Universitat de Lleida

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TESI DOCTORAL

Estrategias de mejora de sostenibilidad en granjas de vacas de leche altamente productoras

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Directora

Dra. Irina Garcia Ispierto

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A la meua mare

i a tota la gent que em rodeja,

per estimar-me i cuidar-me.

If you can’t fly, then run

if you can’t run, then walk

if you can’t walk, then crawl,

but whatever you do

you have to keep moving forward.

Martin Luther King Jr.

AGRADECIMIENTOS / AGRAÏMENTS

Quan s'acostava el final dels estudis del Doble Grau en Veterinària i Ciència i Producció Animal a la Universitat de Lleida pensava que no em veia dins del món laboral, que no em veia a una clínica o a qualsevol empresa, sigui d'animals de companyia o de producció, i que tampoc em veia com a veterinari. De fet, a dia d'avui, la síndrome de l'impostor encara persisteix, amb menys intensitat, però persisteix. Llavors, vaig pensar en la possibilitat de cursar un màster. El màster que em va cridar molt l'atenció va ser el Màster Universitari en Zoonosi i Una Sola Salut (One Health) que s'imparteix a la Universitat Autònoma de Barcelona però vaig descartar ràpidament aquesta opció per molts motius, el motiu de més pes l'econòmic. Davant el mar de dubtes que em trobava em vaig preguntar quines matèries de la carrera em van agradar més i la que sobreeixia va ser la de Reproducció Animal, impartit íntegrament per la professora Irina Garcia. Llavors és quan li vaig dir a la meua mare que volia fer un Doctorat, i que amb l'única persona que el volia fer era amb la Irina. Des que la Irina em va dir que sí han passat moltes coses i he ampliat encara més el meu cercle. Un cercle que no sé si és d'amistat o ni tan sols professional, però el que està clar és que s'ha ampliat, i que sense ell aquesta tesi no s'hagués pogut realitzar.

A la meua mare i a tota la família, que sense entendre ben bé, fins i tot a dia d'avui, el què he estat fent durant tota aquesta època m'han donat un suport i una estima que m'ha donat l'energia necessària per realitzar aquesta tesi. Confien més amb mi que jo mateix. A les meues amistats. Les de Salàs de Pallars que, segurament sense ser-ne conscients, han esdevingut una força cabdal. A tots ells i elles, amb unificació, gràcies. Les de Tremp i, molt especialment, a l'Anna. Per entendre'm, respectar-me i estimar-me com mai ningú no ha fet. Per regalar-me només bons moments, per riure'ns de la vida quan hi ha maldades, per estar-hi sempre, inclús quan no m'ho mereixo, gràcies i mil gràcies. A les de Lleida, als meus Xaiets que, tot i que els camins de la vida ens porten a paratges diferents, sempre estan presents en el meu dia a dia. En especial a la Jordina, que entre salsa i salsa, m'ha inspirat en aquesta tesi. Als nous descobriments, a les amistats d'un cap de setmana o d'un dia. Totes les experiències viscudes en el transcurs d'aquest projecte han contribuït a la realització d'aquesta tesi.

A la Mònica Llobera, una professional en la que m'he emmirallat, a la que sempre que li he demanat ajuda mai m'he l'ha negada i que sense cap mena de dubte és un dels descobriments més bonics durant la tesi. Com també ho és la Mònica Pando, una professional envejable amb la qual gaudeixes compartint temps amb ella tant dins com fora de la granja. I, parlant de granges, en aquest agraïment no pot faltar la meva gratitud a les granges que he estat treballant, en especial, a la Granja Allué i a la Granja Fontanals. Obrir les portes d'una granja no és fàcil, i més en els temps que corren, però no només m'han obert les seues portes sinó que m'han fet sentir com a casa. Al Ronald i Fernando per compartir els seus coneixements amb mi. A tots ells i elles, gràcies.

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Gràcies per, literalment, obrir-me les portes de casa teua, per deixar-me conèixer als teus pares, sogres, a l’Axel, la Lia i el Pol Jr. Per descobrir-me al Pol, el teu marit. Una família que desprèn amor, generositat i molt bon rotllo. Són molts moments viscuts, molts aprenentatges personals i professionals i em quedo amb tots ells, sense excepció. No hi ha res més gratificant i bonic en el món professional que treballar colze a colze amb algú que admires professionalment i que, de seguida, també admires personalment. Així doncs, crec que amb això sí que ens posarem d’acord quan dic que de tot el que hem viscut, de tots els aprenentatges que he après de tu, del creixement professional que m’has guiat donant-me la llibertat que necessitava, el que em quedo és amb el respecte mutu, el tractar-nos de tu a tu, la nostra amistat. I, per suposat, les nostres bogeries, que seguiran un cop marqui el punt final d’aquest apartat.

SUMMARY

The critical situation of the dairy cattle sector in this country causes the need to improve its sustainability. Thus, the main objective of this thesis was to contribute to improving the sustainability of intensive farms of highly productive dairy cows through reproductive management. The research included in this thesis is divided into four studies. In the first study, the possibility of double ovulation was analysed in cows with an ovulatory size follicle (10 mm or more) and a follicle smaller than 10 mm at estrus. The results were that the smaller follicle has the capacity to ovulate alone or with the dominant follicle and can give rise to a single or twin pregnancy. This indicates that a follicle of deviation size may ovulate in the presence of a follicle of ovulatory size. The second study focused on evaluating the possible incidence of freemartins on the gestation and birth of calves after a twin reduction at day 28-44 of gestation. The results indicated that there was no effect of the male twin on the normal development of the gonads and genital tract of the female co-twin during the late embryonic period after twin reduction. The objective of the third study was to determine whether the transfer of a single frozen-thawed in vitro-produced embryo could prevent twin pregnancy without reducing the fertility of a cow. The result was that the IVP embryo transfer prevented twin pregnancy with no affects on the fertility of the cow. Finally, in the fourth study, the that affect uterine size and position at 30-36 days postpartum were identified. The most relevant finding was that the sex of the calf is a determining factor for the size and uterine position of the cow.

RESUMEN

La situación crítica a la que se encuentra el sector del vacuno de leche en este país provoca la necesidad de mejorar su sostenibilidad. Así pues, el objetivo principal de esta tesis fue contribuir en la mejora de la sostenibilidad de las granjas intensivas de vacas lecheras altamente productoras mediante el manejo reproductivo. La investigación incluida en esta tesis se divide en cuatro estudios. En el primer estudio se analizó la posibilidad de la doble ovulación en vacas con un folículo de tamaño ovulatorio (10 mm o más) y un folículo menor de 10mm al estro. Los resultados fueron que el folículo de menor tamaño tiene la capacidad de ovulación solo o con el folículo dominante, pudiendo dar a lugar a una gestación simple o gemelar. Esto indica que un folículo de tamaño de desviación puede ovular en presencia de un folículo de tamaño ovulatorio. El segundo estudio se centró en avaluar la posible incidencia de los freemartins en la gestación y nacimiento de terneras después de una reducción de gemelos a día 28-44 de gestación. Los resultados indicaron que no había efecto del macho gemelo en el normal desarrollo de las gónadas y el tracto genital de la hembra gemelo durante el periodo embrionario tardío después de una reducción gemelar. El objetivo del tercer estudio fue determinar si la transferencia de un embrión congelado producido *in vitro* puede prevenir la gestación gemelar sin reducir la fertilidad. El resultado fue que la transferencia de dicho embrión previene la gestación gemelar sin afectar negativamente a la fertilidad de la vaca. Finalmente, en el cuarto estudio se identificó los factores que afectan al tamaño y posición uterina a 30-36 días postparto. El hallazgo más significativo fue que el sexo del ternero es determinante para el tamaño y posición uterina de la vaca.

RESUM

La situació crítica a la que es troba el sector del vacú de llet en aquest país provoca la necessitat de millorar-ne la sostenibilitat. Així doncs, l'objectiu principal d'aquesta tesi va ser contribuir en la millora de la sostenibilitat de les granges intensives de vaques lleteres altament productores a través del maneig reproductiu. La investigació inclosa en aquesta tesi es divideix en quatre estudis. En el primer estudi es va analitzar la possibilitat de la doble ovulació en vaques amb un fol·licle de mida ovulatòria (10 mm o més) i un fol·licle menor de 10 mm a l'estre. Els resultats van ser que el fol·licle de menor mida té la capacitat d'ovulació sol o amb el fol·licle dominant, podent donar lloc a una gestació simple o gemel·lar. Això indica que un fol·licle de mida de desviació pot ovular en presència d'un fol·licle de mida ovulatòria. El segon estudi es va centrar en avaluar la possible incidència dels freemartins en la gestació i naixement de vedelles després d'una reducció gemel·lar a dia 28-44 de gestació. Els resultats van indicar que no hi havia efecte del mascle bessó en el normal desenvolupament de les gònades i el tracte genital de la femella bessona durant el període embrionari tardà després d'una reducció gemel·lar. L'objectiu del tercer estudi va ser determinar si la transferència d'un embrió congelat produït *in vitro* pot prevenir la gestació gemel·lar sense reduir la fertilitat. El resultat va ser que la transferència de l'embrió congelat evita la gestació gemel·lar sense afectar negativament a la fertilitat de la vaca. Finalment, en el quart estudi es va identificar els factors que afecten a la mida i posició uterina a 30-36 dies postpart. La troballa més significativa va ser que el sexe del vedell és determinant per a la mida i posició uterina de la vaca.

ÍNDICE

INTRODUCCIÓN GENERAL

1. Contexto actual

En las últimas décadas la mejora genética del vacuno lechero ha causado un aumento de la producción lechera por vaca y una disminución de la cabaña a nivel mundial (Stevenson & Britt, 2017). A su vez, la eficacia reproductiva se ha visto alterada negativamente en forma de infertilidad y trastornos reproductivos (López-Gatius, 2003). Esta situación, sumada al aumento de los costes de producción, la presión social respecto al bienestar animal, la falta de relevo generacional y el bajo precio de la leche (Figura 1), ha provocado que las granjas de vacuno lechero en España se encuentren en una situación crítica. Por ello, su sostenibilidad está en riesgo.

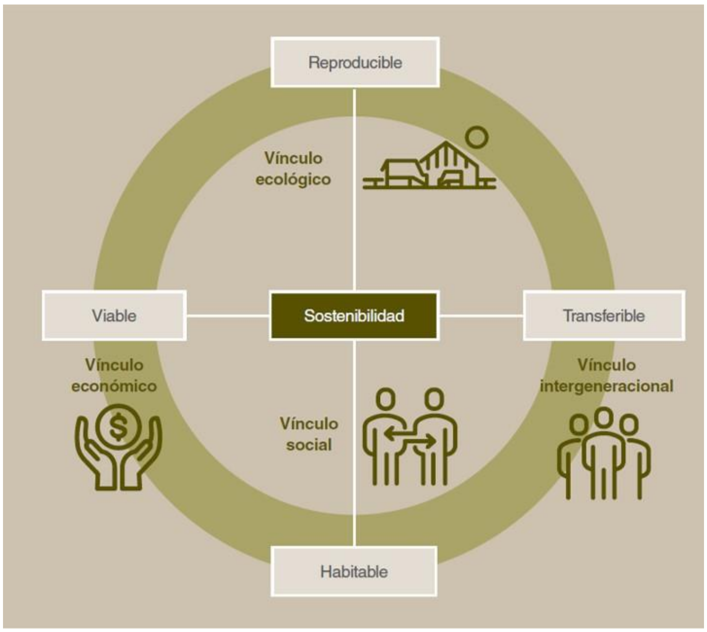

Figura 1. Pilares del concepto de sostenibilidad en granja. Adaptación de Landais, 2002

Según datos del Ministerio de Agricultura, Pesca y Alimentación del Gobierno de España, en los últimos años (periodo 2015-2020) ha disminuido considerablemente el número de ganaderos que se dedican a la producción de leche, el número de explotaciones totales y el censo de vacas lecheras en todo el territorio. Así pues, es necesario mejorar la eficacia reproductiva en las granjas lecheras para que su actividad perdure de manera sostenible.

2. Fisiología de la doble ovulación y gestación

La vaca es un animal monovular, pero en los últimos 30 años las dobles ovulaciones y los partos gemelares se han incrementado significativamente (De Rensis et al., 2021). En numerosos estudios se ha observado que el ovario derecho tiene mayor actividad funcional que el izquierdo (Sellars et al., 2006; Silva del Río et al., 2009), pero aún se desconoce su explicación. De hecho, alrededor del 60% de ovulaciones ocurren en el ovario derecho (Saiduddin et al., 1967; López-Gatius et al., 2005) y un 59-68% de las gestaciones se establecen en el cuerno uterino derecho (Erdheim, 1942). El crecimiento folicular es un proceso dinámico en el que el incremento de concentraciones de LH y FSH durante el ciclo mantiene el crecimiento de los folículos, y el incremento de valores de LH durante la fase folicular induce la estimulación de los cambios estrogénicos preovulatorios que provocan la ovulación (Savio et al., 1988). Las oleadas foliculares transcurren a lo largo del ciclo y, en vacas lecheras, se producen de dos a tres oleadas por ciclo (Knopf et al., 1989). Durante cada oleada, un folículo es seleccionado y domina sobre los otros: después de la fase de dominancia, este folículo o bien ovulará o bien se atresiará (Sirois & Fortune, 1988).

La fase de la desviación folicular es el comienzo de la diferencia mayor en la tasa de crecimiento entre los dos folículos más grandes, cuando el segundo más grande consigue su máximo diámetro (Ginther et al., 1996). Cuando hay una absencia de esta desviación tiene lugar la codominancia de dos o más folículos que provoca ovulaciones múltiples (Kulick et al., 2001). Actualmente, la incidencia de este tipo de ovulaciones en granjas de vacas lecheras de alta producción es de más del 20% del total de ovulaciones

(Fricke & Wiltbank, 1999; Macmillan et al., 2018). Asimismo, en un experimento de Bleach y colaboradores (2004) se observó que las dobles ovulaciones tenían mayor frecuencia en vacas con tres oleadas foliculares que en aquellas con dos o cuatro o más. Además, durante el periodo temprano posparto ocurren más frecuentemente en vacas multíparas que en primíparas, y las multíparas que experimentan dobles ovulaciones producen más leche (Kusaka et al., 2017) y tienen la duración del estro menor (Lopez et al., 2005). Por lo tanto, para que se produzca una doble ovulación intervienen muchos factores relacionados con la fisiología reproductiva y las consecuencias también pueden ser variadas.

El mecanismo de la multiovulación se presenta como un aumento en el número de folículos dependientes de gonadotropinas: cuando más folículos más probabilidad de multiovulación (Vinet et al., 2012). De hecho, hay una tendencia significativa a que sigan ovulaciones múltiples en secuencia (Kidder et al., 1952). Las dobles ovulaciones augmentan significativamente la probabilidad de gestación gemelar (López-Gatius et al., 2005).

En granjas lecheras de alta producción las gestaciones dobles pueden sobrepasar el 18% (Andreu-Vázquez et al., 2012), mientras que los partos dobles pueden superar el 12% dependiendo del rebaño (Silva del Río et al., 2007). A diferencia del vacuno de carne, en la producción lechera los gemelos no son deseables ya que causan pérdidas económicas muy importantes (Lee & Kim, 2007). Dichas consecuencias económicas provienen de varios factores: pérdidas embrionarias (López-Gatius et al., 2004), abortos, retenciones de placenta, distocias, nacidos muertos y freemartins (Echternkamp & Gregory, 1999; Echternkamp & Gregory, 1999b), además de aumentar la tasa de reposición (Gregory et al., 1990; Bicalho et al 2007). Asimismo, la mayoría de las complicaciones clínicas requieren del uso de medicamentos y, especialmente, de antibióticos, en un contexto actual cuyo objetivo es la reducción del uso de antibióticos debido al desafío de la resistencia a antimicrobianos tanto en animales como en humanos (Longfukang et al., 2023). Por lo tanto, es una necesidad la reducción de partos gemelares en vacas lecheras de alta producción por el bienestar animal, la sostenibilidad de las explotaciones y la salud global de nuestro planeta.

3. Estrategias de manejo reproductivo

Actualmente existen dos sendas para la reducción de partos dobles: por un lado, la reducción de ovulaciones múltiples y, por otro lado, la reducción de las gestaciones gemelares.

3.1. Reducción de ovulaciones múltiples

En el primer caso se debe tener en cuenta que, debido a la selección genética, las condiciones en granja (nutrición, manejo) y los protocolos de sincronización que se utilizan resulta difícil su eficacia en la práctica rutinaria. Una opción es acortar el tiempo de tratamiento hormonal e incrementar la progesterona antes del empleo del protocolo a tiempo fijo de la inseminación artificial (FTAI) (Garcia-Ispierto & López-Gatius, 2021). Otra opción es la utilización de la técnica de la punción folicular de un folículo codominante: puede ser ecoguiada (López-Gatius & Hunter, 2018) o no ecoguiada (Garcia-Isperto & López-Gatius, 2020) (Figura 2).

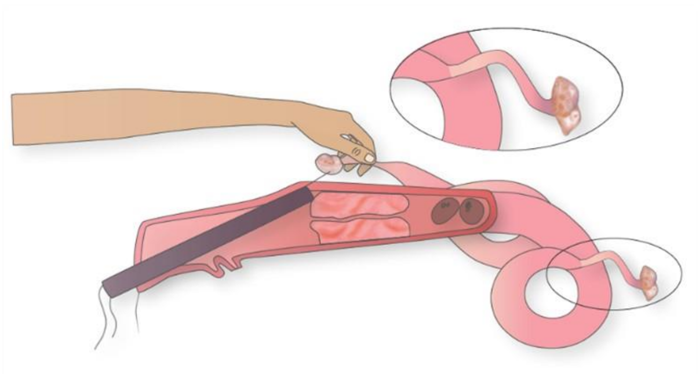

Figura 2. Esquema representativo de la técnica de la punción folicular no ecoguiada. Fuente: propia.

3.2. Reducción de gestaciones gemelares

En el segundo caso, hay dos formas para llevarlo a cabo: reducción embrionaria o transferencia de embriones. Para la reducción embrionaria (eliminación de un embrión de los dos gestantes) se han descrito hasta 3 técnicas que se llevan a cabo a día 28-41 de gestación: rotura manual de la vesícula amniótica (Andreu-Vázquez et al., 2011), aspiración del fluido atlanto-amniótico ecoguiado (López-Gatius, 2005) y administración de PG2α intraluteal en uno de los dos cuerpos lúteos (López-Gatius & Hunter, 2016). Por otro lado, la transferencia de embriones es otra alternativa a la disminución de la incidencia de partos dobles en granjas de vacas de leche de alta producción. Existen tres tipos de embriones para ser transferidos, cuyas técnicas de aplicación en granja son diferentes: *in vivo*, vitrificados y congelados (Thibier, 2005) (Figura 3).

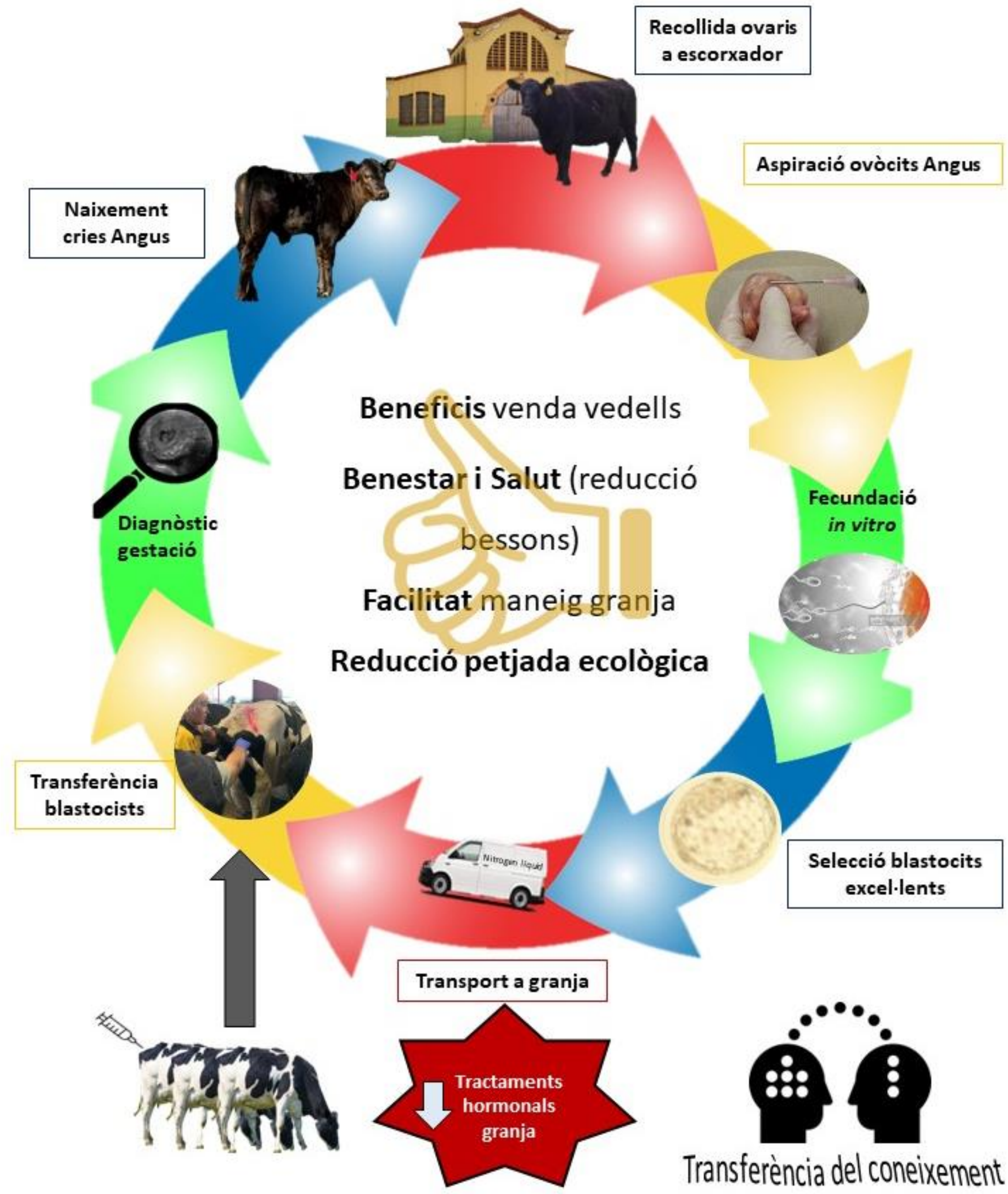

Figura 3. Representación de los pasos para realizar una transferencia de embriones congelados de la raza Angus producidos in vitro en vacas lecheras. Fuente: propia

3.3. Manejo posparto

En la situación actual, en el que la sostenibilidad de las explotaciones de vacas de leche está en un momento crítico, cobra más relevancia que nunca el tener un buen manejo en granja basado en el control de los índices productivos y reproductivos. Recientemente, Young et al., (2017) describió unos criterios de clasificación de las vacas posparto según la posición y el tamaño del útero para la mejora de la fertilidad. Como es bien sabido, el periodo posparto es un punto muy crítico debido a la involución uterina y retorno a la ciclicidad (Gier & Marion, 1968) y a las complicaciones clínicas que puedan manifestarse (Garcia-Ispierto et al., 2007). Así pues, un buen manejo posparto, conociendo los factores que pueden afectar negativamente a su desarrollo y reduciéndolos al máximo, mejora la eficacia reproductiva de la granja.

La presente tesis doctoral se basa en la mejora de la sostenibilidad de las granjas de vacas de leche de alta producción mediante el estudio y evaluación de factores que la afectan y propone el uso de técnicas que sean practicables de manera rutinaria en el control reproductivo del vacuno de leche.

OBJETIVOS GENERALES

El objetivo principal de la presente tesis doctoral es contribuir en la mejora de la sostenibilidad de las granjas intensivas de vacas lecheras altamente productoras. Para conseguir dicho objetivo se fijaron los siguientes subobjetivos:

1. Estudio de las fisiopatología reproductiva y análisis de sus efectos sobre el rendimiento reproductivo.
2. Evaluación de los factores y efectos de la doble ovulación y de la posible gestación gemelar que se pudiera derivar.
3. Estudio y determinación de la terapéutica de dobles gestaciones en la visita reproductiva semanal en granjas comerciales de alta producción.

METODOLOGÍA GENERAL

Animales

La población de estudio fue extraída de los siguientes rebaños:

- Artículo 1: explotación comercial de vacas de leche Holstein-Frisona altamente productoras localizada al nordeste de España (latitud 41.13 N, longitud – 2.4 E). Media de vacas lactantes: 14.500; media anual de producción de leche: 12.450 kg por vaca; tasa de eliminación: 31%.
- Artículo 2: explotación comercial de vacas de leche Holstein-Frisona altamente productoras localizada al nordeste de España (latitud 41.13 N, longitud – 2.4 E). Media de vacas lactantes: 1282; media anual de producción de leche: 12.181 kg por vaca; tasa de eliminación: 33%.
- Artículo 3: 3 explotaciones comerciales de vacas de leche Holstein-Frisona altamente productoras localizadas a 10 km de la ciudad de Lleida. Media de vacas lactantes: 6688; media anual de producción de leche: 11,950 kg por vaca; media tasa de eliminación: 33%
- Artículo 4: explotación comercial de vacas de leche Holstein-Frisona altamente productoras localizada al nordeste de España (latitud 41.13 N, longitud – 2.4 E). Media de vacas lactantes: 1225; media de producción anual: 14.965; tasa de eliminación: 33%.

Todos los animales de estudio eran alimentados con raciones completas y agrupados según el número de lactación (primíparas versus multíparas) en instalaciones con cubículos y patios. Para mitigar las temperaturas extremas de calor propias de la zona, todas las explotaciones disponían de ventiladores y/o aspersores de agua, además de enriquecimiento ambiental para la reducción del estrés. El ordeño de las vacas se realizaba 3 veces al día.

Manejo reproductivo

En todas las explotaciones se realizaba una visita reproductiva semanal en la que se recogían datos para los estudios. Los rebaños estaban sujetos al programa reproductivo FTAI y estro espontáneo, dependiendo de la casuística reproductiva y, además, en el estudio 3 se realizó transferencia de embriones congelados. La FTAI consistía en administrar un dispositivo interno de liberación de hormona en vagina de CIDR que contenía 1,38 gramos de progesterona (Zoetis España SL, España). Dicho dispositivo se retiraba a los 5 días y, en el momento de su retirada, se administraba una dosis de cloprostenol (500 µg i.m.; PGF Veyx Fore, Ecuphar, España). Seguidamente, a las 24 y 60 horas después de la retirada del CIDR, se administraba una segunda dosis de cloprostenol y una dosis de GnRH, en este caso, se trataba de Depherelin (análogo de la GnRH: 100µg de acetato de gonadorelina [6-D-Phe] i.m.; Gonavet Veyx, Ecuphar, España). En el estudio 3 se sustituyó la dosis de Depherelinе por una dosis de gonadotropina coriónica humana (hCG) (3000 IU hCG i.m.; Veterin corion, Divasa-Farmavic, España). Los embriones utilizados en el mismo estudio fueron proporcionados por la empresa Embriovet SL (España), con el número identificador UE ES11ET05B en posesión de certificado sanitario que rige la legislación de la UE. La totalidad de los animales de estudio disponían de podómetros para la detección del celo natural (AfiFarm System; Afikim, Israel), excepto en las vacas de los. Para la definición de los distintos parámetros de estudio se utilizó un ecógrafo modo B de 5-10 MHz (E.I. Medical IVEX LITE; E.I. Medical Imaging, Estados Unidos).

Análisis estadístico

En todos los estudios se utilizó el programa PASW Statstics for Windows Version 18.0 (SPSS Inc., Estados Unidos) y los resultados se expresaban en media ± desviación estándar. Se determinó la significancia estadística a $p < 0.05$ y la tendencia estadística a $p < 0.10$. Se crearon modelos estadísticos según el estudio.

CAPÍTULO I

Follicular Size Threshold for Ovulation Reassessed. Insights from Multiple Ovulating Dairy Cows

Fernando López-Gatius, Mònica Llobera-Balcells, Roger J. Palacín-Chauri, Irina Garcia-Ispierto and Ronald H. F. Hunter

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Simple Summary: The selection of a single ovarian follicle able to differentiate and ovulate is a phenomenon common to monovular species including humans. The selected follicle acquires the capacity to ovulate when it reaches a diameter of about 10 mm. In cows with a single follicle of ovulatory size, the probability of ovulation significantly increases with follicle diameter. However, two or more follicles of ovulatory size are often present at estrus. In cows with one follicle of ovulatory size and another follicle of 7-9 mm, the small follicle may, under certain circumstances, ovulate producing a pregnancy.

Abstract: In *Bos. taurus* cattle, follicular deviation to dominance begins when the selected ovulatory follicle reaches a mean diameter of 8.5 mm. The dominant follicle acquires the capacity to ovulate when it reaches a diameter of about 10 mm. In this study, data derived from 148 cows in estrus with one follicle of ovulatory size and another of 7-9 mm, reveal that the small follicle has the capacity to ovulate alone or with the dominant follicle; thus, giving rise to a single or twin pregnancy. This indicates that a follicle of deviation size may ovulate in the presence of a follicle of ovulatory size.

Keywords: bovine; follicular size; ovulation failure; pregnancy rate; twins.

1. Introduction

Cattle breeds are generally considered monovular. There are, nevertheless, circumstances in which there is an increased incidence of double ovulation and thus of twin pregnancy. In dairy cattle, it is widely accepted that twinning compromises both the health of cows [1-3] and herd economy [4-6]. The economic impacts of twin pregnancies are likely to rise as multiple ovulation rates, particularly in older cows, have increased substantially over the past 30 years along with increased milk productivity [7-9]. Genetic progress and improvements in nutrition and management practices have led to a continuous increase in milk yield [10-12] suggesting that the multiple ovulation rate will continuo to rise in parallel with milk production. These are cogent reasons to examine the factors that cause multiple pregnancies, as the mechanisms underlying multiple ovulations remain to be elucidated [12].

During the estrous cycle of cattle, two or three waves of follicular growth take place, of which only the last wave is ovulatory. Each wave leads to the development of a large dominant follicle and smaller subordinate follicles [13-15]. Follicular deviation to dominance begins when the largest follicle reaches a mean diameter of 8.5 mm [15]. The deviation mechanism consists of the reduction or cessation of growth of the subordinate follicles while the largest follicle increases in size. The process finishes either with ovulation or atresia of the dominant follicle [13-15]. During the ovulatory wave, the dominant follicle acquires the capacity to ovulate when it reaches a diameter of about 10 mm [16]. In cows with a single follicle of ovulatory size, the probability of ovulation significantly increases with follicle diameter [17,18]. However, two or more follicles of ovulatory size are often present at the time of artificial insemination (AI) and multiple corpora lute (CLs) may be recorded in over 50% of older cows [19].

Multiple ovulations involve the presence of at least two ovulatory follicles in either the same ovary or in both ovaries. Cows with two or more ovulatory follicles at estrus may show different ovulation patterns to those with a single ovulatory follicle. In a recent study examining 316 cows with two follicles of ovulatory size at AI, individual follicle diameter could not be related to the likelihood of ovulation [18]. In the latter study, only the smaller follicle ovulated in 40.5% of cows [18]. This event of ovulation of only the smaller follicles could be related to two processes: first, a second deviation may occur in cows with co-dominant follicles [20]; and second, during a follicular wave there could be a switch in diameter rank between the dominant and largest subordinate follicle just before or at deviation [21]. However, another possibility could be associated with the ovulation of follicles smaller than 10 mm in multiple ovulating cows, this being the minimum diameter, linked to a capacity to ovulate in monovular cows [16]. The observation of unexpected additional CLs seven days after AI in cows with a single follicle of ovulatory size [18,22] reinforces this idea. The objective of the present study was to assess the possibility of double ovulation in cows with a single follicle of ovulatory size (10 mm or more) and a follicle smaller than 10 mm at estrus.

2. Materials and Methods

2.1. Cows and Herd Management

The study population was derived from a Holstein dairy herd in northeastern Spain (latitude 41.13 N, longitude – 2.4 E) in which repeat breeder cows were synchronized for fixed-time AI (FTAI) 5-11 days after the last confirmed estrus. Cows were considered repeat breeders when they did not become pregnant after 3 AI in the absence of detectable anatomic abnormalities [23,24]. Pedometers were used to confirm estrus (AfiFarm

System; Afikim, Israel) in both FTAI and spontaneous estus animals. Cows ≥ 60 days in milk showing estrus during the previous 3 weeks and with no CL were recorded as anestrous cows and received the same FTAI protocol at the same time as repeat breeders. The remaining cows were inseminated either at spontaneous estrus or following another FTAI protocol. During the study period (October 2020 to July 2021), the mean number of lactating cows and mean annual milk production was 145 and 12,450 kg per cow, respectively. The mean annual culling rate was 31%. Cows were grouped according to parity (primiparous versus pluriparous) and fed complete rations.

The herd was subjected to a weekly reproductive health program on the day of FTAI. The study population was selected among cows ready for service and inseminated in the weekly visit after ultrasound scanning: FTAI repeat breeders, FTAI anestrous cows, and cows inseminated at spontaneous estus (first, second, or third AI). In this way, most of the cows should have a single ovulatory follicle at AI, both repeat breeders [25] and cows inseminated during early lactation [26]. Cows were included if they ere healthy, confirmed by a body condition score of 2.5-3.5 on a scale of 1-5 [27], produced ≥ 30 kg of milk per day, and were free of clinical signs of diseases from insemination days -7 to +28. In all cows, the presence of a follicle of ovulatory size (10 mm or more) in the absence of luteal structures, a uterus that was highly turgid and contractile to the touch, and copious vaginal fluid, were used as a reference to confirm estrus by ultrasound and palpation per rectum [28]. Cows were inseminated by two technicians using frozen-thawed semen from 8 bulls. If a cow returned to estrous before pregnancy diagnosis, its status was confirmed by examination per rectum, and the animal was inseminated at his time and recorded as non-pregnant. A cow was included only once in the study.

2.2. Fixed-Time AI Protocol and Ultrasound Exams

Repeat breeders and anestrous cows were synchronized for FTAI using a controlled internal drug-releasing device (CIDR, containing 1.38 g of progesterone (P4); Zoetis Spain SL, Spain). The CIDR was left in place for 5 days, and these animals were also given cloprostenol (500µg i.m.; PGF Veyx Forte, Ecuphar, Spain) on CIDR removal. Then, 24 and 60 h later, the cows received a second cloprostenol and a GnRH dose (using the GnRH analogue dephereline: 100µg gonadorelin acetate [6-D-Phe] i.m.; Gonavet Veyx, Ecuphar, Spain), respectively. Cows were inseminated 72 h after CIDR removal [29].

Ovarian follicular structures larger than 6 mm in diameter and the absence or presence of one or more CLs were assessed by ultrasound immediately before AI and 7 days later using a portable B-mode ultrasound scanner equipped with a 5-10 MHz transducer (E.I. Medical IBEX LITE; E.I. Medical Imaging, Loveland, CO, USA).each ovary was scanned in several planes, moving the transducer along its surface to identify follicular and luteal structures; measurements and the number and location of both were recorded. Ovulation, confirmed by the presence of at least one mature CL, was assessed on Day 7 post-AI. A lack of high pixel intensity associated with a young CL [30,31] was used as reference to confirm the state of CL maturity. Follicular diameter was measured on the widest image of the follicle and calculated as the average of the greater and lower diameter measurements. The dimensions of a CL were recorded as the mean of two measurements approximating the greatest length and width. As the presence of a central cavity is not functionally important [32-34], cavity CL were measured just like solid CL. Scanning was also performed along dorsal/lateral surface of each uterine horn for pregnancy diagnosis on Day 28 post-AI. Twin pregnancies were registered as unilateral (both embryos in the same uterine horn) or bilateral (one embryo in the right horn and its co-twin in the left horn).

2.3. Data collection and Statistical Analyses

The pregnancy rate was defined as the percentage of cows that became pregnant out of the total number of cows in the corresponding group. The following data were recorded in each animal: parturition and AI dates; status (repeat breeder, anestrus or spontaneous estrus); lactation number (partity, primiparous vs. pluriparous); the number and location of follicles and CL (unilateral vs. bilateral in cows with two structures); days in milt at AI (DIM; <90 days postpartum versus ≥90 days postpartum); milk production at AI (mean production in the three days before AI) (low producers < 45 kg vs. high producers ≥ 45 kg); sire; AI technician; follicular size at AI; ovulation failure (absence of at least a mature CL seven days after AI); CL size seven days after AI; double ovulation (presence of two CLs seven days after AI); pregnancy 28 days post-AI for ovulating cows; and presence of twins in pregnant cows. The threshold for milk production was set as the median value of production recorded in primiparous cows. Three follicular groups were established: cows with a single follicle, cows with two bilateral follicles (one follicle in each ovary), and cows with unilateral follicles (two follicles in the same ovary). AI dares were used to assess the effects of season on subsequent reproductive performance. In our geographical region, there are only two clearly differentiated seasons: warm (May to September) and cool (October to April) [35,36]. Temperatures for study period were from October 2020 to April 2021: 28 days with minimum temperature < 0ºC and 3 days with maximum temperature >251C (15.25 ± 2.3); from May to July 2021: 0 days with minimum temperature < 0 ºC and 75 days with maximum temperature > 25 ºC (29.8 ± 5.2).

The software package PASW Statistics for Windows Version 18.0 (SPSS Inc., Chicago, IL, USA) was used for data processing. Significance was set at $p < 0.05$. variables are expressed as the mean ± standard deviation (S.D.). overall reproductive performance in the groups was compared using the chi-square test (percentages) or ANOVA and Tukey post-hoc tests (means ± SD). Possible correlation between follicular size and CL size was examined in single-ovulating and bilateral double-ovulating cows.

The effects of the presence of a small follicle on ovulation and pregnancy rates were analyzed by binary logistic regression. Two regression analyses were performed using ovulation and conception after AI as the dependent variables. The factors entered in the models as independent variables were repeat breeding, anestrus, follicular group (three classes: single, and bilateral or unilateral in cows with a small follicle), season of AI (warm), parity (pluriparous), days in milk (>90 days) and milk production at AI (high producers). For dependent variable conception rate, only ovulating cows were included. Possible interactions between the presence of small follicles and the variables milk production, season, and parity were also examined. Regression analyses were conducted according to the method of Hosmer and Lemeshow [37].

Two further binary regression models were built using the presence of a small follicle as the dependent variable for all cows, and ovulation of the small follicle for cows with this structure. The factors entered in the modes were those considered for the dependent variable ovulation of the small follicle, the site with respect to the follicle of ovulatory size (bilateral vs. unilateral) and diameter difference between follicles were added as factors.

3. Results

Twelve cows with no follicles of ovulatory size (10 mm or more), 164 cows with two or more follicles of ovulatory size, and 139 cows with luteal structures were removed from the study. The final study population was comprised of 434 cows with a single follicle of ovulatory size along with the presence or not of a single follicle between 7 and 9 mm: 246 repeat breeders, 61 anestrous cows, and 127 cows showing spontaneous estrus. Of the 434 cows, 286 (65.9%) had a single follicle, 99 (22.8%) had two bilateral follicles (one follicle in each ovary) and 49 (11.3%) had two unilateral follicles (two follicles in the right or left ovary). All cows were submitted to AI. Mean milk production and days in milk at the time of AI, as well as the number of lactations and Ais, were 50 ± 9 kg, 133 ± 82 days, 3 ± 1.3 lactations, and 4 ± 2.1 Ais, respectively (mean ± SD). The independent variables recorded for each follicular group and their effects on each dependent variable are shown in Table 1.

Follicular diameter was significantly ($p < 0.0001$) greater for follicles of pre-ovulatory size than small follicles. As high positive correlation ($r = 0.86$; $p < 0.0001$) was recorded between follicle diameter and CL size in single-ovulating plus bilateral double-ovulating cows, CL derived from small follicles were identified in cows with two unilateral follicles. Of the total of 148 small follicles, 51 (34.5%) ovulated: 33 in the bilateral and 18 in the unilateral group. Ovulation occurred only in the small follicle in 17 cows: 12 in cows with bilateral and 5 in cows with unilateral follicles. CL size was significantly ($p < 0.0001$) greater for CLs derived from follicles of ovulatory size than those derived from small follicles. According to chi-square tests, cows with two follicles showed a significantly ($p < 0.001$) greater proportion of double ovulations (34 / 142, 16.9%) referred to the total of ovulating cows, and of twin pregnancies (5 / 44, 11.4%) referred to the total of pregnant cows, compared with cows with one follicle, in which both parameters were 0%.

Table 1. Independent variables recorded at the time of AI and effects of the three ovulatory follicle states on each dependent variable (n = 434).

Follicular Status [(a)]	One Follicle (n = 286)	Two Bilateral Follicles (n = 99)	Two Unilateral Follicles (n = 49)
Independent variables [(b)]			
Parity (pluriparous)	155 (54.2%)	53 (53.5%)	28 (57.1%)
Milk production (≥45 kg)	140 (49%)	60 (60.6%)	24 (49%)
Days in milk (≥90 days)	223 (78%)	68 (68.7%)	36 (73.5%)
Season (warm period: May–September)	91 (31.8%)	33 (33.3%)	12 (24.5%)
Repeat breeding	174 (60.8%)	48 (48.5%)	24 (49%)
Spontaneous estrus	82 (28.7%)	28 (28.3%)	17 (34.7%)
Diameter (mean ± SD) of follicles of ovulatory size (≥10 mm)	19.8 ± 6.1	19.7 ± 6.2 *	19 ± 6 *
Diameter (mean ± SD) of small follicles (7–9 mm)		7.9 ± 0.5 **	7.8 ± 0.8 **
Size (mean ± SD) of CL derived from follicles of ovulatory size	23.7 ± 7.5	23.7 ± 7.3 *	22 ± 8.2 *
Size (mean ± SD) of CL derived from small follicles		10.8 ± 0.8 **	11.6 ± 1.1 **
Dependent variables [(c)]			
Ovulation failure	11/286 (3.8%)	4/99 (4%)	2/49 (4.1%)
Double ovulation [(d)]	0/275 (0%) ***	21/95 (22.1%) ****	13/47 (27.7%) ****
Conception rate [(d)]	84/275 (30.5%)	33/95 (34.7%)	11/47 (23.4%)
Twin pregnancy [(e)]	0/84 (0%) ***	3/33 (9.1%) ****	2/11 (18.2%) ****

[(a)] One follicle: cows with a single follicle of ovulatory size (10 mm or more); two bilateral follicles: cows with a single follicle of ovulatory size in an ovary and a follicle between 7 and 9 mm in the contralateral ovary; two unilateral follicles: cows with a single follicle of ovulatory size in an ovary and a follicle between 7 and 9 mm in the same ovary. [(b)] Values with different superscripts within columns denote significant differences detected by ANOVA and Tukey post-hoc tests (*, **: $p < 0.0001$). [(c)] Values with different superscripts within rows denote significant differences detected by the chi-square test or Fisher's exact test (***, ****: $p < 0.001$). [(d)] In ovulating cows. [(e)] In pregnant cows.

3.1. *Ovulation and Conception Rates*

Seventeen cows (3.9%) failed to ovulate. These cows were subjected to a further FTAI protocol at ovulation diagnosis and their data were excluded from the subsequent analyses but maintained for the analyses of ovulation failure rate and presence of a small follicle. Based on binary logistic regression procedures, no factors were noted to affect ovulation. No young CLs were detected in ovulating cows. Of the 417 ovulating cows, 128 (30.7%) became pregnant. Table 2 provides the pregnancy rate, odds ratio, and 95% confidence interval for the total population of ovulating cows. The final model included only repeat breeding. Impacts of follicular group, anestrus, parity, days in milk, season and milk production were not significant, so these factors were not included in the final model. Using non-repeat breeders as the reference, repeat breeding resulted in a pregnancy rate significantly reduced ($p < 0.0001$) by a factor of 0.4. Nine conceptuses were derived from small follicles: 6 (3 as co-twins) from the bilateral and 3 (2 as co-twins) from unilateral group. One cow, in the unilateral group, became pregnant from its small follicle (9 mm), underwent ovulation failure of the follicle of ovulatory size (18 mm) and only a CL of 12 mm was observed 7 days post-AI.

Table 2. Odds ratios of the pregnancy rate variables included in the final logistic regression model (n = 417).

Factor	Class	*n*	% Pregnancy	Odds Ratio	95% Confidence Interval	*p*
Repeat breeding (>3 AI)	No	72/179	40.2	Reference		
	Yes	56/238	23.5	0.4	0.26–0.6	<0.0001

R^2 Nagelkerke = 0.14.

3.2. *Presence and Ovulation of Small Follicles*

Of 434 cows with a follicle of ovulatory size, 148 (34.1%) had a small follicle. Table 3 provides the incidence of the presence of a small follicle, odds ratio, and 95% confidence interval for the total population of cows. The final model included only repeat breeding. The impacts of anestrus, parity, days in milk, season, and milk production were not significant and these factors were not included in the final model. Taking non-repeat breeders as the reference, repeat breeding resulted in an incidence of the presence of a small follicle that was significantly (p = 0.001) reduced by a factor of 0.6. No factors were associated with the ovulation of a small follicle.

Table 3. Odds ratio for the small follicle presence variables included in the final logistic regression model (n = 434).

Factor	Class	*n*	% Pregnancy	Odds Ratio	95% Confidence Interval	*p*
Repeat breeding (>3 AI)	No	76/188	40.4	Reference		
	Yes	72/246	29.3	0.6	0.41–0.91	0.001

R^2 Nagelkerke = 0.15.

4. Discussion

In this study, we examined the effects of the presence of a small follicle (7-9 mm) in lactating dairy cows with a follicle of ovulatory size (10 mm or more) at estrus. To our knowledge, the presence and ovulation of a small follicle and possible associated factors following either natural or synchronized estrus have not been previously analysed. A total of 34.5% (51/148) of the small follicle ovulated, with an associated pregnancy rate of 17.6% (9/51). Under our working conditions, there was no influence synchronization, anestrus, repeat breeding, site with respect to the follicle of ovulatory size (bilateral vs. unilateral), and diameter difference between follicles on ovulation of the small follicle. Although we were unable to find a significant correlation between the variables analyzed and the ovulation of such follicles, we did observe a strong link between the presence of a small follicle and repeat-breeder syndrome.

Follicles with 7-9 mm sies accompanying a dominant follicle of ovulatory size at estrus are often considered as atretic. Follicular selection to dominance depends on a transient increase in plasma concentrations of follicle-stimulating hormone (FSH) [38-40]. The selected follicle continues development in the setting of a profound drop in FSH level and may escape atresia, which is the fate of all other follicles that lack the capacity to make use of the low FSH concentrations [41-43]. As a dominant follicle matures, there is a transfer of dependency from FSH to LH supporting to establishment of dominance [13,14,44].

The selection of a single ovarian follicle for differentiation and ovulation is a phenomenon shared by monovular species including humans [39,45,46]. However, the presence of two or more co-dominant follicles (follicles of ovulatory size) in high producers can exceed 50% at the time of estrus [18]. In these cases, the largest follicles is not necessarily the one most likely to ovulate. Ovulation only of the smaller follicle may occur in over 40% of ovulating cows [18], 10-30% being an accepted figure among cows, mares, and women [46-47]. The rate of switching of dominance to the small follicle seems similar in the present study. Hence, ovulation only of the small follicle is observed in 12% (17 / 142) of ovulating cows. However, our results also suggest that follicles of deviation diameter (7-9 mm) behave differently to the smaller follicle in cows with two co-dominant follicles of ovulatory size. In prior work, we were able to relate double ovulation along with ovulation of the smaller follicle to the least size between the larger and smaller follicle in a study population of 316 cows with only two co-dominant follicles (cows with three or more follicles of ovulatory size were removed) [18]. This pattern could not be demonstrated here as the diameter difference between follicles was not found to influence ovulation of the small follicles. Studies are needed to elucidate mechanisms related to the ovulation of follicles of deviation size.

It is well documented that a positive correlation exists between follicular diameter at estrus and the size of its associated CL [45-51]. The high positive correlation in cows with bilateral follicles allowed us to identify the ovulation of small follicles in the unilateral group. Irrespective of this, double ovulation was recorded in 23.9% (34/142) of ovulating cows with two follicles, and twin pregnancies in 14.7% (5/34) of cows experiencing double ovulation. This double ovulation rate is within reported ranges of 12-30% [7,8,26,29,52,53]. We can, therefore, view the presence of deviation-size follicles at estrus as an additional factor favoring twin pregnancies. In fact, an increased proportion of small ovulatory follicles (8-10 mm) has been associated with multiple ovulations (from 2 to 6 ovulations) in beef cow breeds selected on the grounds of five generations of multiple pregnancies [49]. Although a high dose of LH was found by some authors to result in 0% ovulation of follicles of neat deviation diameter (7-9 mm) in dairy cows [16], we would expect the high presence of small ovulatory follicles besides two or more ovulatory-size follicles at estrus in dairy cattle. In effect, an increased level of follicular recruitment and growth has been described for cows [54] and women [55] delivering twins.

As we might expect, the presence of small follicles was reduced in repeat breeders. Abnormal follicular dynamics is a common reproductive pattern related to repeat-breeder syndrome [56-58] and a low double ovulation rate has been associated with this disorder [25,26]. Repeat breeding emerged here as the only factor influencing the conception rate, and 56.7% (246/434) of cows in our study population were classified as repeat breeders.

5. Conclusions

In the presence of a dominant follicle of ovulatory size (10 mm or more) at estrus, follicles measuring between 7 and 9 mm can ovulate alone or along with the dominant follicle, leading to single or twin pregnancies. In dairy cattle, the lower follicular size limit for ovulation needs revising.

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Data Availability Statement: The data presented in this study are available on request. These data are not publicly available to preserve the data privacy of the commercial farm.

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CAPÍTULO II

Twin reduction in the late embryonic period prevents the condition of freemartin in dairy cattle

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Abstract

The condition of freemartinism occurs in the bovine heterosexual twin foetuses and refers to the resulting infertile female. Vascular anastomoses of the foetal membranes are the major reason of this anomaly. This study examines whether single born heifers following induced twin reduction at 28-34 days of pregnancy could develop normal reproductive functions. The study population derived from 367 lactating dairy cows carrying: unilateral twins (n = 178), bilateral twins (n = 174) or triplets (n = 15), in which manual embryo reduction was performed. The final study population was constituted of 95 single born twin females that reached 12 month of life and entered into the AI period. Of these heifers, 40 have had one unilateral co-twin, 49 one bilateral co-twin, and 6 two contralateral co-twins. A total of 1688 heifers inseminated during the same period were used as controls to compare the rates of pregnancy at 15 months of age, culling due to infertility and birth before the age of 24 months. No differences were detected between groups. With an accepted incidence rate of 50% heterosexuality for all tin sets, we should assume that half of our study population had a male co-twin. Our results indicate lack of effect of the male co-twin on normal development of the gonads and genital tract of his female partner during the late embryonic period.

Keywords: cattle, dairy cattle, double ovulation, leuko-chimerism, twinning rate

1. Introduction

The bovine freemartin remains the best known rather than best understood example of abnormal sexual differentiation in mammals (Hunter, 1995). This abnormality was already observed at the dawn of our era by Varro and Columella (Hunter, 1979). The freemartin condition develops in a female foetus co-twin to a male, which sharing a common placental circulation undergoes a partial sex-reversal of her gonads and becomes sterile (Hunter, 1995). There is a masculinization of the female genital tract and gonads to varying degrees (Padula, 2005). Despite of this, a normal female may be expected between 3% and 20% of heterosexual twin births (Hunter, 1995). The period of gestation is divided into embryonic, from conception to the end of differentiation (approximately 45 days) and foetal, from completion of differentiation to parturition (Committee on Bovine Reproductive Nomenclature, 1972). Fused embryonic circulations are already developed during the late embryonic period in twin pregnancies (Echternkamp, 1992). Twins are diagnosed in the late embryonic period, 28-34 days post-artificial insemination (AI), in almost 33% (697/2173) of pregnancies in older cows (Garcia-Ispierto & López-Gatius, 2018). It has been suggested that twin pregnancies diagnosed before 36 days post-AI may be susceptible to developing into a single born freemartin (Padula, 2005). Since twins are undesirable in dairy cattle, twin reduction by manual rupture of the amniotic vesicle is currently performed at pregnancy diagnosis in commercial dairy herds under our surveillance (López-Gatius, 2020). The objective of the present study was to evaluate the possible incidence of freemartins in single born heifers following twin reduction at 28-34 days of pregnancy.

2. Materials and Methods

This study was performed on a commercial Holstein-Friesian dairy herd in north-eastern Spain (41.13 latitude, 0.24 longitude). During the study period (January 2017 to December 2019), the mean number of lactating cows in the herd was 1282, and mean annual milk production was 12,181 kg per cow. All animals were reared within the herd. Only semen from Holstein-Friesian bulls was used in the herd and pregnancy diagnosis was performed by ultrasound 28-34 days post-AI.

The study population derived from 367 cows carrying: unilateral twins (*n* = 178), bilateral twins (*n* = 174) or triplets (*n* = 15), in which manual embryo reduction was performed. In triplet pregnancies, two embryos were located into one uterine horn, on which amnion rupture was carried out, and the remaining embryo was located on the contralateral horn. Embryo reduction was not practised in six further cows bearing unilateral triplets or four cows with quadruplets. Pregnancy loss was registered in 33% (121/367) of cows following embryo reduction. Of the 246 cows maintaining gestation, 114 delivered calves. The final study population was constituted of 95 single born twin females that reached 12 months of life and entered into the AI period. Of these heifers, 40 have had one unilateral co-twin, 49 one bilateral co-twin, and 6 two contralateral co-twins. A total of 1688 heifers inseminated during the same period were used as controls to compare the rates of pregnancy at 15 months of age and culling due to infertility before the age of 24 months. The genital organs of all heifers were examined at 12 months of age.

3. Results

Derived from 2197 singleton births with a female newborn, 1783 heifers entered into the AI period: 95 single born twin females (83.3%: 95/114) and 1688 controls (81%: 1688/2083). The genital organs of all heifers were normal in form and size at 12 months of age. The rates of pregnancy and culling were for single born twins 95.8% (91/95) and 4.2% (4/95), respectively, and 95% (1604/1688) and 4.9% (82/1688) for controls. No differences between the proportions were detected using the Chi-squared test.

A hyperechoic line from embryo to embryo representing chorionic membrane apposition between twins (López-Gatius & Garcia-Ispierto, 2010) was observed in all unilateral twin pregnancies at pregnancy diagnosis.

4. Discussion

Reproductive function of the twin born females was not influenced by twin reduction, much less the 12-month-old heifers showed signs of sterility associated with the freematin condition. Placental fusion leading to vascular anastomosis of allantois-chorionic blood vessels allow transplacental flow of cells between co-twin foetuses. Such twins are blood chimaeras (Owen, 1945; Peretti et al., 2008). There is some evidence of chimerism among single birth bulls (Pollock, 1974) and single born freemartin heifers (Kadokawa et al., 1995). Such type of chimerism may arise from the demise of the heifer co-twin or, if vice-versa, of the bull co-twin, at some stage after the asnastomoses of chorion blood vessels. Although cytogenetic tests were not performed in the present study, with an accepted incidence rate of 50%

heterosexuality for all twins sets (Foote, 1977; Hunter, 1995; Padula, 2005), we should assume that half of our study population had a male co-twin. The level of anastomoses could not be determined, but the hiyperechoic line from embryo to embryo representing chorionic membrane apposition between unilateral twins suggests an intimate contact between conceptuses at twin reduction. Irrespective of possible embryo-to-embryo cell colonization or twin chimerism, testicular differentiation begins in the male co-twin at Day 40 of gestation and then influences the reproductive development of his female co-twin (Hunter, 1995). Our results indicate lack of effect of the male co-twin on normal development of the gonads and genital tract of the female during the late embryonic period.

Author contributions

Garcia-Ispierto and López-Gatius conceptualized the study and wrote the manuscript; Palacín-Chauri obtained the data, validated and edited the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

Data availability

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

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CAPÍTULO III

Direct transfer of a single frozen-thawed in vitro-produced embryo to avoid twin pregnancy in dairy cows

Fernando López-Gatius, Roger J. Palacín-Chauri and Irina Garcia-Isperto

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Abstract

Fertility of cow receiving fixed-time artificial insemination (FTAI) was compared with cow in which a single frozen-thawed in vitro-produced (IVP) embryo was fixed-time transferred (FTET) to avoid twin pregnancy. The study population was comprised of 596 lactating dairy cows synchronized for oestrus: 440 were fixed-time inseminated (AI cows), and 156 were given GnRH treatment at the time of embryo transfer (ET cows) 8 days post-oestrus. Of the 596 cows, 235 (39.4%) became pregnant: 175 (39.8%) AI cows and 60 (39.8%) ET cows. Twin pregnancy was recorded in 16% of the AI pregnant cows (28/175), whereas no ET cows had twins (0/60). Significant interaction ($p<.01$) was observed between breeding technique (FTAI vs FTET) and repeat (RB) or not repeat breeding (NRB) for the likelihood of pregnancy. This meant that using RB AI cows as reference, the odds ratio for pregnancy in RB ET cows was 2.2 ($p = .04$). In conclusion, transfer of a frozen IVP embryo proved useful to prevent the risk of twin pregnancy without affecting fertility.

Keywords: double ovulation, repeat breeding, twinning

1. Introduction

Twin pregnancies are undesirable in dairy herds as they considerably compromise the cow's general health and productive lifespan. Recently, the transfer of a single fresh in vitro-produced (IVP) embryo to a non-inseminated cow prevented twin pregnancies without compromising the fertility (López-Gatius et al., 2022). Transfer of frozen-thawed IVP embryos rather than fresh embryos can make the process easier under on-farm conditions (Dochi, 2019). The objective of the present study was to determine whether the transfer of a single frozen-thawed IVP embryo could prevent twin pregnancy without reducing the fertility of a cow.

2. Materials and Methods

This study was performed on three commercial Holstein-Friesian dairy herds kept on farms 10 km apart in north-eastern Spain. During the study period (October 2020 to December 2021), the overall average of lactating cows in the herds was 6688. Mean annual milk production was 11,950 kg per cow.

The herds were maintained on a weekly reproductive health programme. All cows with no oestrous signs 60 days post-partum (not repeat breeders: NRB) and those deemed negative at pregnancy diagnosis (repeat breeders (RB) or NRB) were synchronized for fixed-time AI (FTAI) using a controlled internal drug-releasing device (CIDR, containing 1.38 g of progesterone [P4]: Zoetis Spain SL, Alcobendas, Madrid, Spain). The CIDR was left in place for 5 days, and these animals were also given cloprostenol (500 µg i.m.; PGF Veyx Forte, Ecuphar, Barcelona, Spain) on CIDR removal. Then, 24 and 60 h later, these cows received a second cloprostenol and a GnRH dose (using the GnRH analogue dephereline: 100 µg gonadorelin acetate [6-D-Phe] i.m.; Gonavet Veyx, Ecuphar, Barcelona

Spain), respectively. Cows were FTAI 72 hours later after CIDR removal (Figure 1).

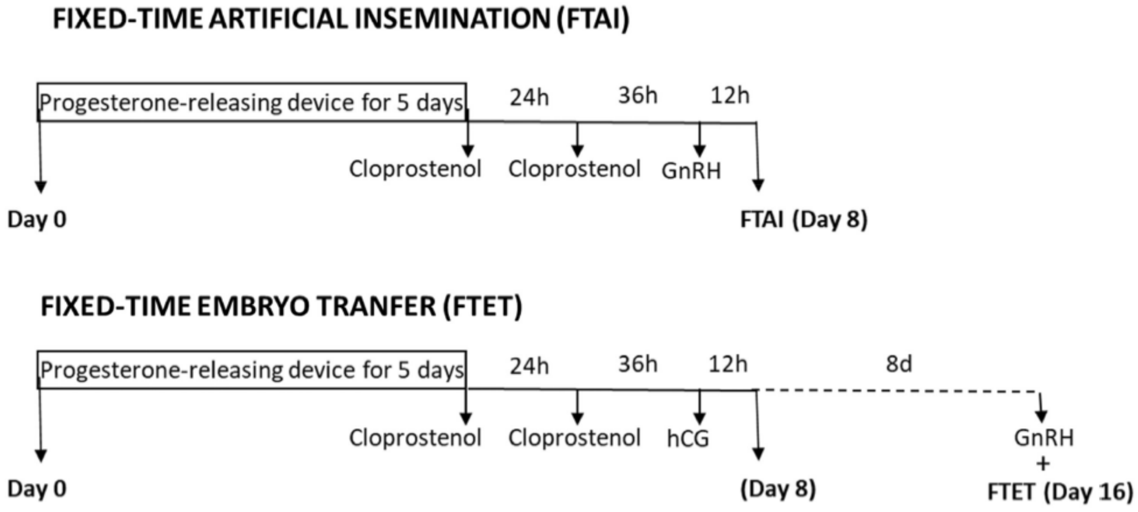

Figure 1. Treatment protocols used to synchronize oestrus for fixed-time artificial insemination or embryo transfer.

Synchronized cows were used as recipients (ET cows) for given weeks of the study period, balancing for the warm and cool period of the year. These cows were treated according to the same P4-based protocol but the dose of GnRH was replaced with human chorionic gonadotropin (hCG) (3000 UI hCG i.m.: Veterin Corion, Divasa-Farmavic, Gurb-Vic, Barcelona, Sapin) (Figure 1) (Garcia-Ispierto et al., 2021). FTAI cows were cows which were synchronized in the weeks before and after the week of synchronization of ET cows. AI cows were selected from 462 synchronized cows of wich 22 were not ready for service at FTAI. ET cows were selected from 165 synchronized cows of which 2 failed to ovulate, and 7 further cows had a very young CL at ET. These 22 AI cows and 9 ET cows were removed from the study. The final study population comprised 596 lactating cows synchronized for oestrus: 440 were FTAI (AI cows), and 156 were FTET (ET cows). ET cows received a GnRH dose at ET (Figure 1). Only healthy cows that were free of detectable clinic disorders during the study period

(Days -7 to 49 of synchronized oestrus) were included. Cows were included only once per lactation in the experiment.

Fresh IVP embryos were first assessed morphologically according to International Embryo Transfer Society (IETS) guidelines and then were slow frozen in 1.5 M ethylene glycol containing 0.1 M sucrose. Freezing rate was -0.5ºC/min from -6ºC (seeding temperature) to -32ºC (Dochi, 2019). Frozen-thawed embryos were transferred to the uterine horn ipsilateral to the CL by the same technician. ALL ET cows were given caudal epidural anaesthesia (2.5-4 ml, 2% lidocaine; Lidocaína clorhidrato 2% Fresenius Kabi, Barcelona, Spain), and the perineum cleaned before ET. Embryos were obtained from 'Embriovet SL', with the number of the EU embryo producer ES11ET05B, followed the traceability legislation determined by the EU, were certified for sanitary requirements, and the individuality of each embryo was known.

Pregnancy was diagnosed by transrectal ultrasound at 28 days post-oestrus and revised 21 days later. Pregnancy loss was recorded when the second examination proved negative. All gynaecological examinations and pregnancy diagnosis were performed by the same operator.

As pregnancy rates following artificial insemination (AI) or IVP embryo transfer (ET) were not significantly different between herds, data were grouped as derived from a single herd and 'herd' considered as a factor in binary logistic regression procedures. Parturition and AI/ET dates; parturition number; breeding technique (AI or ET); milk production at oestrus; AI number; pregnancy; presence of twins and pregnancy loss were recorded for each animal. In terms of AI number, ET was taken as equivalent to an AI. Dates of AI/ET were used to assess the effects of the weather period of year, warm (May to September) versus cool (October to April).

The effect of breeding technique on the pregnancy and pregnancy loss rates was analysed by binary logistic regression (Hosmer & Lemeshow, 1989). Significance was set at $p<.05$. The factors entered in the model as independent dichotomous variables (where 1 denotes presence and 0 denotes absence; data between parentheses: Total, AI and ET cows, respectively) were parity (multiparous: 78.9, 79.5 and 76.9% of cows), weather period (warm: 50.8, 50.7 and 51.3% of cows), repeat breeding (>3 AI: 29.9, 30 and 29.5% of cows) and milk production (>45 kg: 46.8, 46.8 and 46.8% of cows). Breeding (AI or ET) was considered a factor in the analyses).

3. Results

Mean milk production at oestrus and AI and parturition number were 45.2 ± 9.8 kg, 2.2 ± 2.0 parturitions, respectively (mean ± SD). Pregnancy was recorded in 235 of the 596 cows (39.4%), 11.9% of which (28/235) suffered pregnancy loss. Twin pregnancy was recorded in 16% of the AI pregnant cows (28/175), whereas no ET cows had twins (0/60).

Table 1. Effects of breeding technique on pregnancy for repeat breeding (n = 596).

Breeding[a]	Repeat breeding	*n*	% pregnancy	Odds ratio	95% confidence interval	*p*
AI	Yes	34/132	25.8	Reference		
AI	No	141/308	45.8	2.5	1.8–4.3	.0007
ET	Yes	20/46	43.5	2.2	1.0–4.6	.04
ET	No	40/110	36.4	1.6	0.9–2.9	.10

[a]Interaction between breeding technique and the repeat-breeding syndrome (>3 AI) was significant ($p<.01$).

The final model included only the interaction between breeding technique (FTAI vs FTET) and repeat (RB) or not repeat breeding (NRB) for the likelihood or pregnancy. Impacts of year, parity, milk production and season were not significant. Using RB AI cows as the reference, AI resulted in an increased pregnancy rate in NRB AI cows, whereas ET did so in RB cows (Table 1).

No factors were associated with the pregnancy loss rate.

4. Discussion

The use of frozen-thawed IVP embryos proved valuable in avoiding the risk of twin pregnancies. The procedure appeared not to influence fertility, and the pregnancy rate was improved in RB cows. In fact, improvement of fertility in RB dairy cows by ET has been extensively reported (Nowicki, 2021). In this situation, our results reinforce previous findings that showed similar results when comparing the use of frozen IVP embryos and AI (Ambrose et al., 1999; Drost et al., 1999).

Author contributions

López-Gatius and Garcia-Ispierto performed the experiment and wrote the manuscript. Palacín-Chauri collected the data and validated the manuscript.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Data availability

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

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CAPÍTULO IV

Cow and management factors and their association with estimates of uterine size and position at the time of insemination of dairy cattle

Roger J. Palacín-Chauri and Irina Garcia-Ispierto

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Abstract

This study examines factors affecting uterine size and position determined at 30-36 days postpartum in dairy cattle. The final study population consisted of 328 dairy cows, all calving during the warm season. Uterus position (pelvis, pelvic-abdominal, abdominal) and uterus size (small, medium, large) was measured by ultrasound on Day 30-36 postpartum. Multiparous cows had a larger uterus positioned in the abdominal cavity (p=.03) and a male newborn was associated with a larger uterus (p= .022). The number of cows with the uterus in the abdominal cavity was higher among multiparous and high producer (p<.0001) cows. High producers were 0.41 times more likely to have a small uterus in a pelvic position. The most important findings of this study was that the delivery of a male dairy, rather than beef, calf was related to the least optimal maternal uterus size and position.

Keywords: bovine, newborn gender, position, postpartum, uterus

1. Introduction

The postpartum period runs from parturition to completion of uterine involution, approximately 28-49 days after parturition. Due to the complexity of this process, veterinarian visits focus on improving uterine involution as this directly linked to postpartum anoestrus. To ensure that a cow is ready to be inseminated after this period, it is important that any necessary therapeutic actions be taken before 30-36 days postpartum.

To assess uterine involution, the most widely used methods are palpation per rectum and transrectal ultrasonography (Krueger et al., 2009). Recently, Young et al. (2017) developed a scoring system based on the size and position of the uterus to assess its involution. In effect, at the time of insemination, a larger uterus with no detectable abnormalities and positioned in the abdominal cavity has been associated with impairment of reproduction (Madureira et al., 2020). This system can be easily implanted during a weekly reproductive visit but more research is necessary to determine its applicability to dairy farms. The aim of the present study was to identify factors that affect uterine size and position, determined at 30-36 days postpartum, in high producing dairy cattle.

2. Materials and Methods

This study was performed in northeastern Spain over the period November 2021 to August 2022. The study population was a dairy herd comprising 1225 high-producing Holstein-Friesian dairy cows housed in free stalls with concrete slatted flooring and cubicles. During the scheduled herd visit, an exam was performed on Days 1-7, 15-21 and 30-36 days postpartum.

During the examination on Day 30-36 postpartum, the size and position of the uterus were recorded. The latter was noted as: (1) uterine horns within the pelvic cavity; (2) uterine horns partially out-side the pelvic cavity; or (3) uterine horns lying outside the pelvic cavity, according to the classification system of Young et al. (2017). Uterine size was measured as described elsewhere (Grunert, 1979). Briefly, the diameter of the uterus was measured by the same veterinarian on an ultrasound image and recorded as: small (S, < 25 mm), medium (M, 25 – 30 mm) or large (L, > 30 mm). the entire reproductive tract was examined by ultrasound using a portable B-mode ultrasound scanner (Easi-scan with a 7.5 MHz transducer). Cows delivering twins were not included in this study. The final study population consisted of 328 dairy cows. Ovarian structures were measured only in multiparous cows (193 cows).

The variables recorded for each cow before parturition were: date of parturition, parity (primiparous vs. multiparous), semen used for artificial insemination (AI) (beef vs. Holstein-Friesian bull), newborn gender (male vs. female), retained placenta, primary metritis. The variables recorded for each cow after parturition were: milk production on Day 30-36 days postpartum (lower producers < 50 kg vs. higher producers ≥ 50 kg), ovarian structures on Day 30-36 postpartum (follicle, follicular ovarian cyst and/or corpus luteum), uterus position (1,2 or 3) and uterus size (S, M or L – only recorded in a subgroup of animals) on Day 30-36 postpartum. All cows calved in the warm season of the year (May – September).

The software package PASW Statistic for Windows Version 18.0 (SPSS Inc.) was used for data processing. Possible significant differences between variables were assessed using the Chi-Square test. Significance was set at $p < .05$. Values are expressed as the mean ± standard deviation (SD). Possible plausible interactions were analysed. A regression analysis relating uterus size to position was conducted according to the method Hosmer and Lemeshow (1989) using the logistic procedure of PASW (mean ± SD).

3. Results

Mean milk production at 30-36 days postpartum, number of lactations and number of inseminations were 48.9 ± 11.4 kg, 2.3 ± 1.6 lactations, and 2.1 ± 1.6 inseminations, respectively (mean ± SD).

In Tables 1 and 2, factors are presented according to uterine size (S, M, L). and uterine position (1, 2, 3) at 30-36 days postpartum, respectively. Multiparous cows had a larger uterus positioned in the abdominal cavity (P = 0.03), male newborns were associated with a larger uterus (p = .02), and there was a positive relationship between uterine position and size (p < .0001) (Table 1). The number of cows with an abdominal uterine position was a greater among multiparous high-producing cows (p < .0001) (Table 2).

Table 1. Variables recorded and effects on uterine size (S = small, M = medium, L = large) determined at 30-36 days postpartum.

Variables	Class	S (n = 72)	M (n = 39)	L (n = 23)
Parity	Multiparous	43 (59.7)[a]	24 (61.5)[a]	19 (82.6)[b]
Milk production	HP	37 (51.4)	18 (46.1)	16 (69.5)
Uterine disorders	Placenta retention	2 (2.8)	1 (2.5)	0 (0)
	Metritis	1 (1.4)	0 (0)	0 (0)
Calf gender	M	24 (33.3)[b]	14 (35.9)[b]	14 (60.9)[a]
AI semen	Beef	24 (33.3)	13 (33.3)	11 (47.8)
Ovarian structures**	Non-cyclic	18/43 (41.9)	15/28 (53.6)	12/23 (52.2)
Uterus position	1	41 (57)[a]	9 (23.1)[b]	2 (8.7)[c]
	2	23 (31.9)	18 (46.1)	7 (30.4)
	3	8 (11.1)[c]	12 (30.8)[b]	14 (60.9)[a]

[a,b,c]Values with different superscripts differ within rows according to Tukey–Kramer tests (p < .05). Uterus position: 1 pelvic, 2 pelvic-abdominal, 3 abdominal. n differs from the total because primiparous cows were excluded.

Abbreviations: HP, high producer (≥50 kg daily); M, male.

**Ovarian structure: non cyclic (non presence of corpus luteum) vs cyclic (presence of corpus luteum).

Table 2. Variables recorded and effects on uterine position (1 = pelvic, 2 = pelvic-abdominal, 3 = abdominal) determined at 30-36 days postpartum.

Variables	Class	1 (*n* = 136)	2 (*n* = 116)	3 (*n* = 75)
Parity	Multiparous	49 (36)[a]	81 (69.8)[b]	62 (82.7)[c]
Milk production	HP	37 (27.2)[a]	67 (57.8)[b]	53 (70.7)[c]
Uterine disorders	Placenta retention	9 (6.6)	9 (7.8)	5 (6.7)
	Metritis	7 (5.2)	3 (2.6)	0 (0)
Calf gender	M	43 (31.6)	43 (37)	30 (40)
AI semen	Beef	28 (20.6)	33 (28.4)	33 (44)
Ovarian structure**	Non cyclic	13/28 (46.4)	16/36 (44.4)	16/32 (50)

[a,b,c]Values with different superscripts differ within rows according to Tukey–Kramer tests ($p < .05$).
n differs to the total because primiparous were excluded.
Abbreviations: HP, high producer (≥50 kg daily); M, male.
**Ovarian structure: non cyclic (non presence of corpus luteum) vs cyclic (presence of corpus luteum).

Table 3 shows odds ratios for the factors linked to a small uterus in pelvic cavity in the final logistic regression model. Accordingly, cows with high milk yields were 0.41 times more likely to have a small uterus in a pelvic position (S1). There were no significant differences between having a large uterus in the abdominal cavity and other interactions.

Table 3. odds ratios of the variable included in the final logistic regression model for factors promoting a small uterus in a pelvic position (SP) in dairy cows (n = 328).

Factor	Class	*n*	% cows	Odds ratio	95% confidence interval	*p*
Milk production	LP	27/171	15.8	Reference		
	HP	14/157	8.9	0.41	0.13–0.9	.009

Note: R^2 Nagelkerke = 0.10.
Abbreviations: HP, high producer (≥50 kg daily); LP, low producer (<50 kg daily).

4. Discussion

In this study, we observed no effect of the semen of AI on uterine size and position. In contrast, a male newborn foetus was related here to a larger uterus than a female one. Accordingly, the use of sexed semen, especially in heifers or primiparous cows, could be a good management practice on dairy farms to improve reproductive performance. Besides improving genetics by calving females from selected heifers, multiparous cows or higher producers, could be inseminated with beef semen as a way of reduce carbon footprint of beef production and increase the profit of the sale of calves.

Milk production significantly affects uterus position and size. Here, all high producers were multiparous cows. Uterine involution period is a secondary indicator of postpartum reproductive performance. It is influenced by the negative energy balance, abnormal calving and age (Lin et al., 2020). Thus, logically, primiparous animals postpartum will have a smaller uterus with both uterine horns within the pelvic cavity (Szelényi et al., 2022). In the present study, the use of sexed semen may have been a factor promoting a smaller uterus as most heifers delivered females. Calf sex is significantly associated with birth weight; male calves are heavier than female calves, which may partially explain their higher birth weight. As a result, at least half of secundiparous cows should also have a small uterus as the ratio male/female using conventional semen in close 50% (Foote, 2003).

Dairy cattle reproduction can be severely affected by a retained placenta. This may provoke uterine lesions not detected by ultrasound decreasing fertility. In the latter study, no effect of previous retained placenta or uterine disorders were found on the size or position of the uterus. Moreover, all the animals in our study calved during the warm season, which is when most postpartum disorders arise (Molinari et al., 2022). To reduce heat stress and its negative impacts on reproductive performance, this farm is equipped with fans and water sprinklers. It therefore seems that our strict management measures are successful at mitigating the known detrimental impacts of heat stress and uterine disorders on reproductive performance.

Author contributions

Conceptualization, methodology and writing-original draft preparation: Irina Garcia-Ispierto. Data collection, statistical analyses, writing-review and editing: Roger J. Palacín-Chauri.

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Conflict of interest statement

None of the authors have any conflict of interest to declare.

Data Availability statement

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

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DISCUSIÓN GENERAL

El concepto de "desarrollo sostenible" fue descrito por primera vez en el informe Brundtland de 1987 como "desarrollo que responde a las necesidades presentes sin comprometer la capacidad de las futuras generaciones de responder a las suyas." Desde entonces se han celebrado numerosas cumbres mundiales con el propósito de reducir los impactos negativos de la actividad humana sobre el planeta: Cumbre de la Tierra (1992), Cumbre de Kyoto (1997), Cumbre de la Tierra (2002), Cumbre de Río (2012), además de conferencias de lucha contra el cambio climático y otras convenciones regionales e internacionales. En septiembre de 2015, la Asamblea General de las Naciones Unidas aprobó la Agenda 2030 donde se recogen 17 Objetivos de Desarrollo Sostenible (ODS) cuyo objetivo principal es "lograr un futuro mejor y más sostenible para todos" (Naciones Unidas, 2015). Todos los ítems de los ODS conciernen al sector ganadero y, especialmente, la salud y el bienestar (2), producción y consumo responsables (12), acción por el clima (13) y vida de los ecosistemas terrestres (15). El aumento de temperaturas debido al cambio climático provoca estrés por calor a cada vez más vacas y durante un periodo de tiempo más largo en todo el mundo, poniendo en peligro la producción de leche a nivel global (Dovolou et al., 2023). Es por ello que el sector debe ser resiliente al cambio climático para seguir produciendo alimento, y una vía es la mejora del manejo reproductivo en granja.

La sostenibilidad reproductiva se tiene que basar en la mejora de los índices productivos y reproductivos de las granjas de leche altamente productoras teniendo en cuenta el impacto económico, social y medioambiental. La mejora de dichos índices disminuye el número de vacas no productivas del rebaño, por lo que se reducen los costes económicos y las emisiones de metano (huella ecológica) y, por lo tanto, aumenta la sostenibilidad en la granja (Diavao et al., 2023). A nivel genético, se ha descrito en rebaños experimentales una heredabilidad de 0,03 de la tasa de ovulación (van Vleck y Gregory, 1996) y de 0,01 en partos dobles (Weller et al., 2008). Al ser unas heredabilidades tan bajas, la mejora genética se debe centrar con el aumento de fertilidad en el rebaño acompañada de una nutrición adecuada a las necesidades fisiológicas en cada etapa productiva, de una detección de gestación temprana y de un control reproductivo exhaustivo (Crowe et al., 2018).

1. Fisiología de las dobles ovulaciones

El crecimiento folicular es un proceso dinámico que está bajo el control sistémico y local (Savio et al., 1988) de distintas hormonas como la GnRH, FSH, LH, progesterona y estrógenos. Así pues, las concentraciones de dichas hormonas durante el ciclo estral influenciaran en la tasa de ovulación, además de su determinismo genético, que está involucrado por un gran número de genes y rutas biológicas distintas (Vinet et al., 2012), de la nutrición y el manejo. Se han hecho varios estudios relacionados con la dinámica folicular centrados en las oleadas foliculares, la codominancia y estrategias de reducción de las dobles ovulaciones.

En un estudio de Kulick y colaboradores (2001) con vacas multíparas, en la primera oleada aparecían más folículos codominantes en comparación con las otras por un aumento transitorio en las concentraciones de FSH y LH 24 horas antes del comienzo de la desviación. Dicho aumento es debido, por un lado, a la disminución de la concentración de inhibina circulante, por el que aumenta la FSH y, por otro lado, a la reducción de progesterona circulante que eleva la frecuencia el pulso LH (Lopez et al., 2005b). En la posdesviación, los niveles de FSH se reducen más notoriamente y la concentración de estradiol es mayor en vacas multíparas que ovulan doble

(Kulick et al., 2001; Lopez et al., 2005). De hecho, la aparición de las dobles ovulaciones está ligada con el metabolismo de los esteroides en el hígado (Kusaka et al., 2017): una mayor producción de leche y niveles altos de ingesta inducen un mayor flujo sanguíneo hepático y un mayor metabolismo hormonal, lo que resulta en una alteración del feedback positivo del estradiol y retrasa la disminución de la concentración de FSH (Fricke y Wiltbank, 1999; Wiltbank et al., 2000). Se han observado diferencias entre vacas lactantes y novillas con respecto a las concentraciones de estradiol: las vacas lactantes tenían una concentración más baja que las novillas, a pesar de tener folículos ovulatorios y CL más grandes, debido seguramente a que el metabolismo de esteroides en vacas lactantes es mayor (Sartori et al., 2004). Además, en el mismo estudio, las vacas lactantes tuvieron mayor incidencia en el fallo ovulatorio después de la luteolisis y de ovulaciones múltiples que las novillas.

A lo largo de los años la literatura científica recoge varios factores que pueden afectar a la incidencia de las dobles ovulaciones en las vacas lecheras altamente productoras, no solamente factores exclusivamente hormonales. Según Lopez y colaboradores (2005) vacas con folículos codominantes producen más leche que vacas con solo un folículo dominante. Otros autores defienden en su estudio que las dobles ovulaciones tienen menos probabilidad de ocurrir en vacas que presentan mayor producción láctea debido al estrés metabólico y el estrés por calor que comprometen la folicogénesis (López-Gatius et al., 2005). De hecho, en el mismo estudio se reporta que el riesgo de fallo ovulatorio es 3,9 veces mayor en verano. Así pues, la literatura científica no tiene muy claro si la producción láctea tiene incidencia en las dobles ovulaciones. En todo caso, en el que sí se pone de acuerdo es que el manejo, la nutrición y las condiciones ambientales son factores que afectan a la incidencia de dobles ovulaciones en vacas de leche.

Existen varias estrategias para reducir las ovulaciones múltiples en vacas lecheras altamente productoras (véase apartado Introducción general) pero aún se precisa de más estudio. Por lo tanto, a nivel clínico, quizás sea de más utilidad conocer qué tratamientos y prácticas de los que se realizan en el día a día de las explotaciones lecheras no afectan negativamente a la ovulación. Por ejemplo, se ha descrito que el tratamiento con Depherelina no incrementa el riesgo de dobles ovulaciones (Garcia-Ispierto et al., 2019). Además, el tratamiento de hCG para inducir la ovulación al final del protocolo de 5 días de progesterona de FTAI tampoco afecta a las ovulaciones múltiples (Garcia-Ispierto et al., 2018). Por lo tanto, el uso de Depherilina y hCG son una buena elección para el manejo reproductivo.

En la visita reproductiva una de las acciones que se realiza es determinar el tamaño de los folículos presentes, y se ha visto que el diámetro individual del folículo no tiene relación con la probabilidad de ovulación en vacas con dos folículos bilaterales, es decir, el desarrollo de dos folículos codominantes está más relacionado con la diferencia pequeña de tamaño que con el diámetro individual de cada folículo (López-Gatius et al., 2018). Ocasionalmente un futuro folículo subordinado es más grande inicialmente que el futuro folículo dominante, pero tiene una tasa de crecimiento menor, de modo que no es el primero en llegar al estadio decisivo (Ginther et al., 1996). El diámetro del folículo ovulatorio no afecta a la relación de preñez e IA en los días 32 y 60, pero folículos más grandes están asociados a pérdidas embrionarias tardías y fetales tempranas en vacas sujetas a protocoles basados en GnRH por FTAI (Colazo et al., 2015). También se ha determinado que en presencia de un folículo dominante de 10 o más milímetros, folículos de 7 a 9 milímetros pueden ovular sin o con el folículo dominante pudiendo dar a lugar a gestaciones gemelares (López-Gatius et al., 2022). Por lo tanto, cobra más importancia el hecho de determinar el tamaño de los folículos durante la visita reproductiva semanal.

La elevación de las concentraciones de LH en la predesviación, junto con el incremento de FSH, puede ser importante a la hora de estimular cambios que son necesarios para permitir al folículo el cambio de folículo subordinado a dominante (Lopez et al, 2005b). La duración del proestro es influenciada por el tamaño del folículo preovulatorio al principio del proestro: folículos de menor tamaño, proestro más largo (Sirois y Fortune, 1988). Finalmente, hay que destacar que las peores tasas de preñez son de vacas que han doble ovulado de forma unilateral (López-Gatius et al., 2005) y que el fallo de fertilización o la pérdida embrionaria temprana, o las dos, tienen más relación con ovulaciones múltiples unilaterales que bilaterales (Hanrahan, 1983).

2. Estrategias de reducción de partos gemelares

La incidencia de los partos dobles en explotaciones de vacas lecheras altamente productoras no es una tasa fija y es el manejo del rebaño que da su control (Kinsel et al., 1998). Al igual que en las ovulaciones múltiples hay autores que sostienen que existe una correlación positiva entre los partos gemelares y la producción lechera (Vinet et al., 2012) y otros que producen menos leche (Andreu-Vázquez et al., 2012b; Kusaka et al., 2017). La literatura científica sí que está de acuerdo en que los partos gemelares producen mayores complicaciones clínicas que los partos simples. Es importante diferenciar las gestaciones gemelares de los partos gemelares ya que la incidencia del primero es mayor (Andreu-Vázquez et al., 2012; Silva del Río et al., 2007) y esto provoca pérdidas económicas importantes que, a menudo, no se contabilizan. La concepción de gemelos monocigóticos apenas tiene impacto ya que solo representa un 4% (Silva del Río et al., 2006). Por lo tanto, las pérdidas embrionarias de las gestaciones dobles son, también, de interés para la reducción de su incidencia.

Se han descrito varias estrategias para la reducción de partos dobles a las que se suman a las ya descritas en la Introducción general, centradas básicamente con el manejo hormonal. Por ejemplo: el protocolo de 5 días de progesterona más eCG reduce los partos dobles en animales cíclicos en comparación al de 9 días (PRID-9) (Garcia-Ispierto y 2014); la inducción de la luteolisis en uno de los dos CL provoca reducción de gemelos espontánea (López-Gatius y Hunter, 2016); la administración de GnRH en el momento del diagnóstico de gestación en vacas gestantes de gemelos unilaterales con un CL reduce las dobles gestaciones (Garcia-Ispierto y López-Gatius, 2018); tratar con PRID en el momento del diagnóstico de gestación no incrementa la tasa de gestación gemelar, al igual que aquellas vacas que conciben con estro natural (Andreu-Vázquez et al., 2012b). Además, recientemente, se ha descrito que la reducción del gemelo macho en el periodo tardío embrionario no afecta al normal desarrollo de las gónadas y genitales del gemelo hembra evitando el freemartinismo en vacas lecheras de alta producción (Garcia-Ispierto et al., 2022), un problema que también tiene un impacto económico que no se debe menospreciar. La transferencia de embriones de manera rutinaria en vacas multíparas resulta una buena estrategia de reducción de partos gemelares porque se evita la fertilización de dos óvulos y, además, la técnica no afecta negativamente a los índices de fertilidad y aumenta la tasa de preñez en vacas repetidoras (López-Gatius et al., 2022). Son muchas las estrategias que se describen para reducir los partos dobles pero lo más importante a nivel práctico es la detección de la doble gestación ya que, a menudo, la incidencia que se cree tener no es real, pero las consecuencias económicas sí.

Las dobles ovulaciones y los partos gemelares afectan de manera muy directa en el desarrollo del posparto. De hecho, en vacas multíparas, las dobles ovulaciones son frecuentes en el periodo posparto temprano, normalmente en la segunda ovulación (Kusaka et al., 2017). Además, las complicaciones clínicas del periparto en partos gemelares son muy comunes: retenciones de placenta, distocias, nacidos muertos y freemartins (Echternkamp y Gregory, 1999; Echternkamp y Gregory, 1999b), además de aumentar la tasa de reposición (Gregory et al., 1990; Bicalho et al., 2007). Sin embargo, a lo contrario que se podría pensar, dichas complicaciones no afectan al tamaño y posición del útero en el momento de la inseminación por lo que parte de la eficacia reproductiva no se ve afectada (Palacín-Chauri y Garcia-Ispierto, 2023). De hecho, en este estudio se demostró que solo afectaba el sexo del feto y la producción láctea al tamaño y posición del útero. Por lo tanto, el uso de semen sexado en novillas puede ser una buena estrategia para aumentar la fertilidad del rebaño.

CONCLUSIONES GENERALES

Las conclusiones generales que se obtienen de la presente tesis doctoral son las siguientes:

1) En el estudio de la codominancia de folículos es importante conocer el tamaño de los folículos en el estro para predecir la probabilidad de que se produzca una ovulación múltiple.
2) La reducción gemelar al periodo embrionario tardío en gestaciones dobles de embriones de distinto sexo previene los efectos del freemartinismo y no afecta negativamente a la función reproductiva.
3) La transferencia de embriones reduce el riesgo de gestaciones dobles sin afectar a la fertilidad.
4) El uso de semen sexado en novillas y primíparas y la trasferencia de embriones de raza cárnica en vacas multíparas mejora la sostenibilidad de la granja.

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