias a largo plazo en la vida postnatal del nuevo individuo, mientras que una alimentación materna restringida en la parte final de la gestación afectará más al crecimiento fetal y, por tanto, al peso al nacimiento. En nuestro ensayo, la subnutrición materna tampoco afectó a los resultados del **test de vitalidad** que se realizó a los terneros inmediatamente después del nacimiento. La raza, en cambio, sí tuvo un efecto significativo, ya que los terneros Pirenaicos tuvieron mejores resultados que los Pardos, debido probablemente a que su menor peso al nacimiento facilitó el proceso del parto y redujo el sufrimiento fetal.

Los valores de los **parámetros hematológicos** de los terneros al nacimiento no se vieron afectados por la subnutrición materna, pero se observó un retraso en la maduración de su sistema hematopoyético. Durante el desarrollo fetal, a consecuencia de un ambiente uterino de hipoxia relativa, los eritrocitos son de mayor tamaño y contienen hemoglobina fetal. A partir del nacimiento, estos eritrocitos se van

substituyendo por nuevos eritrocitos de menor tamaño que contienen hemoglobina B (Brun-Hansen et al., 2006). En nuestro estudio, en los terneros procedentes de madres control se observó durante los primeros días de vida una reducción fisiológica de los valores de hematocrito, hemoglobina, hemoglobina corpuscular media y volumen corpuscular medio, mientras que en los terneros procedentes de madres subnutridas no llegó a observarse dicha reducción. Estos resultados indicarían que una subnutrición fetal en la gestación temprana ha podido incidir también en el desarrollo embrionario del sistema hematopoyético, que empieza a partir de la tercera semana post-concepción y se mantiene durante toda la gestación (Tchernia, 1989).

Los **parámetros metabólicos** y **endocrinos** de los terneros al nacimiento también se vieron afectados por la alimentación materna durante la gestación temprana. En los terneros procedentes de madres subnutridas se observó una disminución en la concentración plasmática de IGF-1. En línea con los resultados descritos por Gallaher et al. (1998), la subnutrición materna pudo haber afectado al sistema de regulación de la IGF-1 durante el desarrollo fetal, alterando los niveles de esta hormona en el recién nacido. Contrariamente, la concentración plasmática de cortisol en terneros procedentes de madres subnutridas estuvo incrementada al nacimiento. La subnutrición materna podría haber aumentado la producción de cortisol en el feto y haber alterado la funcionalidad del eje hipotalámico-hipofisario-adrenal. Estos resultados nos demuestran como a través de un estímulo o situación ambiental durante el desarrollo gestacional, el embrión o feto desarrolla estrategias adaptativas (en las que la epigenética juega un papel fundamental) que modificarán sus respuestas fisiológicas ante un estímulo. Sin embargo, si estos cambios fenotípicos persisten durante la vida adulta del animal, nos encontraremos ante un individuo con una fisiología que no se adaptará a las condiciones habituales de las explotaciones ganaderas, aumentando la posibilidad de desarrollar enfermedades crónicas como un síndrome metabólico, adiposidad o diabetes.

En nuestro estudio, la subnutrición materna no afectó a la transferencia de inmunidad pasiva y, a pesar de que el calostro procedente de madres Pirenaicas subnutridas presentó una menor concentración de Ig G, no se vieron afectadas las concentraciones plasmáticas de **Ig G** y **M** de los terneros a las 48 horas de vida. Estos resultados indican que la concentración de inmunoglobulinas presentes en el calostro es elevada *per se,* para asegurar la correcta transferencia de anticuerpos aún en el caso de que por diferentes circunstancias se reduzca su concentración. Por otro lado, estos resultados también nos indican que la subnutrición materna no afectó al desarrollo durante la gestación de los mecanismos de absorción de las células del epitelio intestinal para poder transferir estos anticuerpos desde el lumen intestinal a la circulación

sanguínea. En nuestro estudio, no se detectaron diferencias en la morbilidad o mortalidad de los terneros analizados en función de su alimentación materna o raza, asumiendo una correcta transferencia de inmunidad pasiva en todos los grupos. No obstante, sí que se estableció una relación positiva entre la concentración de anticuerpos presentes en el calostro y en el plasma de los terneros y el crecimiento de éstos durante la lactación.

A la tercera semana de lactación los terneros Pirenaicos procedentes de madres subnutridas registraron un **consumo de leche** inferior al de sus homólogos del grupo control, a pesar de que no había diferencias en la producción de leche de sus respectivas madres. Esto indicó que, especialmente en la raza Pirenaica, una restricción alimentaria durante la gestación podría haber limitado el desarrollo del sistema digestivo, ya fuera durante el desarrollo fetal, o durante las primeras semanas de vida, reduciendo su capacidad de ingesta y con evidentes consecuencias en su posterior desarrollo. Estos terneros Pirenaicos tuvieron un menor crecimiento durante la lactación, con un peso al destete un 19% inferior respecto a los terneros Pirenaicos del grupo control. Durante la primera semana de vida no se registraron diferencias entre las **medidas morfométricas** de los terneros, pero al destete los valores de estas medidas fueron menores en aquellos terneros procedentes de madres subnutridas. Estos resultados indican que la subnutrición causó una desaceleración en el desarrollo corporal de los terneros, especialmente en la raza Pirenaica. Un menor peso del ternero al destete, a consecuencia de un retraso en su crecimiento, generará un impacto económico inmediato en aquellas explotaciones de vacas nodrizas que, una vez destetados, optan por vender sus terneros para su engorde en otras explotaciones.

Estas diferencias de crecimiento se vieron reflejadas en los **perfiles metabólicos** y **endocrinos** de los terneros. En el caso de la IGF-1, hormona relacionada directamente con el crecimiento muscular y el desarrollo corporal, la concentración plasmática de los terneros Pirenaicos procedentes de madres subnutridas fue inferior a la de sus homólogos del grupo control durante los dos primeros meses de lactación. Además, hasta donde llega nuestro conocimiento, es la primera vez que se describe una relación transgeneracional entre la concentración plasmática de IGF-1 de la madre durante el primer tercio de la gestación, condicionada por la dieta, y la de su ternero durante la lactación 6 meses más tarde. Como se ha indicado anteriormente, la subnutrición materna durante la gestación temprana induciría cambios en la programación fetal del individuo para adaptarse a ese ambiente uterino restringido, aumentando la probabilidad de desarrollar patologías o alteraciones metabólicas durante su vida adulta (Fleming et al., 2012; Velazquez, 2015).

En nuestro estudio, el retraso de la maduración del sistema hematopoyético en los terneros subnutridos, la alteración de sus perfiles endocrinos y su reducida capacidad de ingestión durante los primeros días de vida (cuando todavía era muy pequeña la influencia que hubiera podido tener en el ternero la producción y composición de leche de su madre u otros factores ambientales) evidenciaron los efectos "congénitos" que la subnutrición materna había provocado durante el desarrollo fetal. La repercusión de estos efectos fue incrementándose durante la lactación, siendo más severos en la raza Pirenaica, de tal forma que al destete los terneros de esta raza procedentes de madres subnutridas manifestaron un importante retraso en su crecimiento y desarrollo.

5.4. En las novillas y su descendencia durante las fases de RECRÍA, GESTACIÓN y LACTACIÓN

La alimentación materna de las vacas durante la gestación temprana tuvo repercusiones a largo plazo en las terneras (a partir de ahora, novillas) durante su recría. Al principio de la recría (4 meses de edad), las novillas procedentes de madres subnutridas tenían un **peso** un 15% inferior a las novillas cuyas madres recibieron una alimentación control. Durante la recría, estas diferencias desaparecieron, con pesos y **medidas morfométricas** similares entre grupos a la llegada a la pubertad (12 meses de edad) y en el momento de la IA (16 meses de edad). La ganancia media diaria de las novillas que procedían de madres subnutridas fue mayor que la de sus homólogas, aunque no de forma significativa. Estos resultados nos indican que el retraso en el desarrollo corporal que sufrieron las novillas pudo ser compensado mediante una adecuada alimentación durante la recría, en línea con los resultados descritos por Freetly et al. (2000).

Consecuentemente, las novillas procedentes de madres subnutridas incrementaron sus necesidades energéticas y metabólicas, como reflejaron sus **perfiles metabólicos** con una mayor concentración plasmática de AGNE, urea y colesterol. Serán necesarios más estudios para determinar si esta alteración metabólica fue puntual y consecuencia únicamente de este crecimiento acelerado, o se debe a una alteración permanente del metabolismo lipídico de estos animales.

La subnutrición fetal no afectó a la **edad a la pubertad**, pero se vio una disminución del **número de folículos antrales** a los 16 meses de edad. Nuestros resultados, en línea con los obtenidos por Mossa et al. (2013), demostraron el efecto que puede ejercer un ambiente uterino adverso sobre la diferenciación de los folículos primordiales durante el desarrollo fetal del ovario, reduciendo el tamaño de la reserva ovárica.

A pesar de esta disminución en el número de folículos antrales, se obtuvo una **tasa de fertilidad** media a la IATF del 80%, sin diferencias entre grupos. Estos buenos resultados se atribuyen al buen estado de desarrollo corporal de las novillas en el momento de la IATF. En nuestro estudio, las novillas tenían un peso superior al 70% de su peso adulto estimado, superando el umbral del 65% del peso adulto recomendado por Gasser (2013). No obstante, esta reducción del número de folículos antrales podría comprometer los rendimientos reproductivos de estas novillas en el futuro. Diferentes autores relacionan un mayor recuento de folículos antrales con una mayor eficiencia y duración de la vida reproductiva de un animal (Rhind et al., 2001; Mossa et al., 2012; Taylor, E. G. et al., 2017), por lo que será necesario seguir analizando los parámetros reproductivos de estas novillas para determinar si la subnutrición materna puede afectar a largo plazo su eficiencia reproductiva.

La subnutrición materna no afectó al peso ni a la **CC** de las novillas en su primer parto, ni al peso de sus terneros al nacimiento, ni a los crecimientos de las novillas y su descendencia durante la siguiente lactación. No obstante, teniendo en cuenta que estos animales no alcanzan su peso y talla adulta hasta los 5 años de edad (Cano et al., 2016), serán necesarios más estudios para determinar si las diferencias de peso encontradas en la lactación se revirtieron definitivamente a la entrada a la pubertad, o pudieron haberse silenciado durante esta fase y reaparecer durante el posterior desarrollo de la novilla. En nuestro ensayo, cabe destacar que, a pesar de las diferencias registradas en los ritmos de crecimiento de las novillas en sus diferentes etapas, la edad al primer parto fue de 25 meses en todas ellas, independientemente de su alimentación materna y raza. En España, la mayoría de las vacas nodrizas tienen su primer parto entre los 2 y los 4 años de edad (MAPA, 2018). El resultado de nuestro estudio pone de manifiesto la importancia de realizar un adecuado y constante manejo alimentario de una ternera, especialmente en la fase de recría, para mejorar la eficiencia reproductiva de una explotación. Por otro lado, nuestros resultados indican que la subnutrición peri-implantacional no tuvo consecuencias en los crecimientos de la descendencia de las novillas durante la lactación (tercera generación), sin embargo, será de gran interés analizar los futuros rendimientos productivos de estos animales. Según nuestras referencias, muy pocos estudios han evaluado los efectos de la subnutrición materna a partir de la segunda generación en el ganado bovino. La dificultad logística, temporal y económica que entraña un estudio de tales características pone de manifiesto el valor de los resultados obtenidos en esta tesis.

De esta Tesis Doctoral se desprende la necesidad de asegurar una adecuada alimentación en la vaca nodriza durante el primer tercio de gestación. La subnutrición

tendrá unas consecuencias directas e inmediatas en la madre y en feto que se está desarrollando. En la madre, esas consecuencias incidirán principalmente en su estado metabólico, repercutiendo en la siguiente lactación y, por tanto, indirectamente en la alimentación del ternero. En el feto gestante, mucho más sensible a los cambios ambientales, las consecuencias implicarán cambios en su fisiología, condicionando su desarrollo postnatal. Además, la base genética del animal modulará la magnitud de estos efectos, siendo la raza Pirenaica más sensible a una restricción alimentaria que la Parda de Montaña. Para lograr una mejora de los actuales índices productivos y reproductivos de la vaca nodriza será necesario que los ganaderos garanticen una adecuada alimentación de las vacas preñadas que a su vez están criando un ternero, introduciendo si es necesario cambios sustanciales en el manejo de sus explotaciones.

6. Conclusiones / Conclusions

6. CONCLUSIONES

1) La subnutrición durante el primer tercio de gestación redujo el peso y la condición corporal de las vacas nodrizas, comprometiendo su estado metabólico y el crecimiento de los terneros que estaban criando. Sin embargo, no afectó al reconocimiento y mantenimiento de la gestación (la expresión de genes estimulados por el interferón tau los días 18 y 21 post-IATF, y las concentraciones de progesterona y PSPB), ni a la tasa de fertilidad.

2) Los cambios en la expresión de los genes OAS1 y MX1 entre el día 18 y 21 post-IATF, la concentración plasmática de progesterona el día 21 y la concentración plasmática de PSPB el día 26 fueron los indicadores más tempranos para diagnosticar el estado de preñez de las vacas.

3) La subnutrición afectó a diferentes parámetros leucocitarios y plaquetarios de las vacas a día 20 post-IATF, especialmente en la raza Pirenaica. Con una alimentación adecuada a sus necesidades a partir del segundo tercio de gestación, las vacas subnutridas habían restablecido sus parámetros hematológicos al final de la gestación.

4) Las vacas subnutridas y las vacas control tuvieron un peso al parto similar gracias a la adecuada alimentación que recibieron durante el resto de la gestación. Sin embargo, las vacas subnutridas presentaron una inferior condición corporal al parto, que repercutió en la mayoría de parámetros productivos del conjunto vaca-ternero durante la lactación.

5) La subnutrición materna durante la gestación temprana no afectó al peso y vitalidad de las crías al nacimiento, ni a la transferencia de inmunidad de la madre al ternero, a pesar de que el calostro de las vacas Pirenaicas subnutridas tuvo una menor concentración de Ig G.

6) La subnutrición materna modificó la programación fetal, repercutiendo en la regulación de los parámetros metabólicos, endocrinos y hematológicos de la descendencia. Los terneros recién nacidos procedentes de madres subnutridas tuvieron una menor concentración plasmática de IGF-1, una mayor concentración de cortisol y un retraso en la maduración de su sistema hematopoyético.

7) Los terneros procedentes de madres subnutridas tuvieron un menor crecimiento durante la lactación, debido a la alteración de sus mecanismos fisiológicos junto con una reducida capacidad de ingestión. Este efecto estuvo más marcado en los terneros Pirenaicos, que tuvieron un menor peso al destete (19% inferior) y una menor concentración plasmática de IGF-1 que sus homólogos del grupo control.

8) En las novillas, las diferencias de peso observadas durante la lactación desaparecieron a partir de los 12 meses de edad (inicio de la pubertad). Las novillas procedentes de madres subnutridas aceleraron su crecimiento en los meses previos a la entrada a la pubertad, comprometiendo su estado metabólico.

9) La subnutrición materna afectó al desarrollo embrionario de las gónadas de las novillas, que sufrieron una disminución en el número de folículos antrales a los 16 meses de edad (IATF). Sin embargo, no se vieron diferencias en la tasa de fertilidad (80%), ni repercusiones en el peso al nacimiento y al destete de la descendencia de las novillas.

10) Durante este ensayo, la raza Pirenaica se mostró más sensible a la restricción alimentaria, poniendo de manifiesto la modulación de los efectos de la subnutrición en función de la base genética de los animales. En una situación de balance energético negativo, la raza Pirenaica priorizó sus necesidades de mantenimiento en detrimento del crecimiento del ternero. Estas características raciales deberían considerarse para optimizar la producción de una explotación ganadera en función de su manejo alimentario.

11) Esta Tesis ha puesto de manifiesto que la restricción alimentaria durante el primer tercio de gestación en las vacas nodrizas disminuirá los rendimientos de los terneros criados al inicio de la gestación, los rendimientos de la descendencia durante la siguiente lactación y puede comprometer el futuro reproductivo de las novillas de reposición. Por tanto, será necesario garantizar una correcta alimentación durante el primer tercio de gestación en las vacas nodrizas para maximizar la eficiencia productiva de una explotación.

CONCLUSIONS

1) Undernutrition during the first third of pregnancy decreased the live weight and the body condition score of suckler cows, compromising their metabolic status and the growth of the reared calves. However, it had no effects on the maternal recognition and maintenance of pregnancy (interferon-tau stimulated gene expression on days 18 and 21 post-FTAI, and progesterone and PSPB concentrations), neither on the fertility rate.

2) OAS1 and MX1 fold changes from day 18 to 21 post-FTAI, plasma progesterone concentration on day 21, and plasma PSPB concentration on day 26 were the earliest indicators to discriminate the pregnancy status in cows.

3) Undernutrition affected some leucocyte and platelet parameters of the cows on day 20 post-FTAI, especially in the Pirenaica breed. Due to an adequate nutrition according to their requirements from the second third of gestation, undernourished cows had their hematological parameters restored at the end of the gestation.

4) Undernourished and control cows had similar live weights at calving due to an adequate nutrition during the rest of gestation. However, undernourished cows had a lower body condition score at calving, which affected most of the cow-calf pair productive parameters during lactation.

5) Early maternal undernutrition had no effects on the calf live weight and vitality at birth, neither on the passive transfer of immunity from dam to calf, although the colostrum from undernourished Pirenaica cows had lower Ig G concentration.

6) Maternal undernutrition modified the fetal programming, affecting the regulation of the metabolic, endocrine and hematological parameters of the progeny. Newborns from undernourished cows had lower plasma IGF-1 concentration, higher plasma cortisol concentration and a later maturation of their hematopoietic system.

7) Calves from undernourished cows had lower growth rates during lactation due to an alteration of their physiological mechanisms together with a reduced intake capacity. This effect was more marked in Pirenaica calves, which had lower weaning weights (19% lower) and lower plasma IGF-1 concentrations than their control counterparts.

8) In heifers, the live weight differences observed during lactation disappeared from 12 months of age (onset of puberty). Heifers from undernourished cows accelerated their growth rate during the months before puberty, which impaired their metabolic status.

9) Maternal undernutrition affected the embryo development of heifers' gonads, which suffered a reduction in the antral follicle count at 16 months of age (FTAI). However, no differences were found in fertility rate (80%), neither in the birth and weaning weights of heifers' progeny.

10) During this study, Pirenaica breed was more sensitive to nutrient restriction, which implies that the effects of undernutrition are modulated by the genetic characteristics. In a negative energy balance situation, Pirenaica breed prioritized the cow maintenance over the calf growth. These breed differences should be taken into account in order to optimize the feeding management of suckler cattle herds.

11) This PhD Thesis highlights that a nutritional restriction during the first third of gestation in suckler cows will decrease the performance of the reared calves at the beginning of the gestation, the performance of the progeny during the following lactation and it can compromise the reproductive performance of the replacement heifers. Therefore, an adequate nutrition during the first third of gestation of suckler cows will be necessary to maximize their production efficiency.

7. Apéndice

7. APÉNDICE

Las revistas donde se han publicado los trabajos que forman parte de esta Tesis Doctoral están incluidas en el área temática *Agriculture, Dairy and Animal Science* (Publicaciones 1, 2, 4 y 5) y *Veterinary Sciences* (Publicación 3) del *Journal Citation Reports* (ISI Web of Science):

1) Noya A., Casasús I., Rodríguez-Sánchez J. A., Ferrer J., Sanz A. (En prensa). "A negative energy balance during the peri-implantational period reduces dam IGF-1 but does not alter progesterone or pregnancy-specific protein B (PSPB) or fertility in suckled cows". Domestic Animal Endocrinology. https://doi.org/10.1016/j.domaniend.2019.106418. Factor de impacto año 2018: 2,302. Cuartil: Q1.

2) Noya A., Serrano-Pérez B., Villalba D., Casasús I., Molina E., López-Helguera I., Sanz A. (2019). "Effects of maternal subnutrition during early pregnancy on cow hematological profiles and offspring physiology and vitality in two beef breeds". Animal Science Journal, 90: 857-869. https://doi.org/10.1111/asj.13215. Factor de impacto año 2018: 1,301. Cuartil: Q2.

3) Serrano-Pérez B., Molina E., Noya A., López-Helguera I., Casasús I., Sanz A., Villalba D. (2020). "Maternal nutrient restriction in early pregnancy increases the risk of late embryo loss despite no effects on peri-implantation interferon-stimulated genes in suckler beef cattle". Research in Veterinary Science, 128: 69-75. https://doi.org/10.1016/j.rvsc.2019.10.023. Factor de impacto año 2018: 1,751. Cuartil: Q1.

4) Noya A., Casasús I., Ferrer J., Sanz A. (2019). "Long-term effects of maternal subnutrition in early pregnancy on cow-calf performance, immunological and physiological profiles during the next lactation". Animals, 9: 936. https://doi.org/10.3390/ani9110936. Factor de impacto año 2018: 1,832. Cuartil: Q1.

5) Noya A., Casasús I., Ferrer J., Sanz A. (2019). "Effects of developmental programming caused by maternal nutrient intake on postnatal performance of beef heifers and their calves". Animals, 9: 1072. https://doi.org/10.3390/ani9121072. Factor de impacto año 2018: 1,832. Cuartil: Q1.

8. Bibliografía

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