UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
DOCTORADO EN CIENCIAS DE LA PRODUCCIÓN Y DE LA SALUD ANIMAL

EVALUACIÓN DEL ESTADO METABÓLICO EN EL ÚLTIMO TERCIO DE GESTACIÓN EN VACAS *Bos indicus* SOBRE EL REINICIO DE LA ACTIVIDAD OVÁRICA POSPARTO

TESIS
QUE PARA OPTAR POR EL GRADO DE:
DOCTOR EN CIENCIAS DE LA PRODUCCIÓN Y DE LA SALUD ANIMAL

PRESENTA:
RAMIRO FERNANDO DIAZ BOLAÑOS

TUTOR PRINCIPAL

CARLOS S. GALINA HIDALGO. FMVZ – UNAM

COMITÉ TUTORAL:

LUIS A. ZARCO QUINTERO. FMVZ – UNAM

TATIANA FIORDELISIO COLL. F. CIENCIAS – UNAM

CIUDAD UNIVERSITARIA CD. MX. ENERO 2018

DEDICATORIAS

A mi esposa, que siempre ha estado a mi lado.

A mis hijas, también participes de este esfuerzo.

A mis padres, que a la distancia son siempre un pilar importante de apoyo.

A mis hermanos, porque hicieron que la distancia sea corta.

Al resto de mi familia, por ser unidos y no tener igual en el mundo.

AGRADECIMIENTOS

A Dios por todo.

A la Secretaria de Educación Superior, Ciencia, Tecnología e Innovación del Ecuador – SENESCYT por la beca a profesores universitarios otorgada para realizar el doctorado.

A la Facultad de Medicina Veterinaria y Zootecnia – FMVZ de la Universidad Nacional Autónoma de México – UNAM por nuevamente acogerme como un alumno más.

A mi tutor Dr. Carlos Galina por no retirarse del mundo de la ciencia.

Al personal técnico- académico del Centro de Enseñanza, Investigación y Extensión en Ganadería Tropical-CEIEGT de la FMVZ – UNAM por su apoyo en el 1er experimento.

Al Dr. Felipe Montiel Palacios, por su ayuda en la realización del 2do experimento en Veracruz.

Al personal técnico-académico del Colegio de Posgraduados campus Tabasco y al personal de apoyo en campo del centro experimental “Km 21” Cárdenas – Tabasco por su excelente acogida durante la realización del 3er experimento.

Al Dr. Agustín Orihuela por su invalorable ayuda en la publicación del primer artículo.

A todas las personas que de una u otra manera participaron y ayudaron en la finalización de los objetivos de esta tesis.

RESUMEN

Se realizaron cuatro experimentos para evaluar el efecto del perfil metabólico durante el último tercio de la gestación y el posparto temprano sobre el reinicio de la actividad ovárica y fertilidad. El primer experimento se diseñó para observar el efecto de tres sistemas de separación de becerros (destete temporal, amamantamiento restringido y amamantamiento continuo) y el perfil metabólico durante el último tercio de gestación y el posparto sobre el reinicio de la actividad ovárica. Se midieron la grasa dorsal (GD), metabolitos sanguíneos como glucosa, triglicéridos, urea y ácidos grasos no esterificados (NEFA´s). Se sincronizó a todos los animales con un dispositivo intra-vaginal liberador de progesterona (CIDR) a los 30 días postparto (dpp) y se retiró 9 días después. La GD se incrementó durante el último tercio de gestación y disminuyó después del parto en todos los animales y no existieron diferencias en la proporción de animales que iniciaron actividad ovárica, ni en los niveles de metabolitos sanguíneos entre los grupos. El segundo experimento tuvo como objetivo probar la hipótesis que los animales que mantengan una reserva estable de grasa durante el peri-parto tienen una mejor oportunidad de gestarse. Se midió la GD de forma similar al anterior experimento y se evaluó el inicio del programa reproductivo <30dpp y ≥30dpp colocándoles un CIDR por 9 días. Se analizó en cada grupo a los animales gestantes al primer servicio (1s), los gestantes después del primer servicio, los no gestantes y los anéstricos. Los animales 1s del grupo <30dpp mantuvieron estable su grasa dorsal y se gestaron con la misma rapidez que los 1s que perdieron GD después del parto e iniciaron su programa reproductivo más tarde (≥30dpp). En el tercer experimento se midió la influencia del índice de temperatura – humedad (ITH) y la variación de la GD sobre la fertilidad y la velocidad de gestación, calculada por los días a riesgo de quedar gestantes (DRC). Este experimento se realizó en dos fincas en dos países distintos: Veracruz – México y Puntarenas – Costa Rica. Los animales de Veracruz soportaron un ITH >74 durante el 57% del tiempo que duró el experimento. Mientras que en Puntarenas lo soportaron el 100% del tiempo. La GD disminuyó tanto en el último tercio de gestación como durante el posparto en ambas fincas. Los días a riesgo de concepción (DRC) se calcularon en base al intervalo parto concepción y los días en que los animales estuvieron con el toro. Los niveles de ITH tienen una relación significativa (R^2= 0.6; P=0.033) sobre la velocidad de gestación. Los animales que perdieron más GD disminuyen el tiempo requerido para estar en riesgo de quedar gestantes. En el cuarto experimento se evaluó el efecto del ITH y la variación del GD sobre el reinicio de la actividad ovárica y el comportamiento reproductivo. Durante este experimento los animales soportaron casi el 60% del tiempo que duró la investigación un ITH >74. Se detectó que entre los meses de marzo y mayo los animales no experimentaron en ningún momento niveles de ITH <74. En este experimento los animales no presentaban una buena condición corporal, por lo que solo aquellos que lograron movilizar grasas después del parto presentaron ciclicidad. Mientras que aquellos que experimentaron un ITH elevado (>74) durante el último tercio de gestación fallaron en ciclar y en presentar algún comportamiento reproductivo (anestro). Los estudios realizados demuestran la importancia que tiene preservar las reservas energéticas durante el último tercio de gestación, ya que después del parto la movilización de grasas será necesaria para poder enfrentar el desbalance energético posparto. Si esta variación no es muy drástica podría no necesitarse realizar un destete temprano de los animales para iniciar la actividad ovárica, pero si debe ser lo suficiente para estimular el reinicio de ciclicidad. A su vez, se debe tomar en cuenta el clima medioambiental existente mientras en animal está en su último tercio de gestación ya que éste puede tener un efecto que retrase el reinicio de la actividad ovárica posparto.

ABSTRACT

Four experiments were conducted to evaluate the effect of the metabolic profile during the last third of gestation and early postpartum on the resumption of ovarian activity and fertility. The first experiment was designed to observe the effect of three systems of calf separation (temporary weaning, restricted suckling and continuous suckling) and the metabolic profile during the last third of gestation and the postpartum period on the resumption of ovarian activity. Backfat thickness (FAT), blood metabolites such as glucose, triglycerides, urea and non-esterified fatty acids (NEFA's) were measured. All animals were synchronized with insertion of a CIDR at 30 days post-partum (dpp), and its withdrawal 9 days later. FAT increased during the last third of gestation and declined after parturition in all animals and there was no difference in the proportion of animals that started ovarian activity or in blood metabolite levels between groups. The second experiment aimed to test the hypothesis that animals maintaining a stable reserve of fat during the peripartum have a better opportunity to become pregnant. The FAT was measured similarly to the previous experiment and the postpartum onset of the reproductive program <30dpp and ≥30dpp was evaluated by placing the CIDR for 9 days. In each group, animals were analyzed for pregnancy at first service, first service (1s), later than the first service (>1s), non-pregnant and anestrous. Those animals 1s in the <30dpp group kept their dorsal fat stable and became pregnant with the same speed as those who lost GD after birth and started their reproductive program later (≥30dpp). In the third experiment the influence of the temperature - humidity index (THI) and the variation of the GD on the conception rate, calculated by the days at risk of becoming pregnant (DRC), and the fertility were measured. This experiment was conducted in two farms in two different countries: Veracruz - Mexico, and Puntarenas - Costa Rica. In Veracruz, animals endured a THI> 74 with a 57 % of the experiment period. While in Puntarenas they endured this THI 100% of the time. FAT decreased both in the last third of gestation and during the postpartum period in both farms. The DRC were calculated based on the interval from parturition to conception and the days in which the animals were joined with the bull. The levels of THI had a significant relation to time to conception. Those animals that lost more FAT decreases the time required to be at risk of becoming pregnant. The fourth experiment evaluated the effect of THI and the variation of FAT on the resumption of ovarian activity and reproductive behavior. During this experiment, the animals endured almost 60% of the duration of the research a THI > 74. It was found that between the months of March to May, animals did not experience at any time, levels of THI <74. In this experiment, the cows did not have a good body condition, so that only those who were able to mobilize fat after parturition showed cyclicity. While those who experienced THI high (> 74) during the last third of gestation in general failed to cycle and did not show any reproductive behavior (anestrus). These studies demonstrate the importance of preserving energy reserves during the last third of gestation since fat mobilization will be necessary to cope with postpartum effects on energy balance. If this variation is not very drastic, early weaning of the animals may not be necessary to initiate ovarian activity, but it should be sufficient to stimulate this restart of cyclicity. In turn, the environmental climate that occurs while the animal is in its last third of gestation must be taken into account, since this can have a delaying effect on the resumption of postpartum ovarian activity.

DECLARACIÓN

El autor da consentimiento a la División de Estudios de Posgrado e Investigación de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de México para que esta tesis esté disponible para cualquier intercambio bibliotecario

Ramiro Fernando Díaz Bolaños

“Somos como enanos a los hombros de
gigantes. Podemos ver más y más lejos
que ellos no por la agudeza de nuestra
vista ni por el tamaño de nuestro
cuerpo, sino porque somos levantados
por su gran altura.”

Bernardo de Chartres,
1130 AD.
(Cita erróneamente acreditada a Sir Isaac Newton)

CONTENIDO

INTRODUCCIÓN

La nutrición de los animales durante la gestación tiene efecto en diferentes procesos fisiológicos tanto en la madre como en la cría. Esto se puede observar en el nacimiento de crías con alta susceptibilidad a enfermedades no transmisibles, como afecciones cardiovasculares, resistencia a la insulina, diabetes tipo 2, obesidad y síndrome metabólico (Armitage *et al.*, 2004; McMillen *et al.*, 2005; Ojeda *et al.*, 2008). Muchas de estas alteraciones están relacionadas al estado nutricional de la madre gestante, en especial durante el último tercio de gestación. En humanos, por ejemplo, determinadas enfermedades crónicas presentes en la vida adulta pueden tener su origen antes del nacimiento (Barker, 1997). Este estado podría estar relacionado con una mala nutrición durante el último tercio de gestación de la madre (Harding, 2001). Así, Martyn *et al.* (1996), demostraron que los niños que nacieron con un bajo peso corporal pueden sufrir de fallas cardíacas relacionadas con problemas circulatorios en los vasos coronarios. Igualmente, los niveles nutricionales en diferentes etapas de la gestación tienen un efecto importante en el desarrollo de la cría. Ravelli *et al.* (1999), indican que una mala nutrición materna en fases tempranas de la gestación se relaciona con una mayor circunferencia de la cintura y una mayor masa corporal en la vida adulta de las hijas. En el caso de los animales domésticos existe evidencia que el desarrollo reproductivo de las novillas puede ser afectado por la nutrición de la madre durante las fases temprana e intermedias de la gestación. En un estudio en vacas lecheras explotadas en sistemas intensivos, Sullivan *et al.* (2009a), encontraron que una dieta con altos niveles de proteína en el segundo trimestre de la gestación reduce la sobrevivencia del folículo antral en las crías a los 23 meses de edad. Asimismo, demostraron que la elevada nutrición en las fases tempranas de la gestación tiene un efecto negativo en la producción de leche de las crías (Sullivan *et al.,* 2009b). Algunas investigaciones enfocadas al estudio del impacto nutricional durante el desarrollo prenatal han llevado a los científicos de producción animal a adoptar la opinión de que el estado nutricional durante la gestación tiene un efecto importante en el bienestar de las crías, no sólo en el nacimiento, sino también en la vida productiva del animal (Swali y Wathes, 2006). Varios estudios sugieren que los animales nacidos en la época de sequía presentan el primer parto a mayor edad que sus pares nacidos en la época de lluvias (Galina y Arthur, 1989).

Uno de los marcadores más comunes para evaluar de manera indirecta el estado nutricional en los bovinos es la condición corporal. Sin embargo, esta variable es muy difícil de evaluar, ya que en las etapas más tardías de la gestación las regiones corporales en las que tradicionalmente se valora la condición corporal están modificadas por el estado de gravidez de la hembra. Por tal razón, en trabajos pilotos se han demostrado que la medición de la grasa dorsal mediante técnicas de ultrasonografía proporciona resultados más confiables sobre el estatus nutricional de los animales (Galindo *et al.,* 2013).

Existen algunos parámetros para determinar el estado metabólico de un bovino gestante. Las mediciones de metabolitos sanguíneos como la glucosa y los ácidos grasos no esterificados (NEFA) son indicadores del metabolismo energético para diferentes estados nutricionales del animal. Corro *et al.* (1999), encontraron que los niveles de estos metabolitos en bovinos de doble propósito varían dependiendo del estado reproductivo de los animales, presentando niveles distintos durante los estados gestacionales del animal y retomando sus niveles óptimos desde el cuarto mes posparto, cuando el balance energético se ha restablecido.

La ganadería bovina de carne en el trópico se caracteriza por un marcado vinculo madre – cría que favorece el cuidado y protección del becerro promoviendo su supervivencia (Von-Keyserlingk *et al.,* 2007). Esta relación es tan estrecha que su separación puede provocar marcado estrés tanto en la madre como en la cría (Pérez-Torres *et al.,* 2016). Por ende, la presencia del becerro puede inhibir la actividad ovárica (Hoffman *et al.,* 1996). En consecuencia, las separaciones temporales de la madre son comunes debido a que reducen el periodo de anestro posparto en vacas (Das *et al.,* 2000). Por tal razón, se han desarrollado métodos que reduzcan o eviten el amamantamiento y el contacto físico entre madre y cría (Galina *et al.,* 2001).

Sin embargo, cada vez están más presentes en nuestra sociedad las organizaciones en pro del bienestar animal que demandan un mejor trato en los animales. La separación los becerros y las madres afrontan situaciones nuevas como cambio de alimentación, lugar de alojamiento y estructuras sociales. Estos cambios son considerados como eventos estresantes para cualquier animal, repercutiendo de manera negativa en la conducta y bienestar de madres y crías (Pérez-Torres *et al*., 2016) afectando la productividad de los animales. El impacto negativo que causa la separación varía de acuerdo con la edad del becerro y a la duración de la separación (Marquezini *et al.,* 2013).

El reinicio de la actividad ovárica en ganado Cebú es uno de los factores que determinan la duración del intervalo entre partos en un rango de 12 a 14 meses. Sin embargo, el efecto del amamantamiento tiene influencia directa sobre el reinicio de la actividad ovárica (Williams, 1990); no obstante que el recambio folicular parece comenzar poco después del parto (Rubio *et* *al.,* 2010). A pesar de que la especie *Bos indicus* manifiesta un anestro posparto más prolongado que la *Bos taurus*, existen varios informes que presentan casos en que los animales muestran signos de estro y ovulación antes de los 60 días posparto (dpp; Molina-Sánchez *et al.*, 2003; Henao *et al*., 2008; Galindo *et al.*, 2013). Así, Henao *et al.* (2001), reportan intervalos entre el parto y el primer estro de 20 a 30 días en vacas Gyr. De hecho, a partir de la primera o segunda semana postparto, se desarrollan ondas foliculares con presencia de folículos dominantes capaces de ovular o sufrir atresia y para el día 45 postparto se han registrado ovulaciones silenciosas y normales (Henao, 2001; Pérez *et al.,* 2001). La segunda ovulación generalmente es precedida por signos normales de estro y marca el final del anestro postparto (Henao *et al.,* 2000; 2001).

Los trabajos realizados en el trópico mexicano por Pérez-Torres *et al.* (2015; 2016), encontraron que el beneficio que se obtiene cuando se separa la cría de la madre acompañado de un tratamiento a base de un dispositivo intra-vaginal liberador de progesterona (CIDR) no justifica el estrés que estos sufren por el destete, pues no encontraron diferencia significativa en el reinicio de la actividad ovárica posparto en separaciones madre - cría por 24, 48 o 72 horas a los días 25 y 45 postparto comparado con un grupo testigo que solamente fue tratado con CIDR. Por el contrario, los animales al ser separados de su madre en el día 25 posparto sufren una pérdida de peso mayor en las separaciones de 24 y 48 horas comparada con aquellos cuya separación fue de 72 horas. En los mismos trabajos se encontró que existe una variación del consumo de leche en los becerros, así como en niveles de cortisol tanto de las madres como de las crías. Estos niveles varían dependiendo del tiempo de separación y del día postparto donde se realizó, demostrando que aún en separaciones con diferencias de 24h existen diversos grados de estrés. Estos resultados son apoyados por evidencia indirecta de hallazgos de este grupo de trabajo realizados en Costa Rica y México que demuestran que el reinicio de la actividad ovárica postparto puede ser tan temprana en el grupo suplementado como en el grupo testigo aún en novillas de primer parto (Soto *et al.,* 2001; Maquívar *et al.,* 2010).

Por otro lado, el efecto climático sobre los procesos productivos y reproductivos en bovinos ha sido objeto de mucho estudio en los últimos años. El estrés calórico es un factor que tiene varios efectos sobre la fisiología reproductiva como la expresión de celo, la tasa de ovulación y la sobrevivencia del embrión (Hansen, 2007; Silva *et al.*, 2013). Igualmente, Villa-Mancera *et al.* (2011), reportan un efecto directo del Índice de Temperatura-Humedad (ITH) sobre la tasa de concepción, obteniendo niveles más bajos en los meses de verano que en los de invierno. De esta manera es posible que las condiciones climáticas en el trópico sean también un factor predominante en el desarrollo reproductivo de los animales durante el posparto.

Por tal razón los objetivos de los trabajos que se presentan en esta tesis son: Valorar el efecto del perfil metabólico durante el último tercio de la gestación y el postparto temprano sobre el inicio de la actividad ovárica en vacas bajo un tratamiento de progesterona y con diferentes métodos de separación de la cría. Evaluar el efecto de los niveles de grasa dorsal durante el último tercio de la gestación y el posparto sobre el reinicio de la actividad ovárica y la fertilidad en vacas *Bos indicus* tratadas con un progestágeno intravaginal antes y después de los 30 días posparto. Medir la influencia del clima, valorado por el ITH, y la variación de la grasa dorsal durante el último tercio de gestación y posparto temprano sobre la fertilidad en vacas. Y, evaluar el efecto del índice de temperatura – humedad sobre el reinicio de la actividad ovárica y comportamiento reproductivo de ganado en condiciones tropicales.

CAPITULO 1

REVISIÓN DE LITERATURA

FISIOLOGÍA POSPARTO

Reinicio de la actividad ovárica posparto

El parto provoca la eliminación de los efectos de retroalimentación negativa que ejercen sobre la hormona liberadora de gonadotropinas (GnRH) el estradiol y la progesterona, permitiendo así, el reinicio de la síntesis y secreción de gonadotropinas. La hormona folículo estimulante (FSH) se libera en la circulación periférica y sus concentraciones incrementan dentro de los 3 a 5 días posparto para mantenerse a intervalos de 7 a 10 días (Crowe *et al.,* 1998). El primero de estos incrementos estimula la primera oleada folicular que generalmente produce un folículo dominante entre los 7 a 10 dpp (Murphy *et al.,* 1990; Crowe *et al.,* 1993). A su vez, la hormona luteinizante (LH) se sintetiza y se almacena de forma más lenta y toma entre 2 a 3 semanas en completar su almacenamiento; por lo tanto, son bajas tanto su concentración plasmática como su frecuencia de pulsos (Griffith y Williams, 1996). La liberación pulsátil de la LH incrementa entre los 10 y 20 días posparto provocando el desarrollo de un folículo dominante. Esto permite el aumento del nivel de estradiol que induce un pico de LH. La capacidad del folículo de liberar estradiol es, a su vez, dependiente de la frecuencia de los pulsos de LH durante la fase de dominancia y de la biodisponibilidad del factor de crecimiento parecido a la insulina tipo I (IGF-I; Austin *et al*., 2001; Canty *et al*., 2006). Por lo tanto, el principal factor impulsor de la ovulación de un folículo dominante durante el período posparto es la frecuencia del pulso GnRH/LH.

La frecuencia de pulsos de LH para provocar la ovulación de un folículo dominante es de 1 por hora. En bovinos productores de carne, el primer folículo dominante y sucesivamente algunos folículos dominantes fallan en ovular y sufren atresia (Murphy *et al.,* 1990, Stagg *et al.,* 1995). En bovinos productores de carne con buena condición corporal, el primer folículo en ovular (aproximadamente a los 30 dpp) es de la oleada 3.2 ± 0.2 en promedio (Murphy *et al.,* 1990). En cambio, en aquellas con baja condición corporal, su ovulación es en la oleada 10.6 ± 1.2 (de entre 70 a 100 dpp; Stagg *et al.,* 1995).

La primera ovulación generalmente se presenta sin la expresión de conducta de estro (Kyle *et al.,* 1992) y en un alto porcentaje (>70%) es seguida por un ciclo corto, que normalmente presenta una oleada folicular. A su vez, esta primera fase lútea es de corta duración debido a la prematura liberación de prostaglandina F2α (PGF2α; Peter *et al.,* 1989) posiblemente provocada por el incremento de estradiol secretado por el folículo dominante post-ovulatorio en los días 5 a 8 del ciclo, provocando la formación temprana de receptores de estradiol y oxitocina (Zollers *et al.,* 1993). Así, el cuerpo lúteo (CL), que secreta bajas cantidades de progesterona, se lisa alrededor de los días 9 a 11 después de la primera ovulación. La segunda ovulación es generalmente asociada con la expresión de estro y seguida de una fase lútea de duración normal generando concentraciones normales de progesterona (Crowe *et al.,* 1998).

La presencia de la cría y sus efectos sobre la liberación de GnRH/LH

Aunque el crecimiento folicular en ganado de carne inicia poco tiempo después del parto (entre 10 y 15 días después del parto; Kamimura *et al.,* 1994; Stagg *et al.,* 1995) solo un bajo porcentaje llegan a ovular (11%; Savio *et al.,* 1990). Este aparente fallo en la ovulación de los primeros folículos dominantes que se desarrollan después del parto está estrechamente relacionado a niveles bajos de GnRH y LH, por lo que la aplicación de un análogo de GnRH provoca la ovulación de estos folículos. Este mismo efecto se obtiene con la separación o destete de la cría. Es frecuente, en el manejo de la ganadería en el trópico, separar a la cría de la madre para estimular la liberación de LH incrementando sus niveles sanguíneos, provocando un pico preovulatorio y desencadenando la ovulación (Gazal *et al.,* 1998; Yavas y Walton, 2000).

Efectivamente, la presencia constante de la cría provoca una disminución de la liberación de GnRH y LH prolongando el anestro posparto. Este mecanismo probablemente esté mediado por el incremento sanguíneo de opioides (Myers *et al.,* 1989). Se ha observado que en muchas especies los péptidos opioides endógenos funcionan como neurotransmisores en el hipotálamo, actuando directa o indirectamente en las neuronas productoras de GnRH para disminuir su secreción y por lo tanto reducir la liberación hipofisiaria de LH (Brooks *et al.,* 1986; Haynes *et al.,* 1989; Barb *et al.,* 1991; Cosgrove *et al.,* 1993). Tratamientos con antagonistas de opioides como la Naloxona, al inicio del periodo posparto incrementan la frecuencia de pulsos de LH (Ahmadzadeh *et al.,* 1998) y disminuye la duración del anestro posparto (Stevenson *et al.,* 1997). A su vez, la separación del becerro provoca una disminución de β- endorfinas en el hipotálamo (Connor *et al.,* 1990), y se ha reportado una correlación negativa entre los niveles de β-endorfinas y las de LH durante el periparto y una correlación positiva con el intervalo posparto (Osawa *et al.,* 1998). A este respecto, Boukhliq *et al.* (1999), indican que un subgrupo de neuronas que secretan GnRH en el área hipotalámica media-basal (MBH) son responsables del incremento de la secreción episódica de LH inducida por un antagonista de opioides en ovejas.

La presencia del becerro y el amamantamiento también pueden hacer su efecto mediante el aumento de la sensibilización del hipotálamo a la retroalimentación negativa del estradiol (E_2;

García-Winder *et al.,* 1984; Zalesky *et al.,* 1990) resultando en una baja secreción de LH. La retroalimentación negativa ejercida por el E_2 tiene como uno de sus principales sitios de acción el núcleo Arcuato (ARC) del hipotálamo. Este es uno de los sitios donde se concentran las neuronas secretoras de GnRH y al no presentar receptores para E_2 reciben señales de un mediador que actualmente es considerado uno de los principales pilares en el control de la secreción de la GnRH, y por consiguiente de la reproducción, la Kispeptina (Smith *et al.,* 2005a; Smith *et al.,* 2005b). Así, las neuronas con el gen *Kiss1*, que codifica a la kispeptina, en el ARC pueden ser conductos para recibir la señalización del E_2 desde las gónadas y transmitir esa información a las neuronas GnRH (Smith *et al.,* 2005a). Sin embargo, las neuronas *Kiss1* en el ARC también expresan varios co-transmisores, que pueden tener funciones importantes e independientes. En especies como la oveja, las neuronas *Kiss1* co-expresan Dinorfina (DYN) y Neurokinina B (Goodman *et al.,* 2007), y ambos han sido implicados en la regulación de la secreción de LH. Así, la DYN actúa a través de receptores para opioides-κ inhibiendo la liberación de LH (Navarro *et al.,* 2009; Nakahara *et al.,* 2013). Por esta razón, se podría pensar que los mecanismos de inhibición de la reproducción mediados por los opioides y por la retroalimentación negativa del E_2 que ejerce la presencia del becerro y el amamantamiento pueden actuar de forma conjunta.

Por otro lado, el amamantamiento provoca un incremento sérico de oxitocina en la madre (Silveira *et al.,* 1993; Tancin *et al.,* 2001). La secreción de la oxitocina es en forma pulsátil ya que las células mioepiteliales de la glándula mamaria se desensibilizan ante su exposición continua (para revisión ver Leng *et al.,* 2005). Su estímulo y secreción en la neurohipófisis también es modulado por acciones autocrinas de factores co-liberados con la oxitocina, como son la dinorfina (véase Brown *et al.,* 2000) y el ATP con su metabolito, adenosina (Noguchi y Yamashita, 2000; Wang *et al.,* 2002). La dinorfina y la adenosina son inhibidores de la secreción de oxitocina (para revisión ver Leng *et al.,* 2005) pero su interacción durante el amamantamiento aún no está clara. La oxitocina es necesaria para la eyección de la leche desde los alveolos en la glándula mamaria. La separación de la cría puede provocar una disminución de los niveles sanguíneos de oxitocina y por ende una disminución de la producción de leche (Negrão, 2008). Por otro lado, el incremento de oxitocina en la sangre puede promover la síntesis y secreción de PGF2α, que estimula por retroalimentación positiva la producción de oxitocina, generándose así un incremento pulsátil de ambas hormonas. Como resultado, en una vaca que se encuentre ciclando, se producirá luteólisis ya que la PGF2α es la hormona con propiedades luteolíticas más potente (Niswender *et al.,* 2000), dando como resultado la imposibilidad de mantener una gestación. Al destetar a la cría se produce una disminución de la oxitocina (Negrão, 2008) evitando que se produzca este efecto.

METABOLISMO Y REPRODUCCIÓN

Estado nutricional de los animales y su efecto sobre la reproducción

Una adecuada nutrición en los animales produce un estado metabólico adecuado para sobrellevar los diferentes eventos reproductivos que sucederán durante el posparto. Este estado nutricional tiene un efecto directo sobre los niveles de hormonas reproductivas, folículo ovárico, función del cuerpo lúteo (CL), desarrollo del ovocito y fertilidad (Boland *et al.,* 2001; Armstrong *et al.,* 2001, 2003; Da Silva *et al.,* 2002). Un animal, cuando entra a un estado de balance energético negativo, debe utilizar sus propias reservas corporales en un esfuerzo para revertir el déficit energético. Esto da como resultado efectos negativos tanto a nivel hipotálamo-hipofisiario como en el sistema reproductivo (Scaramuzzi *et al.,* 2006).

Mientras que la FSH y la LH son críticas para el crecimiento folicular, ovulación y luteinización, sus acciones son dependientes de otras vías de señalización, incluidas hormonas metabólicas como insulina (Richards *et al.,* 2002) y leptina (Ryan *et al*., 2002; Hamm *et al.,* 2004). Así, por ejemplo, en ovejas con baja condición corporal se presentan niveles de LH disminuidos (Tatman *et al.,* 1990). Además, en ovejas desnutridas se presenta una supresión de la expresión del ARNm de LH (Kile *et al.,* 1991). Mientras que a largo plazo la restricción de alimento causa anestro debido a niveles insuficientes LH (Rhodes *et al.,* 1996), a corto plazo sus efectos provocan una disminución del pico preovulatorio de LH (Kiyma *et al.,* 2004). Por el contrario, en novillas crónicamente desnutridas las concentraciones séricas de FSH fueron mayores (Rhodes *et al.,* 1996; Mackey *et al.,* 2000). Este comportamiento distinto entre los niveles de LH y FSH podría deberse a sus diferencias en su almacenamiento y secreción. La LH es sintetizada y almacenada en la adenohipófisis en cantidades mayores que la FSH y espera la señal de GnRH para ser liberada en forma pulsátil. En cambio, la FSH, aunque sintetizada en menores cantidades, es secretada constantemente (Farnworth, 1995; McNeilly *et al*., 1995; Bernard *et al.,* 2010).

Por otro lado, el ayuno puede provocar que los niveles de estradiol sean bajos (Adams *et al.,* 1997; Kiyma *et al.,* 2004). La disminución de los niveles de E_2 después de una restricción alimenticia en corto plazo puede deberse a una disminución del desarrollo folicular ovárico causado por la supresión de las concentraciones séricas de gonadotropina (Gougeon, 1996). Adicionalmente, Adams *et al.* (1994), reportaron que las ovejas desnutridas no sólo exhibían menos secreción de E_2, sino una disminución simultánea de la tasa de depuración metabólica de esta hormona, lo que puede explicar la concentración periférica más baja observada en los animales con restricciones nutricionales (Adams *et al.,* 1994, 1997).

Se ha reportado una relación directa entre el nivel nutricional y las concentraciones de progesterona (P_4) en ovejas y novillas. Así, los animales a los que se les sobrealimentó presentaron mayores niveles de P_4 que los animales control o con desnutrición (Kaminski *et al.,* 2015). Sin embargo, también se ha demostrado una relación inversa entre el estado nutricional y la P_4 con una disminución de la nutrición que resulta en un aumento de las concentraciones de P_4 en las ovejas (Kiyma *et al.,* 2004), cerdas (Miller *et al.,* 1999) y vacas (Rabiee *et al.,* 2001, Ferraretto *et al.,* 2014). Este comportamiento se debe posiblemente a la tasa metabólica de P_4 y su depuración (Kiyma *et al.,* 2004; Smith *et al*., 2006). Existe evidencia que sugiere que los animales que reciben una nutrición inadecuada pueden tener un catabolismo prolongado de P_4 (Kiyma *et al.,* 2004). Cambios nutricionales alteran el metabolismo de los esteroides provocando una demora en la recuperación de las reservas corporales (Smith *et al.,* 2006). En vacas, al inicio del catabolismo del tejido adiposo se libera P_4 almacenada incrementando sus niveles sanguíneos. Es decir que animales que pierden peso y movilizan grasas pueden incrementar la P_4 periférica liberada de la grasa (Hamudikuwanda *et al.,* 1996). Cabe señalar que el aumento de los niveles de P_4 en animales subalimentados no es por un incremento de su síntesis en glándulas adrenales ya que la concentración del cortisol no se altera (Kiyma *et al.,* 2004), sino, posiblemente, por su liberación desde el tejido lipídico por la movilización de sus reservas de grasa. El sobrealimentar a un animal también puede tener efectos en el metabolismo de las hormonas reproductivas y, por consiguiente, en la reproducción. Parr (1992), demostró que las ovejas sobrealimentadas presentaban concentraciones de P_4 más bajas que el grupo control y se atribuyó a un cambio en la tasa de depuración de la P_4 y no a la disminución en la secreción de esta hormona. En bovinos, el incremento de la alimentación en materia seca (DMI), provocó un incremento en el flujo sanguíneo hacia el hígado, lo que se asoció con un mayor metabolismo de P_4 y E_2 (Sangsritavong *et al.,* 2002) disminuyendo sus concentraciones periféricas.

Efecto del estado nutricional sobre la función ovárica

Los efectos del estado nutricional sobre la reproducción no solo se centran en las alteraciones que provoca en el eje hipotálamo-hipófisis, sino también en los órganos reproductivos. Así, los cambios en las hormonas metabólicas provocados por alteraciones en la nutrición de los animales tienen efectos directos sobre el ovario (Parr, 1992; Gong *et al.,* 2002; Viñoles *et al.,* 2010). De hecho, se ha observado que la nutrición tiene efectos directos sobre la foliculogénesis, calidad del ovocito, tasas de ovulación, función lútea y fertilidad en varias especies de animales (Borowczyk *et al.,* 2006; Grazul-Bilska *et al.,* 2012).

Algunos estudios han demostrado que el aumento de la energía y/o proteína en la dieta resulta en un mayor número de folículos reclutados (Xu *et al.,* 1989; Abecia *et al.,* 2006). Por el contrario, otros investigadores han observado que la sobrealimentación y la subalimentación no presentan diferencias en el número de folículos antrales pequeños y grandes comparado con los grupos control (Abecia *et al.,* 1995, 1997; Borowczyk *et al.,* 2006). Sin embargo, la restricción de nutrientes se ha asociado con la reducción de la ovulación en ovejas tratadas y no tratadas con FSH (Lassoued *et al.,* 2004). También un bajo consumo de nutrientes de tres a seis días antes de la ovulación se ha asociado con una reducción del tamaño del folículo dominante (Murphy *et al.,* 1991) y de las ovulaciones para novillas tratadas con prostaglandina (Mackey *et al.,* 1999). Igualmente, hay evidencia donde se indica que existen momentos específicos durante la vida de la oveja madura en donde los efectos nutricionales son más críticos. Por ejemplo, si la restricción de nutrientes ocurre cuando los folículos están pasando la fase primordial e inician su crecimiento, lo cual sucede 6 meses antes de la temporada de empadre. Esto resulta en un reducido número de folículos ováricos y, por lo tanto, escasas ovulaciones al momento de la monta (Robinson *et al.,* 2006).

Efectos del estado nutricional sobre la calidad del ovocito

Los efectos del consumo de nutrientes sobre la calidad del ovocito son con frecuencia contradictorios sin observar efectos positivos o negativos en rumiantes y otras especies (Boland *et al.,* 2001; Lozano *et al.,* 2003; Peura *et al.,* 2003; Grazul-Bilska *et al.,* 2012). Se ha observado que una alimentación con alto grado nutricional puede mejorar la calidad del ovocito en ovulaciones espontáneas en ovejas. Lo contrario se observó en animales tratados con FSH, que tienen una tasa de ovulación más baja comparada con animales a los que se les administró dietas en base de harinas para mantenimiento de energía (Yaakub *et al.,* 1997). La sobrealimentación en ovejas tratadas con FSH resultó en efectos negativos sobre la calidad del ovocito determinados por la tasa de división *in vitro* (Lozano *et al.,* 2003; Grazul-Bilska *et al.,* 2012). Aunque las razones por las que se presentan estos cambios no se comprenden bien, se ha postulado que un exceso de componentes degradables del rumen causa concentraciones elevadas de amoníaco en el líquido folicular, lo que causa un ambiente perjudicial para el ovocito (Rooke *et al.,* 2004). Yaakub *et al.* (1997), reportaron un desprendimiento de los gránulos de cromatina del núcleo en ovocitos de los animales no estimulados con FSH esto explicaría la baja tasa de división celular. Algunos autores han reportado que la subalimentación tiene un impacto negativo en la calidad de los ovocitos en ovejas superovuladas observándose bajos niveles de división en las células (Yaakub *et al.,* 1997; Borowczyk *et al.,* 2006; Grazul-Bilska *et al.,* 2012). En vacas lecheras se ha observado que altas concentraciones de ácidos grasos no esterificados (NEFA´s), los cuales son elevados en animales con bajo estado nutricional, reducen la proliferación de las células de la granulosa y retrasan la maduración de los ovocitos (Jorritsma *et al.,* 2004). Por el contrario, otros trabajos de investigación han observado que una buena proporción de ovocitos se consideraron viables en ovejas desnutridas tratadas con FSH cuando se compararon con ovejas sobrealimentadas (Lozano *et al.,* 2003). Estas diferencias en los resultados reportados podrían deberse a las diferentes especies y razas de animales utilizados y a la duración de los tratamientos nutricionales.

El exceso o reducción del consumo de energía resulta en cambios del contenido de lípidos endógenos en el ovocito (Metwally *et al.,* 2007; Robker *et al.,* 2011). Los ovocitos de los mamíferos tienen una reserva de lípidos que, aunque varía entre especies y aún no está bien comprendida su función, parece ser vital para el desarrollo embrionario temprano (Dunning y Robker, 2012). Las gotas de lípidos están compuestas por un núcleo lipídico neutro encerrado por una capa de fosfolípidos y, además, existe una capa externa que regula su tamaño (Dunning y Robker, 2012). Se cree que la acumulación de lípidos dentro del ovocito proporciona energía de sostén a un embrión listo para implantarse (Ferguson y Leese, 2006). Las reservas de triglicéridos disminuyen durante la maduración de los ovocitos *in vitro* en vacas (Ferguson y Leese, 1999) y en cerdas (Sturmey y Leese, 2003). Por lo tanto, se ha postulado que una mayor reserva citoplasmática de lípidos podría ser ventajosa para el ovocito a medida que se somete a procesos de alta exigencia energética como la fertilización y división celular (Castaneda *et al.,* 2013). Por otro lado, un exceso de las concentraciones intracelulares de lípidos conduce a cantidades elevadas de ácidos grasos libres, los cuales están sujetos a daño oxidativo y a la acción de peroxidasas lipídicas que, en última instancia, provocarían una alteración en el retículo endoplasmático (RE) y la mitocondria (Borradaile *et al.,* 2006). Los daños en RE por exposición a altas concentraciones de ácidos grasos libres causan cambios en sus funciones y conducen a la producción y acumulación de proteínas libres (Diakogiannaki *et al.,* 2008). Esto activaría un incremento del metabolismo proteico produciendo una degradación proteínica y disminución de la función celular (Rutkowski *et al.,* 2004) y si no se corrige, puede conducir a apoptosis celular (Kaufman *et al.,* 1999). Igualmente, los estudios realizados en ratones hembras obesas han demostrado que la obesidad induce un desarrollo más lento del blastocisto y una proporción alterada del trofectodermo y de la masa celular interna (Minge *et al.,* 2008). Estos ovocitos también presentan una mayor concentración de lípidos, alteraciones en la función mitocondrial, y muestran signos de estrés en el RE (Chan *et al.,* 2015). Sin embargo, no está claro si los efectos nutricionales sobre los ovocitos ocurren durante la foliculogénesis o es provocado inmediatamente después de la concepción (Dunning y Robker, 2012).

Efectos sobre el desarrollo temprano del embrión

Los niveles de fertilización y desarrollo embrionario temprano se ven afectados por el estado nutricional de la madre. Posiblemente estos efectos son los causantes de la pérdida temprana de las gestaciones o alteraciones en el crecimiento temprano del embrión en diferentes especies (Han *et al.,* 2008; Long *et al.,* 2009). Se ha observado una reducción de ovocitos fertilizados y un desarrollo reducido del paso entre mórula a blastocisto después de un programa de fertilización *in vitro* en ovinos con restricción nutricional (Abecia *et al.,* 1995; Borowczyk *et al.,* 2006; Grazul-Bilska *et al.,* 2012). Además, se ha evidenciado que la desnutrición produce una disminución del desarrollo embrionario durante las primeras dos semanas después de la fertilización *in vitro* en ovejas (Abecia *et al.,* 1997).

La sobrealimentación también provoca una disminución en la división celular después de la fertilización *in vitro* en ovejas (Borowczyk *et al.,* 2006; Zhu *et al.,* 2010). McEvoy *et al.* (1995) informaron que la alimentación excesiva en ovejas superovuladas resultó en un número reducido de embriones que se desarrollaron a la etapa de blastocisto. Además, Abecia *et al.* (2006) plantearon la hipótesis de que "la sobrealimentación durante el reclutamiento folicular y la maduración del ovocito imparte un legado de retraso del desarrollo y pérdida embrionaria". Esta diversidad de resultados entre la sobre y/o la sub – alimentación de la madre en las fases tempranas del embrión son el objetivo de más investigación.

INCREMENTO DE TEMPERATURA AMBIENTAL Y REPRODUCCIÓN

Caracterización del clima medioambiental

Analizar el entorno climático del animal es complejo, especialmente en condiciones al aire libre. En tales condiciones, la temperatura ambiental sola no puede ser considerada una medida representativa del ambiente térmico que rodea al animal; la humedad relativa, la radiación solar y la velocidad del viento también deben ser tomados en cuenta. Por tal razón, se han diseñado índices que puedan representar la influencia del intercambio de calor entre el animal y su medioambiente. El intercambio de calor puede evaluarse directamente con medidas fisiológicas (temperatura rectal, cloacal y de piel, jadeo y la producción de calor) o indirectamente del rendimiento de los animales (tasa de crecimiento, producción de leche o huevo, etc.) que están relacionadas con la capacidad o incapacidad del animal para hacer frente a cambios drásticos de la temperatura ambiental. Varios índices derivados de mediciones meteorológicas han sido desarrollados y revisados por Hahn *et al.* (2009). Estos índices van desde una simple medición de temperatura ambiental a un índice que toma en cuenta la temperatura del aire, humedad relativa, radiación solar y la velocidad del viento. Por ejemplo, la temperatura efectiva para los animales a pastoreo se calculó a partir de temperatura del aire (temperatura del bulbo seco) y de las radiaciones directas e indirectas (temperatura del globo negro; Yamamoto *et al.,* 1994) y del bulbo húmedo/seco o índice de temperatura-humedad (ITH) estimado para cerdos, aves y rumiantes (Hahn *et al.,* 2009). Estos índices climáticos tienen limitaciones porque reflejan las condiciones promedio en la instalación y no el microambiente alrededor de un animal, pero definitivamente son las mediciones más confiables que se tienen al alcance en este momento para la evaluación del efecto climático en el animal (Dikmen y Hansen, 2009).

En la práctica, las respuestas de los animales varían según la duración y la intensidad del desafío térmico. En países templados, los cambios a corto plazo en las funciones fisiológicas, de comportamiento e inmunológicas son necesarios para sobrevivir a los eventos estresantes agudos tales como las olas de calor del verano. La gravedad de estos desafíos térmicos cortos depende de la magnitud (intensidad × duración) de los episodios de ola de calor y la posibilidad de recuperación durante el período frío de la noche. En contraste, bajo condiciones tropicales y subtropicales, los animales son desafiados por el calor la mayor parte del tiempo. Las respuestas termorreguladoras a largo plazo propias a la aclimatación al calor incrementan los estándares fisiológicos, que en la mayoría de los casos van acompañados por una reducción en la productividad de los animales. Estas respuestas se producen dentro de la vida del animal e incluyen una reducción de la tasa metabólica, cambios en el sistema cardiovascular, alteración eficiente en la pérdida de calor (respuesta vasomotora: vasodilatación), cambios en la respuesta del comportamiento y en la morfología general del animal. En bovinos y en cerdos, la mayor parte de la aclimatación ocurre dentro de los 3 a 4 días después del inicio de un desafío térmico (Nienaber y Hahn, 2007; Renaudeau *et al*., 2010). Sin embargo, Renaudeau *et al*. (2010), demostraron que el tiempo para la aclimatación total, toma varias semanas y varía con la magnitud del desafío de calor que se presente. En aves de corral, se ha reportado un patrón similar de aclimatación; es decir, varios días para la respuesta vasomotora, pero varias semanas para cambios cruciales en el sistema cardiovascular (volumen plasmático, Yahav *et al*., 1997) o actividad endocrina (actividad de la glándula tiroidea; Yahav, 2009; Yahav *et al.,* 2009).

Efectos del estrés calórico en la reproducción

Efecto en el crecimiento folicular

El incremento de la temperatura ambiental puede afectar el desarrollo y función de los ovarios. Se ha observado que, en vacas lecheras, la elevada temperatura reduce el tamaño de los folículos dominantes de la primera y segunda oleada folicular (Badinga *et al.,* 1993, Wilson *et al*., 1998a, b). Esto atenúa la dominancia que se refleja en un aumento de folículos de mayor tamaño (Wolfenson *et al.,* 1995). Roth *et al.* (2000), mostraron una disminución pronunciada en la concentración plasmática de inhibina y un aumento en la concentración plasmática de FSH en asociación con un mayor número de folículos de tamaño medio. También se encontró una reducción de la concentración plasmática de inhibina en el búfalo cíclico en la India durante el verano (Palta *et al.,* 1997). Estas alteraciones podrían explicar el incremento de dobles ovulaciones y de gestaciones gemelares durante el verano (Ryan y Boland, 1991). Además, se ha reportado en vacas con estrés térmico la aparición temprana del folículo preovulatorio y la extensión de su período de dominancia (Wolfenson *et al.,* 1995). Esto puede tener una importancia fisiológica importante porque la duración de la dominancia del folículo preovulatorio está asociada negativamente con la tasa de concepción (Mihm *et al.,* 1994). El estrés calórico también afecta la cantidad de folículos antrales pequeños de aproximadamente 0.5 a 1 mm de diámetro (Wolfenson *et al*., 1995; Roth *et al.,* 2000). Lo anterior se pudo observar en vacas Gyr que en un período de 28 días de estrés calórico se redujo la viabilidad de los folículos durante 105 días (de S. Torres-Júnior *et al.,* 2008), lo que indica que las alteraciones ocurren en el estado de folículo antral pequeño. Estas observaciones revelan un retraso en el crecimiento y desarrollo folicular (Roth *et al.,* 2001a, b). Sin embargo, las etapas foliculares que son susceptibles al estrés térmico no han sido definidas con precisión.

Efecto en el medio endócrino

Las alteraciones en el desarrollo folicular provocadas por una elevada temperatura están estrechamente ligadas a cambios en el medio hormonal. Una reducción de la capacidad esteroidogénica de los folículos bajo estrés térmico se caracteriza por una menor actividad de la aromatasa de las células de la granulosa y una disminución de la concentración de estradiol en el fluido folicular del folículo dominante (Badinga *et al.,* 1993). Las concentraciones de E_2 en el líquido folicular son más bajas en verano que en invierno (Wolfenson *et al.,* 1997). Estas alteraciones pueden mantenerse hasta las estaciones siguientes reflejadas en niveles bajos de estradiol en el líquido folicular hasta finales de verano y niveles crecientes durante el otoño

(Roth *et al.,* 2001b; 2004). Sin embargo, debe tenerse en cuenta que el efecto del estrés térmico sobre la esteroidogénesis folicular es de naturaleza transitoria porque el contenido de estradiol en el fluido folicular aumenta durante el otoño (Roth *et al.,* 2004). Entre los efectos potencialmente adversos del estrés térmico asociado con niveles bajos de estradiol se encuentran: 1) La reducida concentración plasmática de E_2 (Badinga *et al.,* 1993; Wolfenson *et al.,* 1995) que afecta la duración e intensidad del estro, incrementando la ovulación silenciosa y el anestro y la reducción del número de montas (Gwazdauskas *et al.,* 1981; Wolfenson *et al.,* 1988); 2) La supresión de la liberación pulsátil de la LH, y el aumento del pico preovulatorio de LH (Wise *et al*., 1988; Gilad *et al*., 1993) lo que podría, a su vez, afectar eventos asociados con la maduración del ovocito y/u ovulación; 3) Desarrollo de folículos no ovulatorios o formación de quistes ováricos; y 4) Alteración del funcionamiento lúteo, es decir reducción de la producción y secreción de P_4 (Wolfenson *et al*., 2000).

Efecto en el cuerpo lúteo (CL)

El efecto del estrés calórico en el CL se refleja en las variaciones de los niveles plasmáticos de progesterona. Aunque algunos estudios informaron una disminución de la concentración de progesterona durante el verano (Howell *et al*., 1994; Jonsson *et al.,* 1997; Wolfenson *et al*., 2002) otros reportan mayores (Younas *et al.,* 1993; Trout *et al*., 1998; Wilson *et al.,* 1998a) o similares (Roth *et al.,* 2000) concentraciones de P_4 en vacas bajo estrés crónico en comparación con aquellas que se encuentran en condiciones normo-térmicas. Debido a que la concentración de progesterona en plasma depende no sólo de su nivel de producción, sino también de la tasa de secreción a la circulación, se ha sugerido que estas variaciones están asociadas con algunas variables fisiológicas y ambientales tales como la disminución del flujo sanguíneo luteal (Lublin y Wolfenson, 1996), el metabolismo de la progesterona en el hígado, el volumen sanguíneo, la liberación de progesterona de la glándula suprarrenal, los grados de hipertermia, la duración de la exposición al calor, la edad de las vacas, la nutrición y el estado de lactancia (Jonsson *et al.*, 1997; Trout *et al.*, 1998). La disminución de los niveles sanguíneos de progesterona está relacionada con el estrés calórico crónico típico del verano y no con el estrés calórico agudo como la exposición solar directa o la exposición al calor de una cámara caliente (Wolfenson *et al.,* 2000, 2002). La evidencia de la supresión directa de la producción de progesterona por alta temperatura se ha documentado en estudios *in vitro*. Las células lúteas obtenidas de folículos dominantes durante el verano produjeron menos progesterona y expresaron menos viabilidad que las obtenidas en el invierno (Wolfenson *et al.,* 1993). Además, las células lúteas obtenidas en invierno e incubadas a 40 °C producen un 30% menos de progesterona que las incubadas a

38 °C. Curiosamente, la producción de P_4 derivada de las células de la granulosa (es decir, células lúteas grandes) fue sólo ligeramente menor en verano que en invierno, mientras que, la producción de progesterona por las células derivadas de la teca (es decir, células lúteas pequeñas) fue notablemente menor en verano que en invierno (Wolfenson *et al.,* 2002). Parece que, bajo el estrés calórico crónico durante el verano, la alteración del funcionamiento del CL es en su mayoría debida a la reducción de la producción de P_4 por las células de la teca, las cuales se observaron más susceptibles a las tensiones ambientales que las células de la granulosa (Wolfenson *et al.,* 1997).

Estas observaciones son muy importantes ya que una alteración en la secreción de P_4 puede comprometer la función reproductiva (Mann *et al*., 1999; Thatcher *et al*., 2001). Bajas concentraciones de progesterona alteran la dinámica folicular y el tiempo de duración de la dominancia (Sirois y Fortune, 1990). Por otra parte, la concentración baja de progesterona puede inducir cambios en la secreción de prostaglandina F2α (PGF2α) endometrial y en la morfología uterina (Shaham-Albalancy *et al*., 1997; 2001) que son esenciales para la supervivencia y desarrollo del blastocisto (Spencer *et al*., 2008). Un aumento tardío de P_4 post-ovulatoria o bajas concentraciones durante la pre-implantación está asociada con una insuficiente producción del interferón tau (τ) y un pobre desarrollo embrionario (Mann y Lamming, 2001; Mann *et al.,* 2006). El interferón τ debe producirse en cantidades suficientes ya que es requerido para la supervivencia embrionaria mediante la prevención de la secreción endometrial de PGF2α y posterior luteólisis (Mann *et al*., 1999). No obstante, aún no está claro si esto último es lo que sucede en las vacas sometidas a estrés térmico.

Efectos en el ovocito

Dado que los ovocitos de mamíferos adquieren su potencial de desarrollo paso a paso durante el desarrollo folicular, las perturbaciones en la fisiología del folículo pueden conducir potencialmente a un ovocito con una competencia reducida para la fertilización y posterior desarrollo. De acuerdo con esto, tanto el estrés calórico inducido como el estacional pueden provocar deterioro no solo en el ovocito ovulado sino también en el resto de la reserva de ovocitos en el ovario (Payton *et al*., 2004; Gendelman y Roth, 2012). Los ovocitos obtenidos de vacas durante el verano presentaron una capacidad reducida para desarrollarse hasta la etapa de blastocisto después de la fecundación (Zeron *et al.,* 2001; Al-Katanani *et al.,* 2002). Gendelman *et al*. (2010) reportaron una disminución en el tiempo de división en los ovocitos colectados durante la temporada de calor (mayo a noviembre) en relación con los recolectados durante la estación fría (diciembre a abril). El retraso en las 2 primeras divisiones embrionarias fue expresado por una mayor proporción de embriones en etapa de 4 células 42 h después de la fecundación *in vitro* y se asoció con una proporción reducida de embriones desarrollándose a blastocistos. Por otra parte, se requiere un período de 2 a 3 ciclos estrales para la recuperación del daño térmico durante el verano y la aparición de ovocitos competentes en el otoño, lo que indica un efecto duradero en la reserva ovárica de ovocitos (Roth *et al.,* 2001a). Aunque el mecanismo por el cual el ovocito pierde su capacidad de desarrollo aun no es claro, parece que es de naturaleza multifactorial. Diferentes estudios proporcionan evidencia del daño inducido por el shock térmico en los diferentes estados de desarrollo del ovocito a través de los siguientes mecanismos:

<u>*Apoptosis en los ovocitos inducida por shock térmico*</u>*:* La exposición de ovocitos a 41 °C durante su maduración, aumentó la proporción de ovocitos que expresan alta actividad de las caspasas 2, 3 y 7 y que muestran fragmentación nuclear (Roth y Hansen, 2004a, b). Igualmente, el shock térmico durante la maduración incrementa la proporción de ovocitos con un potencial de membrana mitocondrial bajo (Soto y Smith, 2009). Kalo y Roth (2011), vinculan las alteraciones inducidas por el calor en la membrana de los fosfolípidos de ovocitos. Estas alteraciones incluyeron hidrólisis de esfingomielina de membrana, generación de ceramida y externalización de fosfatidilserina. Al usar inhibidores específicos de la formación de ceramida se alivió en cierta medida la presencia de apoptosis provocada por el shock térmico en el ovocito (Kalo y Roth, 2011). A su vez, el uso de anti-apoptóticos como esfingosina-1 fosfato para modular la formación de ceramida aumentó la proporción de ovocitos fértiles y que se desarrollaron a blastocistos. La ceramida se puede producir *de novo* a partir de la condensación de serina con palmitoil-CoA (Jenkins *et al.,* 2002) y, por lo tanto, la manipulación de la dieta que afecte la composición de ácidos grasos de la membrana o que reduzcan la formación de ceramida podrían mejorar la calidad de los ovocitos. La suplementación *in vitro* de aceite de palma con sales de calcio de ácidos grasos de cadena larga aumentó la proporción de ovocitos que se desarrollan a blastocistos (Fouladi-Nashta *et al.,* 2007). Sin embargo, la administración de ácidos grasos en el alimento a vacas lactantes durante el verano no mejoró la competencia de desarrollo de los ovocitos que fueron sometidos a maduración *in vitro* y fertilización (Bilby *et al.,* 2006). Por otro lado, el uso de inhibidores de caspasas también ha logrado la reducción del efecto perjudicial del shock térmico en el desarrollo del ovocito (Roth y Hansen, 2004a). Péptidos anti-apoptóticos redujeron la proporción de ovocitos con ADN fragmentado (Soto y Smith, 2009).

Estrés oxidativo inducido por shock térmico en los ovocitos: Las alteraciones inducidas por calor en el funcionamiento mitocondrial mencionadas anteriormente están también asociadas con el estrés oxidativo. Por lo tanto, el estrés oxidativo inducido por hipertermia se considera como uno de los mecanismos mediante los cuales el estrés térmico ambiental altera el rendimiento reproductivo de las vacas lactantes. El balance entre factores oxidantes y antioxidantes juega un papel importante sobre el ovocito: la reanudación espontánea de la meiosis es inhibida por los antioxidantes y puede ser inducida por un aumento en las especies de oxígeno reactivo (ROS; Takami *et al.,* 1999). Por otro lado, altos niveles de ROS dentro del folículo están asociados con un aumento de los defectos citoplasmáticos y la segregación cromosómica anormal (Van Blerkom *et al.,* 1997). Elevada temperatura corporal incrementa el ROS intracelular en ovocitos bovinos (Nabenishi *et al.,* 2012) y disminuye la concentración del antioxidante citosólico glutatión en ovocitos y cigotos de ratón (Matsuzuka *et al*., 2004). Además, Ozawa *et al.* (2002), informaron que la exposición de los cigotos al shock térmico ya sea directamente o a través de la hipertermia materna, dio como resultado niveles reducidos de glutatión en asociación con una temprana pérdida embrionaria. Se han realizado experimentos en ratones, utilizando algunas moléculas antioxidantes como el galato epigalocatequina, un componente flavonoide del té verde, o la melatonina, potente eliminador de ROS, resultando en un incremento de la proporción de ovocitos fértiles que se dividen y se desarrollan a embriones, disminuyendo la muerte embrionaria temprana provocada por hipertermia (Matsuzuka *et al*., 2005; Roth *et al.,* 2008). Aréchiga *et al.* (1998) informaron que suplementar a las vacas con β-caroteno durante al menos 90d comenzando aproximadamente 15 días después del parto mejora la tasa de gestación. Igualmente, García-Ispierto *et al*. (2013), informaron que el tratamiento de vacas lecheras con implantes de melatonina antes del parto mejoró su rendimiento reproductivo en el verano. Por otro lado, Zeron *et al*. (2001), reportaron que durante el verano la composición de la membrana del ovocito se caracteriza por la disminución de ácidos grasos poliinsaturados, por lo que cuando se suplementó con estos ácidos grasos, aumentó la proporción de ovocitos viables (Zeron *et al.,* 2002). En conjunto, manipular el estado de oxidación es susceptible a estrategias para superar los efectos adversos del estrés por calor sobre la fertilidad. Parece que se requiere una administración a largo plazo en lugar de periódica para proteger la reserva de folículos ováricos y sus ovocitos del estrés oxidativo inducido por hipertermia (Roth, 2015).

CAPITULO 2

EXPERIMENTO 1

Reinicio de la función ovárica, el perfil metabólico y la condición corporal en vacas Brahman *(Bos indicus)* no es afectada por la combinación de la separación de la cría y el tratamiento de la progesterona.

Artículo publicado en:

Animal Reproduction Science 2017;185:181–187.

Animal Reproduction Science 185 (2017) 181–187

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Resumption of ovarian function, the metabolic profile and body condition in Brahman cows (*Bos indicus*) is not affected by the combination of calf separation and progestogen treatment

Ramiro Díaz[a], Carlos S. Galina[a], Ivette Rubio[b], Manuel Corro[b], José Luis Pablos[c], Ana Rodríguez[a], Agustín Orihuela[d,*]

[a] *Departamento de Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, México DF, 04510 Mexico*

[b] *Centro de Enseñanza investigación y Extensión en Ganadería Tropical, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Tlapacoyan, Veracruz, 93650 Mexico*

[c] *Departamento de Genética y Bioestadística, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, México DF, 04510 Mexico*

[d] *Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, 62210, Mexico*

ARTICLE INFO

Keywords:
Metabolites
CIDR
Body condition
Fat thickness
Reproduction
Calf welfare

ABSTRACT

To evaluate the effect of different calf separation procedures after a progestogen treatment on the resumption of ovarian function, body condition and metabolic profile, 59 multiparous Brahman cows grazing on a mixed grass pasture were studied. No supplementation was given at any time. Body condition score (BCS), fat thickness (FAT) and blood metabolites were measured fortnightly from the beginning of the last trimester of gestation until 96 days postpartum. At 30 days postpartum all animals received a progesterone (P4)-releasing device (CIDR) which was withdrawn 9 days later when prostaglandin F2α was applied. At this time, treatments TW ($n = 28$), where calves were separated from their dams for 48 h; RS ($n = 21$), calves were allowed to suckle once a day for 1 h; and continuous suckling (CS; $n = 10$). Ovarian function was assessed by blood concentrations of progesterone on days −14, −9, 10, 13, 30 and 33 after CIDR removal. At the end of the experimental period, an average of 20% of the cows had not initiated estrous cycles. There were no changes of FAT or BCS during the last trimester of pregnancy in all cows ($P > 0.05$). During the postpartum period cows of all groups lost ($P < 0.05$) BCS and FAT with a nadir at 60–80 days postpartum, regardless of treatment. At 10 days after CIDR withdrawal the percentage of cows having ovulations was 75, 61 and 80 ($P > 0.05$) for TW, RS and CS groups. Blood metabolites follow a similar pattern in the three groups. With the conditions of the present study, the method of calf separation after a progestogen treatment, does not affect the resumption of ovarian function or metabolic profile.

1. Introduction

The two major factors influencing the onset of postpartum ovarian function in cows are their nutritional status and calf presence (Montiel and Ahuja, 2005; Galindo et al., 2013; Diskin and Kenny, 2016). Earlier studies found that nutrition is as important in the prepartum as in the postpartum periods. The transition between these two periods brings many challenges affecting early lactation

---

[*] Corresponding author.
E-mail address: aorihuela@uaem.mx (A. Orihuela).

http://dx.doi.org/10.1016/j.anireprosci.2017.08.018
Received 11 April 2017; Received in revised form 18 July 2017; Accepted 18 August 2017

and the onset of ovarian function. Several reports have concluded that an insufficient diet in the latter part of pregnancy, affects fertility even though nutritional values of feed sources improved during lactation (Wettemann et al., 2003; Watanabe et al., 2013; Crowe et al., 2014). Inconsistent with these findings, if the pasture consumed during the 6th or 7th month of gestation is of good quality, the cows have a greater probability of becoming pregnant (Samadi et al., 2013).

The complexity of energy metabolism makes it difficult to use reliable indicators to assess the nutritional status of a cow and make assumptions about body reserves. One method usually applied, is the metabolic status to measure blood metabolites related to energy metabolism such as glucose, triglycerides, non-esterified fatty acids (NEFAs) and urea (Cozzi et al., 2011; Anderson et al., 2015; Brscic et al., 2015). Other methods are body condition score (BCS) and measurement of body fat thickness (FAT). This latter measurement has been used recently as an accurate objective indicator of subcutaneous fat and reliable predictor of body energy reserves. (Ayres et al., 2009; Galindo et al., 2013). Regardless of the method used to assess the nutritional status of the cow, however, fluctuations in the metabolic reserves seem to induce different responses to reproductive treatments in postpartum cows under tropical conditions (Maquivar et al., 2010).

Historically, restricting suckling in beef cattle has been considered to affect the interval from parturition to first estrus and ovulation after calving (Mukasa-Mugerwa et al., 1991). Also, calf presence could inhibit ovarian follicular growth and ovulation (Hoffman et al., 1996; Duffy et al., 2000; Sinclair et al., 2002). Mackey et al. (2000), have shown that calf separation for short periods tends to stimulate the restoration of ovarian function after calving and, thus, earlier conception. The effect of restricted suckling could be potentiated with the use of a progestogen implant (Pérez-Torres et al., 2015; Mondragón et al., 2016).

Thus, the objective of the present study was to evaluate the onset of ovarian function and changes in the metabolic profile of postpartum Brahman cows subjected to a progestogen treatment and different calf separation methods. It was hypothesized that cows in which continuous suckling by calves occurred would be affected more greatly by metabolic profile and thus there would be a delay in re-initiation of ovarian function after calving.

2. Materials and methods

The present study was conducted in a herd of Brahman cows at the Centre for Teaching, Research, and Extension in Tropical Animal Husbandry belonging to the Faculty of Veterinary Medicine of the National Autonomous University of Mexico, located in the State of Veracruz, Mexico at 20° 4′ N and 97° 3′ W. The climate is hot and humid in the absence of a defined dry season. The study was conducted between February and July. During this period, average ambient temperature was 24.93 ± 2.04 °C and relative humidity 94.77 ± 2.75%.

2.1. Ethical statement

The Animal Care Internal Committee of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico approved the methods used during the present research in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. Animals

Multiparous Brahman cows ($n = 59$) in the last trimester of pregnancy were selected. They averaged four parturitions and were stocked at a rate of 1.25 animal units per hectare (AU = 450 kg of live weight/ha). Pastures were composed of Star grass (*Cynodon nlemfuensis*), *Paspalum* spp and *Axonopus* spp. In addition, mineralized salt and water were offered *ad libitum*.

2.3. Fat thickness (FAT) and body condition score (BCS)

The body condition score and FAT measurements were performed twice each month from the beginning of the last trimester of gestation until 96 days postpartum. The BCS was evaluated using the scale 1–9 (1 = emaciated, 9 = obese) as indicated by Wagner et al. (1988). The assessment of FAT was conducted using an ultrasonic device (Aloka SSD 500, Tokyo, Japan) with a convex transducer of 3.5 MHz frequency. The FAT was measured in the thurl area located midway between the tuber coxae (hooks) and the tuber ischiae (pins), 2–3 cm above the greater trochanter of the femur (Schröder and Staufenbiel, 2006), following immobilization of the animal.

2.4. Blood metabolites

Blood samples were collected for glucose, urea, triglycerides and NEFA analysis at the same time as readings were taken for BCS and FAT. Blood samples were taken from the coccygeal vein or artery, using a 10 ml Vacutainer™ tube without anticoagulant and stored at 4 °C for 3 h. Samples were then centrifuged at 3000 rpm for 15 min. Serum was stored in vials at −20 °C until assayed. Glucose, urea and triglycerides were measured in duplicate with commercial kits (BioSystem®; Barcelona-Spain). The coefficient of variation intra-assay in the samples analyzed in one test was 5.2% for glucose, 6.8% for urea and 7.8% for triglycerides. The NEFAs were also measured using a commercial kit (Wako®; Tokyo- Japan), and the resulting intra-assay coefficient of variation was 9.4%.

2.5. Evaluation of resumption of postpartum ovarian function

At an average of 30 days postpartum (confidence interval of 90% around a mean), all animals received a progesterone (P4)-releasing device (Eazy-breed CIDR™, 1.9 g of natural P4 in silicone, Zoetis®, México) which was withdrawn 9 days later when a dose of 25 mg of prostaglandin $F_{2\alpha}$ (Dinoprost, Lutalyse, Zoetis®, México) was applied. At the time of CIDR withdrawal, animals were allocated into 1 of 3 experimental groups. Temporal weaning (TW n = 28), where calves were separated from their dams for 48 h and returned to their dams; restricted suckling (RS n = 21), where calves were allowed to suckle once a day for 1 h and the rest of the day remained separated from their dams in a distant enclosure; continuous suckling (CS n = 10), where calves remained with their dams at all times. All cows were kept as a single herd in the previously described pasture, where measures were obtained from each cow. Onset of ovarian function was assessed by measuring blood concentrations of P4 on day 14 and 9 before CIDR placement and days 10, 13, 30 and 33 after removal of the device. Progesterone concentrations were determined by a solid phase radioimmunoassay in 100 µl of serum using commercial kits (Pharmaceuticals, Diagnostic Division). The intra- and inter-assay coefficients of variation were 7.41% and 6.18%, respectively. A P4 concentration of greater than 1 ng/ml in two or more successive samples, was indicative of luteal ovarian function (Pulido et al., 1991).

2.6. Statistical analyses

The assessment of differences of FAT and blood metabolites during the last trimester of pregnancy and the postpartum were conducted using Student "t" test. A correlation coefficient was used to determine the relationship between FAT and BCS. Variations of FAT and blood metabolites in the TW, RS and CS groups were evaluated using a mixed linear model ANOVA for repeated measures according to the treatment (TW, RS and CS) and physiological state (last trimester of gestation, until 96 days postpartum followed by Bonferroni adjustments for multiple comparisons. Logistic regression analyses were performed to assess the effect of metabolic status in the last trimester of pregnancy and during the postpartum period on the resumption of ovarian function. The dependence of these variables was evaluated using Chi-square tests for categorical variables. All statistical analyses were performed on IBM SPSS 22 and JMP 6.0 statistical packages. P-values < 0.05 were considered statistically different.

3. Results

3.1. Fat thickness and body condition score

At calving, all cows had an average BCS and FAT of 4.97 ± 1.01 and 0.29 ± 0.04 cm, respectively. There were no changes of FAT and BCS during the last trimester of pregnancy ($P > 0.05$). During the postpartum period, however, cows of all groups had a decrease ($P < 0.05$) in BCS and FAT attaining the nadir at 81 days postpartum. During the last trimester of pregnancy and the postpartum period, the relationship between FAT and BCS was minimal ($P < 0.05$) r = 0.12 and r = 0.02, respectively. From CIDR placement until the end of the experiment, there was no difference in BCS and FAT among treatment groups (Fig. 1).

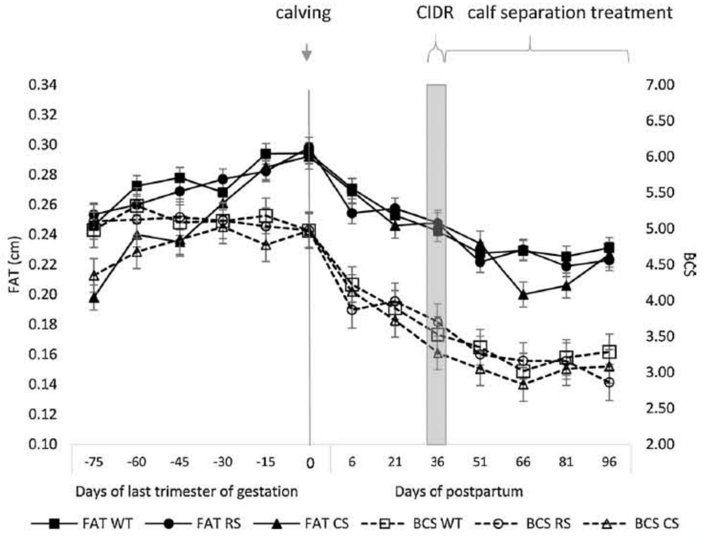


Fig. 1. Fat thickness (FAT) and body condition scores (BCS) in *Bos indicus* cows during the last trimester of pregnancy and early postpartum, while subjected to temporal weaning (TW), restricted suckling (RS) or continuous suckling (CS).

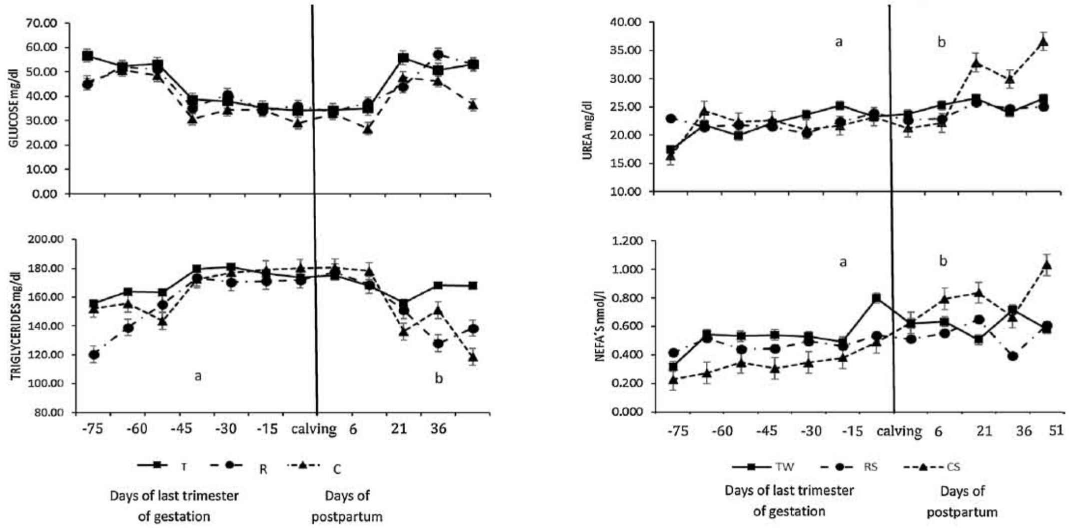


Fig. 2. Blood concentration of glucose, urea, triglycerides and non-sterified fatty acids in *Bos indicus* cows during the last trimester of pregnancy and early postpartum, while subjected to temporal weaning (TW), restricted suckling (RS) or continuous suckling (CS).

^{a,b}Represent statistical differences ($P < 0.05$) between averages values of concentrations obtained before and after parturition

3.2. Blood metabolites

The concentrations of blood metabolites were similar ($P > 0.05$) for the three groups (Fig. 2). Concentrations of triglycerides, urea and NEFAs were different between the last trimester of pregnancy and the postpartum period ($P < 0.05$). Concentrations of urea and NEFAs increased ($P < 0.05$) from the first period to the next with urea values being 21.82 compared with 24.60 mg/dl, and NEFA concentrations 0.47 compared with 0.61 nmol/L for the last trimester of pregnancy and postpartum, respectively. Triglyceride values decreased from 168.29 to 161.54 mg/dl ($P < 0.05$) between these two periods. In all the experimental groups, however, there were no differences in the glucose concentrations ($P > 0.05$) between the last trimester of pregnancy and early postpartum (44.02 ± 8.29 and 44.65 ± 9.83 mg/dl. Nevertheless, within each period, glucose concentrations varied. During the last third of gestation approximately 45–30 days before parturition, the concentrations decreased (52.76 compared with 36.26 mg/dl, respectively ($P > 0.05$)), remaining unchanged until 36 days postpartum ($P > 0.05$), when a significant increase ($P < 0.05$) was detected (33.4 compared with 49.16 mg/dl; Fig. 2).

3.3. Resumption of postpartum ovarian function

Less than 20% of cows had ovarian luteal function by day 31 postpartum with no differences among treatments ($P > 0.05$). The greatest proportion of animals expressing estrous cycles was observed during 10 days after CIDR withdrawal (55%, 44% and 80% for TW, RS and CS, respectively; $P > 0.05$), therefore, there was a cumulative percentage of cows having ovulations at about 51 days postpartum (day 10 after CIDR withdrawal) of 75%, 61% and 80% ($P > 0.05$) for TW, RS and CS, respectively. At the end of the experimental period, an average of 20% of all cows had not initiated estrous cycling regardless of treatment. It is noteworthy that animals that were estrous cycling prior to CIDR placement had a greater probability of the expression of estrous cycles continuing ($P = 0.01$). Fig. 3 depicts the relative frequencies of the onset of ovarian function after calving.

Table 1 provides data for the results of regression analysis between FAT and blood metabolites at 51 days postpartum on the onset of ovarian function. Triglycerides and FAT affected the CS group, urea and NEFAs affected the RS group, while glucose affected the TW and CS groups.

4. Discussion

During the postpartum period cows of all groups had decreases in BCS and FAT similarly, attaining the nadir at 60–80 days postpartum. Similarly, 10 days after CIDR withdrawal, the percentage of cows having ovulations was similar, regardless of calf separation treatment.

Results of the present study provide evidence that Brahman cattle maintained on pastures, maintained their FAT and BCS during the last third of gestation, with a decrease of both variables from parturition until about 80 days postpartum. This use of energy reserves evidenced by metabolic blood changes, however, does not prevent cows from responding to a CIDR treatment. This treatment induced the same rate of initiation of ovarian luteal function, regardless of the presence of the calf or suckling frequency.

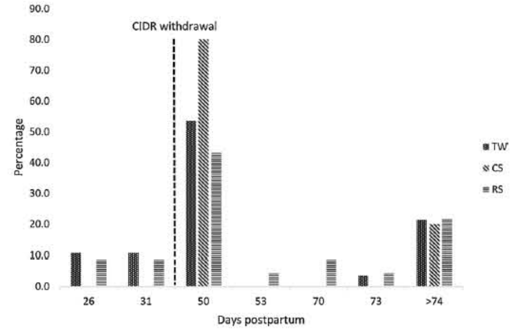


Fig. 3. Percentage of cows subjected to temporal weaning (TW), restricted (RS) and continuous suckling (CS) that initiate ovarian function at different postpartum days.

Table 1
Effect of FAT and blood metabolites levels on the onset of ovarian activity about 51 days postpartum (10 days after CIDR withdrawal) on temporal weaning (TW), restricted suckling (RS) and continuous suckling (CS) groups.

	TW	RS	CS
Fat thickness	NS	NS	0.005
Glucose	0.044	NS	0.002
Triglycerides	NS	NS	0.017
Urea	NS	0.025	NS
Non-esterified fatty acids	NS	0.039	NS

*NS = Non-significant difference ($P > 0.05$).

During the present experiment, FAT concentrations and BCS remained similar during the last third of gestation in all animals, decreasing at parturition and during the postpartum period, regardless of treatment. This phenomenon seems to be characteristic in animals that are not supplemented during this period (Mondragón et al., 2016), reflecting the physiological changes in the energy metabolism occurring during and after parturition, when animals use their reserves, leading to a negative energy balance (Bell, 1995; Grummer et al., 2004). These findings are supported by the changes in blood metabolites observed in the present experiment, suggesting that maintaining FAT and BCS during the prepartum period, are indicative of sufficient energy intake to promote resumption of ovarian function. In contrast, in other studies where animals were supplemented, FAT was increased (Maquivar et al., 2010). Perhaps different results might be found when cows maintain or increase FAT or BCS after parturition.

There were similar patterns for blood metabolites in the three groups during the experiment, with these patterns remaining consistent during the last trimester of gestation and with a significant decrease at parturition and during the following days. Several investigations (Richards et al., 1989; Rusche et al., 1993; Bell, 1995; Radunz et al., 2010; Lee et al., 2012; Folnožić et al., 2015) evaluated metabolic concentrations before and after parturition, obtaining similar results to those of the current research. In addition, during the present study glucose concentrations were similar before and after parturition. Similarly, Lee et al. (2012) reported no changes in glucose concentrations when measured 21 or 10 days before parturition or during early postpartum (Lake et al., 2006). In addition, results in reports for *B. taurus* cows are also consistent with results in the present study, indicating that glucose concentrations may increase after the third week postpartum (Weber et al., 2016), in an apparent recovery from energy utilization incurred during parturition. The concentration of triglycerides in the postpartum period was, however, less than in the last third of gestation, which is consistent with the findings of Guédon et al. (1999), where triglyceride concentrations decreased when compared between the last third of gestation and postpartum. It was suggested that changes in triglyceride concentrations are directly related to the physiological state of the animal. Thus, Meikle et al. (2004) reported that during lactation, cows enter in a negative energy balance, which among other processes, induce fat mobilization as an energy supply. Furthermore, the lipolysis generated in this period leads to an increase in the concentration of NEFAs in blood (Bines and Hart, 1982; Richards et al., 1989). Konigsson et al. (2008) reported that concentrations of NEFAs were greater in anestrous animals compared with those that had initiated luteal function. In this previous study, concentrations of NEFAs were also assessed in first calving dairy cows from a week before until 7 weeks after calving and it was reported that the greater concentrations of NEFAs were found in the postpartum similar to what occurred in Brahman cattle in the present study. Postpartum concentration of urea was greater than that during the last trimester of gestation in the present study. According to Watanabe et al. (2014), postpartum supplementation increases blood concentrations of urea almost immediately after feeding. The relationship between plasma concentrations of urea and the fertility of the animals has been demonstrated in several studies, (Marinov 1981; Ferguson et al., 1993; Cottrill et al., 2002; Gath et al., 2012). A direct relationship between the metabolic status of animals during the last third of pregnancy and at 51 days postpartum, and the onset of postpartum ovarian function was also observed in the present study. Marinov, in 1981, established that cows with blood urea concentrations of about 25 mg/dl have a greater conception rate than cows with greater concentrations. In addition, Gath et al.

(2012), reported that greater concentrations of urea during the postpartum period, increased the pregnancy rate when embryos of 8 or more cells were used for embryo transfer. In the present investigation, the average blood urea during the postpartum period was 24.60 mg/dl, which could affect the conception rate of the animals after a reproductive treatment, and explain the increase from 21.82 in the last trimester of gestation to 24.60 mg/dl during the postpartum period, when cows are more likely to become pregnant.

Suckling affects the duration of the anestrus postpartum (Murphy et al., 1990; Crowe et al., 1993; Duffy et al., 2000). In the present study, however, there was no effect of the presence of the calf or frequency of suckling on the onset of postpartum ovarian function, suggesting that energy loss due to lactation in these animals is not as severe as to affect the results. Fröberg et al. (2007) reported that dual-purpose cows under restricted suckling had a lesser value of milk fat than cows without a calf. Possibly, the effects of the udder emptying coupled with the recovery time, can cause a physiological stimulus triggering lipid metabolism. In contrast, the P4 implant used in the present study had a positive effect on the onset of ovarian function. Most animals of TW, RS and CS groups initiated estrous cycling within 10 days after CIDR withdrawal. There was 20% of the experimental animals, however, that did not reinitiate ovarian function until after the end of the experiment. In a previous study, Diaz et al. (2002) found that 29% of animals remained anestrus for the whole duration of the experiment. Recently, Pérez-Torres et al. (2015), reported similar results. Furthermore, Stagg et al. (1998) and Sinclair et al. (2002) also reported that about 15% of cows failed to respond to the removal of the suckling/maternal calf bond, which typically had prolonged postpartum anestrous intervals and could be described as being in what is referred to as "deep" anestrus (Sinclair et al., 2002). Why 1/5 of the herd remained anestrus whilst the rest has the possibility of becoming pregnant prior to 100 days postpartum remains a question to be elucidated. In addition, the importance of nutrition is also clear from the study of Bishop et al. (1994) where they reported that only 40% of cows in a BCS of less than five (scale 1–9) had ovulations by day 60, following weaning at 35 days postpartum. The available evidence, therefore, suggests that progestogen treatment together with calf isolation or restricted suckling might be management options to shorten the postpartum anestrous interval in beef cows, but with a lesser and more variable response expected in cows that have a lesser BCS at calving. The length of the sampling period was perhaps too short to see an effect on blood metabolites, in spite of the fact that measurements began almost 90 days prior to calving and continued to approximately 40 days post parturition. Thus, it is possible to conclude that with the conditions of the present study, the method of calf separation after a progestogen treatment, does not affect the resumption of ovarian function or metabolic profile of postpartum Brahman cows.

Conflict of interest statement

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved experimental animals, has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from aorihuela@uaem.mx.

Acknowledgements

The authors are grateful for the assistance to the administrative staff of the Centre for Teaching, Research, and Extension in Tropical Animal Husbandry belonging to the Faculty of Veterinary Medicine of the National Autonomous University of Mexico for their assistance in the housing and care of animals. Also, the authors would like to thank MVZ. Daniela Briseño and MVZ. Karen Córdova Hernández for their help in data collection. Funding for this study was supported by the Ministry of Higher Education, Science, Technology and Innovation (SENESCYT) of Ecuador with in the program of scholarships for higher education.

References

Anderson, J.L., Kalscheur, K.F., Clapper, J.A., Pwerry, G.A., Keisler, D.H., García, A.D., 2015. Feeding fat from distillers dried grains with solubles to dairy heifers: II. Effects on metabolic profile. J. Dairy Sci. 98, 5709–5719.

Ayres, H., Ferreira, R.M., de Souza Torres Jr, J.R., Demetrio, C.G.B., de Lima, C.G., Baruselli, P.S., 2009. Validation of body condition score as a predictor of subcutaneous fat in Nelore (*Bos indicus*) cows. Lives. Sci. 123, 175–179.

Bell, A.W., 1995. Regulation of organic nutrient late pregnancy metabolism during transition from to early lactation. J. Anim. Sci. 73, 2804–2819.

Bines, J.A., Hart, I.C., 1982. Metabolic limits to milk production, especially roles of growth hormone and insulin. J. Dairy Sci. 65, 1375–1389.

Bishop, D.K., Wettemann, R.P., Spicer, I.J., 1994. Body energy reserves influence the onset of luteal activity after early weaning of beef cows. J. Anim Sci. 72, 2703–2708.

Brscic, M., Cozzi, G., Lora, I., Stefani, A.L., Contiero, B., Ravarotto, L., Gottardo, F., 2015. Reference limits for blood analytes in Holstein late-pregnant heifers and dry cows: effects of parity, days relative to calving, and season. J. Dairy Sci. 98, 7886–7892.

Cottrill, B., Biggadike, H.J., Collins, C., Laven, R.A., 2002. Relationship between milk urea concentration and the fertility of dairy cows. Vet. Rec. 151, 413–416.

Cozzi, G., Ravarotto, L., Gottardo, F., Stefani, A.L., Contiero, B., Moro, L., Brscic, M., Dalvit, P., 2011. Reference values for blood parameters in Holstein dairy cows:

effects of parity, stage of lactation, and season of production. J. Dairy Sci. 94, 3895–3901.

Crowe, M.A., Goulding, D., Baguisi, A., Boland, M.P., Roche, J.F., 1993. Induced ovulation of the first postpartum dominant follicle in beef suckler cows using a GnRH analogue. J. Reprod. Fertil. 99, 551–555.

Crowe, M.A., Diskin, M.G., Williams, E.J., 2014. Parturition to resumption of ovarian cyclicity: comparative aspects of beef and dairy cows. Animal 8, 40–53.

Diaz, G.S., Galina, C.S., Basurto, C.H., Ochoa, G.P., 2002. Efecto de la progesterona natural con o sin la adición de benzoato de estradiol sobre la presentación de celo, ovulación y gestación en animales tipo *Bos indicus* en el trópico mexicano. Arch. Med. Vet. 34, 235–244.

Diskin, M.G., Kenny, D.A., 2016. Managing the reproductive performance of beef cows. Theriogenology 86, 379–387.

Duffy, P., Crowe, M.A., Boland, M.P., Roche, J.F., 2000. Effect of exogenous LH pulses on the fate of the first dominant follicle in postpartum beef cows nursing calves. J. Reprod. Fertil. 118, 9–17.

Ferguson, J.D., Galligan, D.T., Blanchard, T., Reeves, M., 1993. Serum urea nitrogen and conception rate: the usefulness of test information. J. Dairy Sci. 76, 3742–3746.

Folnožić, I., Turk, R., Đuričić, D., Vince, S., Pleadin, J., Flegar-Meštrić, Z., Valpotić, H., Dobranić, T., Gračner, D., Samardžija, M., 2015. Influence of body condition on serum metabolic indicators of lipid mobilization and oxidative stress in dairy cows during the transition period. Reprod. Domest. Anim. 50, 910–917.

Fröberg, S., Aspegren-Güldorff, A., Olsson, I., Marin, B., Berg, C., Hernández, C., Galina, C.S., Lidfors, L., Svennersten-Sjaunja, K., 2007. Effect of restricted suckling on milk yield, milk composition and udder health in cows and behaviour and weight gain in calves, in dual-purpose cattle in the tropics. Trop. Anim. Health Prod. 39, 71–81.

Galindo, J., Galina, C.S., Estrada, S., Romero, J.J., Alarcón, M., Maquivar, M., 2013. Effect of changes in body weight, body condition and back fat during last month of pregnancy on the reproductive efficiency of *Bos indicus* cows in the tropics of Costa Rica. J. Vet. Med. 3, 22–28.

Gath, V.P., Crowe, M.A., O'Callaghan, D., Boland, M.P., Duffy, P., Lonergan, P., Mulligan, F.J., 2012. Effects of diet type on establishment of pregnancy and embryo development in beef heifers. Anim. Reprod. Sci. 133, 139–145.

Grummer, R.R., Mashek, D.G., Hayirli, A., 2004. Dry matter intake and energy balance in the transition period. Vet. Clin. N. Am-Food A 20, 447–470.

Guédon, L., Saumande, J., Dupron, F., Couquet, C., Desbals, B., 1999. Serum cholesterol and triglycerides in postpartum beef cows and their relationship to the resumption of ovulation. Theriogenology 51, 1405–1415.

Hoffman, D.P., Stevenson, J.S., Minton, J.E., 1996. Restricting calf presence without suckling compared with weaning prolongs postpartum anovulation in beef cattle. J. Anim. Sci. 74, 190–198.

Konigsson, K., Savoini, G., Govoni, N., Invernizzi, G., Prandi, A., Kindahl, H., Veronesi, M.C., 2008. Energy balance, leptin, NEFA and IGF-I plasma concentrations and resumption of postpartum ovarian activity in Swedish Red and White breed cows. Acta Vet. Scand. 50, 3.

Lake, S.L., Scholljegerdes, E.J., Hallford, D.M., Moss, G.E., Rule, D.C., Hess, B.W., 2006. Effects of body condition score at parturition and postpartum supplemental fat on metabolite and hormone concentrations of beef cows and their suckling calves. J. Anim. Sci. 84, 1038–1047.

Lee, H.H., Katsuya, K., Ryotaro, M., Hisashi, I., Miyamoto, A., Kawashima, C., Haneda, S., Miyake, Y.I., Matsui, M., 2012. Slow recovery of blood glucose in the insulin tolerance test during the prepartum transition period negatively impacts the nutritional status and reproductive performance postpartum of dairy cows. J. Vet. Med. Sci. 74, 457–464.

Mackey, D.R., Sreenan, J.M., Rochet, J.F., Diskin, M.G., 2000. The effect of progesterone alone or in combination with estradiol on follicular dynamics, gonadotropin status, and estrus in beef cows following calf isolation and restricted suckling. J. Anim. Sci. 78, 1917–1929.

Maquivar, M.G., Galina, C.S., Galindo, J.R., Estrada, S., Molina, R., Mendoza, G.D., 2010. Effect of protein supplementation on reproductive and productive performance in *Bos indicus* x *Bos taurus* heifers raised in the humid tropics of Costa Rica. Trop. Anim. Health Prod. 42, 555–560.

Marinov, S., 1981. Urea content in the blood serum and fertility in cows. Vet. Med. Nauki 18, 32–38.

Meikle, A., Kulcsar, M., Chilliard, Y., Febel, H., Delavaud, C., Cavestany, D., Chilibroste, P., 2004. Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reprod 127, 727–737.

Mondragón, V., Galina, C.S., Rubio, I., Corro, M., Salmerón, F., 2016. Effect of restricted suckling on the onset of follicular dynamics and body condition score in Brahman cattle raised under tropical conditions. Anim. Reprod. Sci. 167, 89–95.

Montiel, F., Ahuja, C., 2005. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. Anim. Reprod. Sci. 85, 1–26.

Mukasa-Mugerwa, E., Tegegne, A., Franceschini, R., 1991. Influence of suckling and continuous cow-calf association on the resumption of post-partum ovarian function in *Bos indicus* cows monitored by progesterone statuss. Reprod. Nutr. Develop. 31, 241–247.

Murphy, M.G., Boland, M.P., Roche, J.F., 1990. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. J. Reprod. Fertil. 90, 523–533.

Pérez-Torres, L., Rubio, I., Corro, M., Cohen, A., Orihuela, A., Galina, C.S., Pablos, J.L., 2015. A pre-synchronization program at early pospartum might increase the chances of *Bos indicus* cows cycling prior to 50 days regardless of the length of calf separation. J. Reprod. Develop. 61, 199–203.

Pulido, A., Zarco, L., Galina, C.S., Murcia, C., Flores, G., Posadas, E., 1991. Progesterone metabolism during storage of blood samples from Gyr cattle: effects of anticoagulant, time and temperature of incubation. Theriogenology 35, 965–975.

Radunz, A.E., Fluharty, F.L., Day, M.L., Zerby, H.N., Loerch, S.C., 2010. Prepartum dietary energy source fed to beef cows: I: Effects on pre- and postpartum cow performance. J. Anim. Sci. 88, 2717–2728.

Richards, M.W., Wettemann, R.P., Schoenemann, H.M., 1989. Nutritional anestrus in beef cows: concentrations of glucose and nonesterified fatty acids in plasma and insulin in serum. J. Anim. Sci. 67, 2354–2362.

Rusche, W.C., Cochran, R.C., Corah, L.R., Stevenson, J.S., Harmon, D.L., Brandt Jr., R.T., Minton, J.E., 1993. Influence of source and amount of dietary protein on performance, blood metabolites, and reproductive function of primiparous beef cows. J. Anim. Sci. 71, 557–563.

Samadi, F., Phillips, N.J., Blache, D., Martin, G.B., D'Occhio, M.J., 2013. Interrelationships of nutrition, metabolic hormones and resumption of ovulation in multiparous suckled beef cows on subtropical pastures. Anim. Reprod. Sci. 137, 137–144.

Schröder, U.J., Staufenbiel, R., 2006. Invited review: methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. J. Dairy Sci. 89, 1–14.

Sinclair, K.D., Molle, G., Revilla I.R. Roche, J.F., Quintans, G., Marongiu, L., Sanz, A., Mackey, D.R., Diskin, M.G., 2002. Ovulation of the first dominant follicle arising after day 21 postpartum in suckling beef cows. Anim. Sci. 75, 115–126.

Stagg, K., Spicer, I.J., Sreenan, J.M., Roche, J.F., Diskin, M.G., 1998. Effect of calf isolation on follicular wave dynamics, gonadotrophin and metabolic hormone changes, and interval to first ovulation in beef cows fed either of two energy levels postpartum. Biol. Reprod. 59, 777–782.

Wagner, J.J., Lusby, K.S., Oltjen, J.W., Rakestraw, J., Wettermann, R.P., Walters, L.E., 1988. Carcass composition in mature Hereford cows: estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66, 603–612.

Watanabe, U., Takagi, M., Yamato, O., Otoi, T., Tshering, Ch., Okamoto, K., 2013. Metabolic profile of Japanese black breeding cattle herds: usefulness in selection for nutrient supplementation to enhance reproductive performance and regional differences. J. Vet. Med. Sci. 75, 481–487.

Watanabe, U., Takagi, M., Yamato, O., Otoi, T., Okamoto, K., 2014. Retrospective surveillance of metabolic parameters affecting reproductive performance of Japanese Black breeding cows. J. Vet. Sci. 15, 283–288.

Weber, C., Schäff, C.T., Kautzsch, U., Börner, S., Erdmann, S., Görs, S., Röntgen, M., Sauerwein, H., Bruckmaier, R.M., Metges, C.C., Kuhla, B., Hammon, H.M., 2016. Insulin- dependent glucose metabolism in dairy cows with variable fat mobilization around calving. J. Dairy Sci. 99, 6665–6679.

Wettemann, R.P., Lents, C.A., Ciccioli, N.H., White, F.J., Rubio, I., 2003. Nutritional- and suckling-mediated anovulation in beef cows. J. Anim. Sci. 81 (E. Suppl. 2), E48–E59.

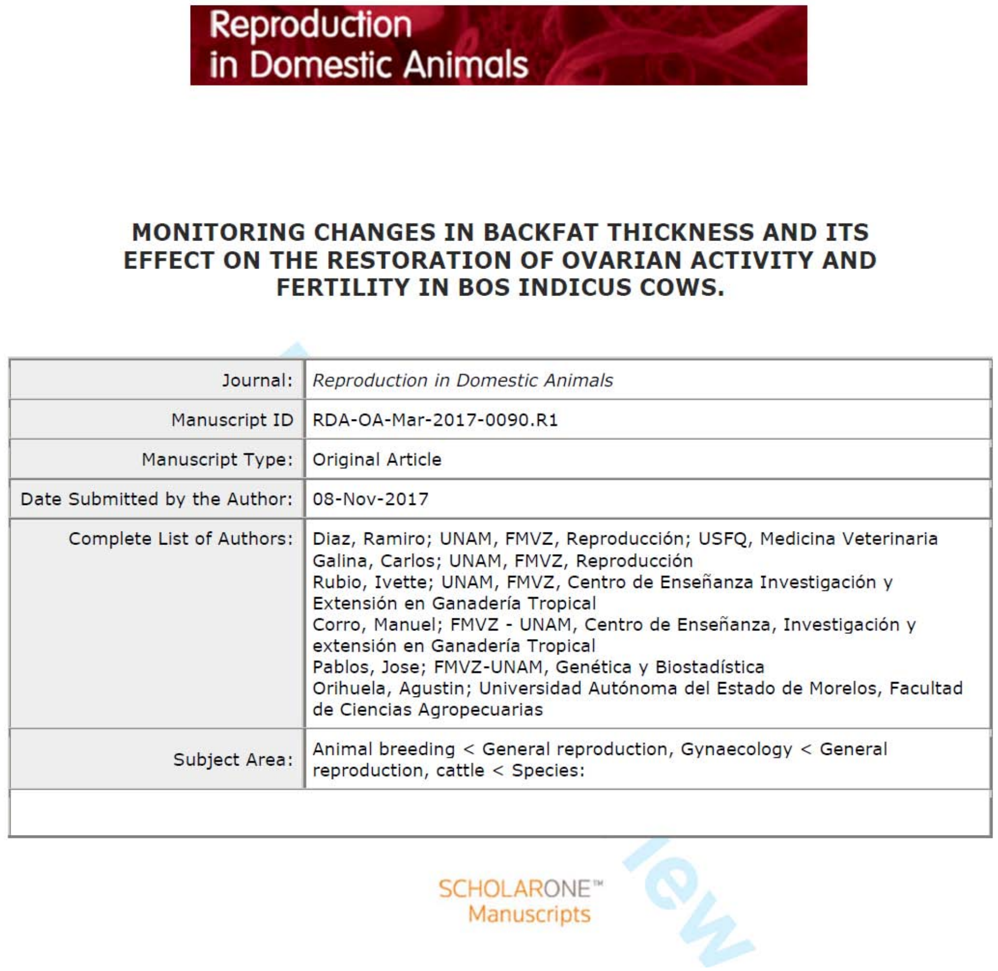
CAPITULO 3

EXPERIMENTO 2

El monitoreo de la grasa dorsal en el periparto en vacas *Bos indicus* podría ayudar en la planificación de un programa reproductivo exitoso en ganado criado en condiciones tropicales.

Artículo publicado en:

Reproduction in Domestic Animals (en prensa)



Reproduction
in Domestic Animals

MONITORING CHANGES IN BACKFAT THICKNESS AND ITS EFFECT ON THE RESTORATION OF OVARIAN ACTIVITY AND FERTILITY IN BOS INDICUS COWS.

Journal:	*Reproduction in Domestic Animals*
Manuscript ID	RDA-OA-Mar-2017-0090.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	08-Nov-2017
Complete List of Authors:	Diaz, Ramiro; UNAM, FMVZ, Reproducción; USFQ, Medicina Veterinaria Galina, Carlos; UNAM, FMVZ, Reproducción Rubio, Ivette; UNAM, FMVZ, Centro de Enseñanza Investigación y Extensión en Ganadería Tropical Corro, Manuel; FMVZ - UNAM, Centro de Enseñanza, Investigación y extensión en Ganadería Tropical Pablos, Jose; FMVZ-UNAM, Genética y Biostadística Orihuela, Agustin; Universidad Autónoma del Estado de Morelos, Facultad de Ciencias Agropecuarias
Subject Area:	Animal breeding < General reproduction, Gynaecology < General reproduction, cattle < Species:

SCHOLARONE™
Manuscripts

MONITORING CHANGES IN BACKFAT THICKNESS AND ITS EFFECT ON THE RESTORATION OF OVARIAN ACTIVITY AND FERTILITY IN BOS INDICUS COWS.

Díaz BR[1], Galina CS[1], Rubio I[2], Corro M[2], Pablos JL[3] Orihuela A[4]*

[1] Departamento de Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México.

[2] Centro de Enseñanza Investigación y Extensión en Ganadería Tropical, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México.

[3] Departamento de Genética y Bioestadística, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México.

[4]Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos,

Contents

With the objective of testing the hypothesis if animals with a stable layer of body fat (FAT) during the peripartum have a better chance to becoming pregnant after calving, fifty-nine multiparous Brahman cows in their last trimester of pregnancy were used. Animals averaged four parturitions and were stocked at a rate of 1.25 animal units per hectare and divided in two groups depending on the time postpartum (dpp) that the intravaginal releasing device CIDR was inserted Group 1 (<30dpp; n=30) received the implant at 25.2 ± 4.21 and withdrawn 9 days later. Group 2 (≥30dpp; n=29), received the CIDR at 38.41 ± 5.8. Animals were AI at detected estrus until 170 dpp and calculated as pregnant at first service or requiring more than one service (1s and >1s), not pregnant but cycling (not pregnant) and those not cycling at all (anestrus). The FAT measurements were taken twice each month from the last trimester of gestation until 96 dpp. The onset of ovarian activity was monitored through blood levels of progesterone (P4) at days 14 and 9 prior to CIDR insertion and days 10, 13, 30 and 33 after CIDR withdrawal. Animals pregnant did not have any major changes in their fat thickness. In contrast, cows pregnant in the group ≥30dpp had changes in their FAT homeostasis, pregnant animals in the 1s and >1s groups did not show differences in dorsal back fat in the last trimester of pregnancy and early postpartum. In contrast, animals not pregnant and in anestrus FAT values decreased considerably after parturition. Overall fertility was 49% but 18% of all the animals remained anestrus losing FAT. Thus, animals with adequate metabolic conditions will have a better chance of pregnancy regardless of the time postpartum when the reproductive program starts.

Introduction

To achieve a calving interval of 365 days, a cow must get pregnant during the first three months after parturition. Poor or inadequate nutrition during the early postpartum is one of the main factors delaying the onset of ovarian activity and fertility (De Rouen *et al.,* 1994; Lents *et al.,* 2008). In the last few years the measurement of dorsal back fat (FAT) is a good indicator of the nutritional status of the animals (Schröder and Staufenbiel, 2006; Singh *et al.,* 2015). In addition, there is evidence that this status during the last trimester of gestation, bears an important effect on reproductive ability of the animal (Short *et al.,* 1990; Dunn and Moss, 1992; Wettemann *et al.,* 2003). Thus, the prompt restoration of ovarian activity is closely linked to the ability of the dam to recuperate from the negative energy balance consequence of the stress related to parturition (Randel, 1990; Short *et al*., 1990; Montiel and Ahuja, 2005; Galindo *et al*., 2013).

Previous reports have shown that hormonal treatments prior to 30 days postpartum could enhance the probability of animals becoming pregnant soon after calving either using artificial insemination (Pérez -Torres *et al.,* 2015, Mondragón *et al.,* 2016) or natural mating (Lamb *et al.,* 2008). Nonetheless, the variability of the response could be due to fluctuations in the metabolic profile of the animals. The objective of this study is to test the hypothesis that animals with a stable layer of FAT during the peripartum have a better chance to becoming pregnant after calving.

Materials and Methods

The present study was conducted in a Brahman herd at the Centre for Teaching, Research, and Extension in Tropical Animal Husbandry belonging to the Faculty of Veterinary Medicine of the National Autonomous University of Mexico, located in the State of Veracruz, Mexico at 20° 4´N and 97° 3´W. The climate is hot and humid in the absence of a defined dry season. Average yearly rainfall is 1840 mm and temperature range between 14 and 35°C. The study was carried out between the months of February and August.

Ethical Statement

The Animal Care Internal Committee of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico approved the methods used during the present work in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Animals

Fifty-nine multiparous Brahman cows in their last trimester of pregnancy were used. All animals averaged four parturitions and were stocked at a rate of 1.25 animal units per hectare (AU=450 kg of live weight/ha). Pastures were conformed mainly by an association of Star grass (*Cynodon nlemfuensis*), *Paspalum* spp. and *Axonopus* spp. In addition, mineralized salt was offered *ad libitum.* A regime of restricted suckling was applied to the calves from 20 dpp.

Back Fat thickness (FAT)

The FAT measurements were performed twice each month from the beginning of the last trimester of gestation until 96 days postpartum (dpp). The assessment of FAT was carried out using an ultrasound device (Aloka SSD 500, Tokyo, Japan) with a convex transducer of 3.5 MHz frequency. The FAT was measured in the thurl area located midway between the tuber coxae (hooks) and the tuber ischiae (pins), 2 to 3 cm above the greater trochanter of the femur (Schröder and Staufenbiel, 2006), following immobilization of the animal using a working chute.

Experimental groups and treatments.

Animals were divided in two groups depending on the time that the intravaginal releasing device was inserted (Eazy-breed CIDR™, 1.9 g of natural progesterone in silicone, Zoetis®, México). Group 1 (<30 dpp; n=30) received the implant at 25.2 ± 4.21 and withdrawn 9 days later. Group 2 (≥30dpp; n=29): received the CIDR at 38.41 ± 5.8. At the moment of CIDR withdrawal, a dose of 25mg of Prostaglandin F2α was given (Dinoprost, Lutalyse, Zoetis®, México).

Postpartum ovarian activity and fertility

The evaluation of the onset of ovarian activity was monitored through blood levels of progesterone (P4) at days 14 and 9 prior to CIDR insertion and days 10, 13, 30 and 33 after CIDR withdrawal. Blood samples were taken from the coccygeal vein or artery, using a 10 ml Vacutainer™ tube without anticoagulant and stored at 4°C for 3h. Samples were then centrifuged at 3000 rpm for 15 min. Serum was stored in vials at -20 °C until assayed. Progesterone concentrations determined by a solid phase radioimmunoassay in 100 μl of serum using commercial kits (Progesterone, Coat-A-Count, Diagnostic Products Corp. Los Angeles, CA). The intra and inter-assay coefficients of variation were 7.41% and 6.18%, respectively. A progesterone concentration above 1 ng/ml in two or more successive samples, was indicative of luteal ovarian activity (Pulido *et al.,* 1991).

All animals were continuously observed for 12h daily during 96 hours after CIDR withdrawal. Artificial insemination with commercial semen was performed when a cow displayed riding behavior using previously validated criteria (Orihuela *et al.,* 1983). Fertility was evaluated based on the percentage of cows pregnant and the time of gestation before the end of the reproductive program, which ensued on average at 170 dpp. Calculations taken into consideration were animals pregnant at first service or requiring more than one service (1s vs >1s). Also, animals not pregnant but cycling (not pregnant) during the AI program and those not cycling at all (anestrus).

Statistical Analyses

A comparison of the number of services in the two groups and the period of days between calving and the last service together with the variation of relative back fat which was determined using the formula: ΔFAT= [initial measure - final measure] / initial measure. The possible differences of FAT during the last trimester of pregnancy and the postpartum were carried out using Student "t" test. Variations of FAT in the <30dpp and ≥30dpp groups were evaluated by ANOVA for repeated measures followed by Bonferroni adjustments for multiple comparisons. Survival analysis was performed using Kaplan-Meier curves as to evaluate the effect of time on the beginning of the reproductive program of the 1s and >1s animals. The proportions of the 1s, > 1s, not pregnant and anestrus cows were compared in the two groups (<30dpp and ≥ 30dpp) and their differences were evaluated with a 95% confidence interval. The dependence of these variables was evaluated using Chi-square tests for categorical variables. All statistical analyses were performed on IBM SPSS 22 and JMP 6.0 statistical packages. P-values <0.05 were statistically different.

Results

Analysis of FAT values showed significant differences when comparing cows in the group <30dpp, 0,27 ± 0.033cm and 0.23 ± 0.022cm for the last trimester of pregnancy and the postpartum period (P=0.00001). Similarly, for the group ≥30dpp, 0.26 ± 0.032cm and 0.24 ± 0.023cm (P=0.027; Fig. 1). In addition, 10 and 21% of the animals started cycling prior to CIDR insertion for the groups <30dpp and ≥30dpp respectively (P>0.05). These cows did not show any changes in FAT in the last trimester of pregnancy and early postpartum (P=0.259 and P=0.233 for <30dpp and ≥30dpp respectively). After CIDR withdrawal 57 and 76% of the animals for the groups <30dpp and ≥30dpp started cycling and ovulated (P<0.05). In this group of animals there was a significant difference in FAT values in the two periods (P=0.001 and P= 0.036 for <30dpp and ≥30dpp). Until the end of the experimental period 67 and 97% of the animals established cyclicity for the groups <30dpp and ≥30dpp respectively (P<0.05). In contrast, 18% of the cows did not resume ovarian activity, these animals were significantly inconsistent in their FAT values (P=0.005).

Overall conception rate was 49%, 27% corresponded to cows with <30dpp and 72% for the other group (P<0.05). As can be seen in figure 2, differences were found in the proportion of pregnant animals conceiving at first service (1s) versus more than one AI (>1s) for groups <30 and ≥ 30dpp. The survival analysis did not depict differences in the time when the animals became pregnant (P<0.05; Fig. 3). Animals 1S of group <30 dpp displayed a gain in total relative fat variation of 25.6% whereas animals 1S of group ≥30 dpp lost 1.2% (P>0.05). In contrast, animals > 1S in the group <30dpp lost 7.6% of backfat, while in the group ≥30dpp gained 2.5% (P> 0.05). On the other hand, not pregnant animals in both groups lost backfat during the duration of the experiment at 19.7% and 2.5% for groups <30dpp and ≥30dpp respectively (P> 0.05; Figure 4). The period between calving and the last service of the a) 1S, b) > 1S and c) not pregnant animals was 56.66, 103.4, 95.75 days respectively for the group <30dpp; and 86.5, 104.1, 119.3 days respectively for the group > 30dpp (figure 4).

On the other hand, as can be observed in table 1, pregnant animals in the 1s and >1s groups did not show differences in dorsal back fat in the last trimester of pregnancy and early postpartum. In contrast, not pregnant and in anestrus animals, FAT values decreased considerably after parturition. In the group of pregnant animals at first service ≥30dpp diminished their FAT values (P=0.014), but the pregnant animals with more than one service and the group not pregnant, did not evidenced FAT changes.

Discussion

The thickness of FAT was similar between the two groups (P>0.05). However, FAT depth decreased after parturition (P<0.05) consequence of the changes occurring immediately after calving driving the animal to a negative energy balance (Grummer *et al.,* 2004). During this period, the animal experiences a metabolic imbalance as the nutritional requirements of the cows are usually less than the intake that the fodder can provide, consequently the animal moves their fat reserve (Gross *et al.,* 2011). Nonetheless, there were differences in the animals, for example, cows that kept their body reserves prior and after calving, started their ovarian activity prior CIDR insertion regardless of their time when they calved. The presence of the first dominant follicle in *Bos indicus* cattle could be observed as early as 10 days (Crowe *et al.,* 2014) although some authors extend this figure to 15 days (Murphy *et al.,* 1990; Savio *et al.,* 1990; Crowe *et al.,* 1993). Although generally in beef cows the first dominant follicle, and frequently a successive number of dominant follicles, generally fails to ovulate and become atretic (Murphy *et al.,* 1990; Stagg *et al.,* 1995). However, in beef cows in good body condition, the first postpartum dominant follicle ovulates generally at ~30 days postpartum (Murphy *et al.,* 1990). Most likely, maintaining a healthy metabolic homeostasis during the peripartum, favors the early onset of ovarian activity. In effect, animals that only ovulated after CIDR removal and cows not cycling at all during the entire duration of the experimental period, did not manage to maintain an equilibrium in body fat reserves.

As expected, the majority of animals, in either group, displaying ovulatory estrus at about 10 days after CIDR withdrawal. This finding is obviously the effect of the drug. Short-term CIDR-based protocols have been the preferred method to synchronize estrus and ovulation in postpartum beef cows (Lamb *et al.,* 2001; Bridges *et al.,* 2008; Wilson *et al.,* 2010). As has been reported, progesterone stimulates the onset of ovarian activity allowing the establishment of follicular waves, with the stable presence of a dominant follicle, which in the right hormonal environment can ovulate (Rhodes *et al.,* 2003). On the other hand, the cows in group ≥ 30 dpp displayed a bigger number of animals ovulating when compared to the group of cows <30 dpp ($P < 0.05$). This is likely the consequence of the time postpartum when the treatment was applied (Rhodes *et al.,* 2003). In the present study, almost 20% of the cows did not cycle during the duration of the experimental period, similar results have been reported previously (Pérez-Torres *et al.,* 2015; Mondragón *et al.,* 2016). These animals coincidently experienced FAT losses after parturition. Which are the correct metabolic indicators to monitor these types of cows? This question remains to be elucidated. A total of 64% of the animals were detected in estrus which falls within the limits of previous research of animals raised under tropical conditions (for review see Galina and Orihuela, 2007).

As anticipated, cows with more than 30 days postpartum on average had a higher number of cows pregnant. This phenomenon is the consequence of the suckling stimulus being minimized as the calves start to use other ingredients for their nutrition (Rasby R., 2007; Pérez-Torres *et al., 2016*). On the other hand, animals calving early, are away from the physiological factors that parturition generates in the organism (negative energy balance, uterine involution, maximum milk production). Thus, these animals present a more favorable condition to become pregnant when entering a late reproductive program (for review see Grummer *et al.,* 2010). In contrast, cows recently calved need to recover for the energy loss after parturition (Bittar *et al.,* 2014). Thus, it is not surprising to either find a larger population of cows not pregnant, or still in anestrus postpartum in the group with less than 30 days after calving. Nonetheless, cows became pregnant earlier did not showed major changes in fat thickness. In effect, Samadi *et al.* (2013), reported that cows properly fed during late gestation would have a better fertility in the early postpartum. In contrast, cows pregnant in the group ≥30dpp did have changes in their FAT homeostasis; the most likely explanation is that other factors, such as the loss of body reserves due to suckling and poor nutrition, have been stabilized (Lamb *et al.,* 2001). This pattern of fertility is evident when comparing pregnant animals in both groups as no differences were found independently of variations in FAT thickness. In addition, animals that became pregnant in both groups did not displayed differences in their total fat variation, (figure 4). Therefore, animals with adequate metabolic conditions will have a better chance of pregnancy regardless of the time postpartum when the reproductive program starts (Shrestha *et al.,* 2005). In conclusion, cows did not show major changes in fat thickness during late prepartum and early postpartum had better opportunity to becoming pregnant regardless of their time postpartum.

Acknowledgement

The authors are grateful for the assistance to the administrative staff of the Centre for Teaching, Research, and Extension in Tropical Animal Husbandry belonging to the Faculty of Veterinary Medicine of the National Autonomous University of Mexico for their assistance in the housing and care of animals. Also, the authors would like to thank MVZ. Daniela Briseño and MVZ. Karen Córdova Hernández for their help in data collection. This study was supported by the Ministry of Higher Education, Science, Technology and Innovation (SENESCYT) of Ecuador with in the program of scholarships for higher education.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Bridges, G.A., Helser, L.A., Grum, D.E., Mussard, M.L., Gasser, C.L., Day, M.L. (2008). Decreasing the interval between GnRH and PGF2alpha from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology, 69, 843-851.
2. Bittar, J.H., Pinedo, P.J., Risco, C.A., Santos, J.E., Thatcher, W.W., Hencken, K.E., Croyle, S., Gobikrushanth, M., Barbosa, C.C., Vieira-Neto, A., Galvão, K.N. (2014). Inducing

ovulation early postpartum influences uterine health and fertility in dairy cows. Journal of Dairy Science, 97, 3558-3569.

3. Crowe, M.A., Goulding, D., Baguisi, A., Boland, M.P., Roche, J.F. (1993). Induced ovulation of the first postpartum dominant follicle in beef suckler cows using a GnRH analogue. Journal of Reproduction and Fertility, 99, 551–555.
4. Crowe, M.A., Diskin, M.G., Williams, E.J. (2014). Parturition to resumption of ovarian cyclicity: comparative aspects of beef and dairy cows. Animal, 8 Suppl 1, 40-53.
5. De Rouen, S.M., Franke, D.E., Morrison, D.G., Wyatt, W.E., Coombs, D.F., White, T.W., Humes, P.E., Greene, B.B. (1994). Prepartum Body Condition and Weight Influences on Reproductive Performance of First-Calf Beef Cows. Journal of Animal Science, 72, 1119-1125.
6. Dunn, T.G., Moss, G.E. (1992). Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock. Journal of Animal Science, 70, 1580–1593.
7. Galina, C.S., Orihuela, A. (2007). The detection of estrus in cattle raised under tropical conditions: what we know and what we need to know. Hormones and Behavior, 52, 32-38.
8. Galindo, J., Galina, C.S., Estrada, S., Romero, J.J., Alarcón, M., Maquivar, M. (2013). Effect of Changes in Body Weight, Body Condition and Back Fat During Last Month of Pregnancy on the Reproductive Efficiency of Bos indicus Cows in the Tropics of Costa Rica. Open Journal of Veterinary Medicine., 3, 22-28.
9. Gross, J., van Dorland, H.A., Bruckmaier, R.M., Schwarz, F.J. (2011). Performance and metabolic profile of dairy cows during a lactational and deliberately induced negative energy balance with subsequent realimentation. Journal of Dairy Science, 94, 1820–1830.
10. Grummer, R.R., Mashek, D.G., Hayirli, A. (2004). Dry matter intake and energy balance in the transition period. The Veterinary clinics of North America Food Animal Practice, 20, 447-470.
11. Grummer, R.R., Wiltbank, M.C., Fricke, P.M., Watters, R.D, Silva-Del-Rio, N. (2010). Management of dry and transition cows to improve energy balance and reproduction. Journal of Reproduction and Development, 56, Suppl, S22-28.
12. Lamb, G.C., Stevenson, J.S., Kesler, D.J., Garverick, H.A., Brown, D.R., Salfen, B.E. (2001). Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. Journal of Animal Science, 79, 2253-2259.
13. Lamb, G.C., Dahlen, C.R., Vonnahme, K.A., Hansen, G.R., Arseneau, J.D., Perry, G.A., Walker, R.S., Clement, J., Arthington, J.D. (2008). Influence of a CIDR prior to bull breeding on pregnancy rates and subsequent calving distribution. Animal Reproduction Science, 108, 269-278.
14. Lents, C.A., White, F.J., Ciccioli, N.H., Wettemann, R.P., Spicer, L.J., Lalman, D.L. (2008). Effects of body condition score at parturition and postpartum protein supplementation on estrous behavior and size of the dominant follicle in beef cows. Journal of Animal Science, 86, 2549-2556.
15. Mondragón, V., Galina, C.S., Rubio, I., Corro, M., Salmerón, F. (2016). Effect of restricted suckling on the onset of follicular dynamics and body condition score in

Brahman cattle raised under tropical conditions. Animal Reproduction Science, 167, 89-95.

16. Montiel, F., Ahuja, C. (2005). Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. Animal Reproduction Science, 85, 1-26.
17. Murphy, M.G., Boland, M.P., Roche, J.F. (1990). Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. Journal of Reproduction and Fertility, 90, 523–533.
18. Orihuela, A., Galina, C.S., Escobar, J., Riquelme, E. (1983). Estrous behavior following prostaglandin f2α injection in zebu cattle under continuous observation. Theriogenology, 9, 795-809.
19. Pérez-Torres, L., Rubio, I., Corro, M., Cohen, A., Orihuela, A., Galina, C.S., Pablos, J.L. (2015). A pre-synchronization program at early pospartum might increase the chances of Bos indicus cows cycling prior to 50 days regardless of the length of calf separation. Journal of Reproduction and Development, 61, 199-203.
20. Pérez-Torres, L., Orihuela, A., Corro, M., Rubio, I., Alonso, M., Galina, C.S. (2016). Effects of separation time on behavioral and physiological characteristics of Brahman cows and their calves. Applied Animal Behaviour Science, 179, 17–22.
21. Pulido, A.L., Zarco, C.S., Galina, C., Murcia, G., Flores, G., Posadas, E. (1991). Progesterone metabolism during storage of blood samples from Gyr cattle: Effects of anticoagulant, time and temperature of incubation. Theriogenology, 35, 965-975.
22. Randel, R. (1990). Nutrition and postpartum rebreeding in cattle. Journal of Animal Science, 68, 853-862.
23. Rasby, R. (2007). Early weaning beef calves. Veterinary Clinics Food Animal Practice, 23, 29–40.
24. Rhodes, F.M., McDougall, S., Burke, C.R., Verkerk, G.A., Macmillan, K.L. (2003). Invited review: treatment of cows with an extended postpartum anestrous interval. Journal of Dairy Science, 86, 1876–1918.
25. Samadi, F., Phillips, N.J., Blache, D., Martin, G.B., D’Occhio, M.J. (2013). Interrelationships of nutrition, metabolic hormones and resumption of ovulation in multiparous suckled beef cows on subtropical pastures. Animal Reproduction Science, 137, 137-144.
26. Savio, J.D., Boland, M.P., Roche, J.F. (1990). Development of dominant follicles and length of ovarian cycles in post-partum dairy cows. Journal of Reproduction and Fertility, 88, 581–591.
27. Schröder, U.J., Staufenbiel, R. (2006). Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. Journal of Dairy Science, 89, 1-14.
28. Shrestha, H.K., Nakao, T., Suzuki, T., Akita, M., Higaki, T. (2005). Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high-producing dairy cows in a subtropical region in Japan. Theriogenology, 64, 855-866.

29. Singh, R., Randhawa, S.N., Randhawa, C.S. (2015) Body condition score and its correlation with ultrasonographic back fat thickness in transition crossbred cows. Veterinary World, 8, 290-294.

30. Short, R.E., Bellows, R.A., Staigmiller, R.B., Berardinelli, J.G., Custer, E.E. (1990). Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. Journal of Animal Science, 68, 799-816.

31. Stagg, K., Diskin, M.G., Sreenan, J.M., Roche, J.F. (1995). Follicular development in long-term anoestrous suckler beef cows fed two levels of energy postpartum. Animal Reproduction Science, 38, 49–61.

32. Wettemann, R.P., Lents, C.A., Ciccioli, N.H, White, F.J., Rubio, I. (2003). Nutritional- and suckling-mediated anovulation in beef cows. Journal of Animal Science, 81, (E. Suppl. 2), E48-E59.

33. Wilson, D.J., Mallory, D.A., Busch, D.C., Leitman, N.R., Haden, J.K., Schafer, D.J., Ellersieck, M.R., Smith, M.F., Patterson, D.J. (2010). Comparison of short-term progestin-based protocols to synchronize estrus and ovulation in postpartum beef cows. Journal of Animal Science, 88, 2045-2054.

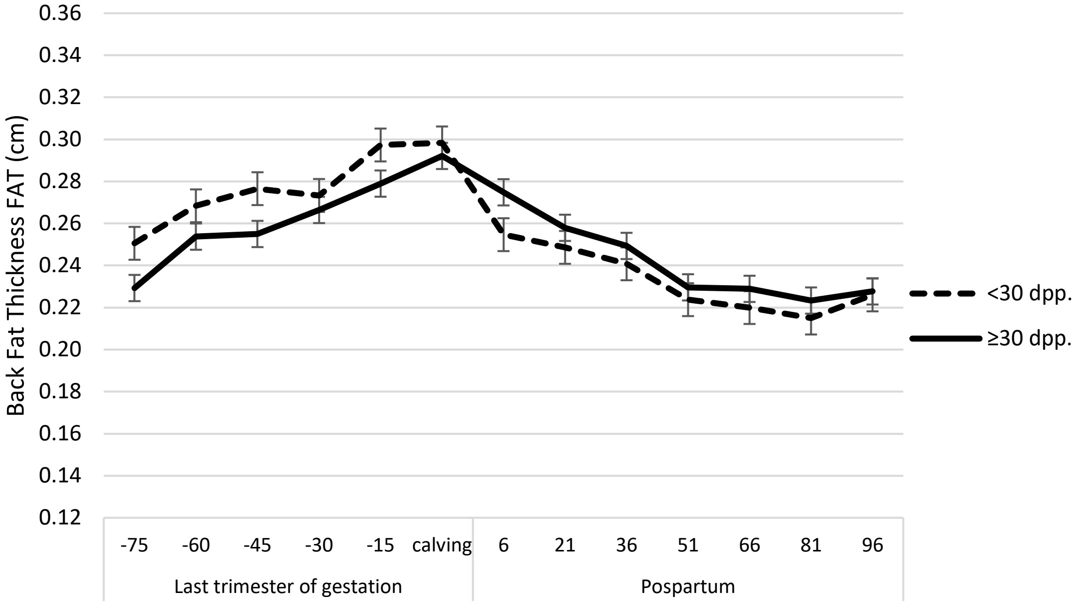


Figure 1: Values for body back fat during the last trimester of pregnancy and the postpartum period in *Bos indicus* (bars indicate standard error). Statistical differences were observed when comparing the last trimester of pregnancy and the early postpartum in the groups <30dpp (P<0.00001) and ≥30dpp (P=0.027). The comparison between groups of cows <30dpp vs ≥30dpp was not significant (P>0.05).

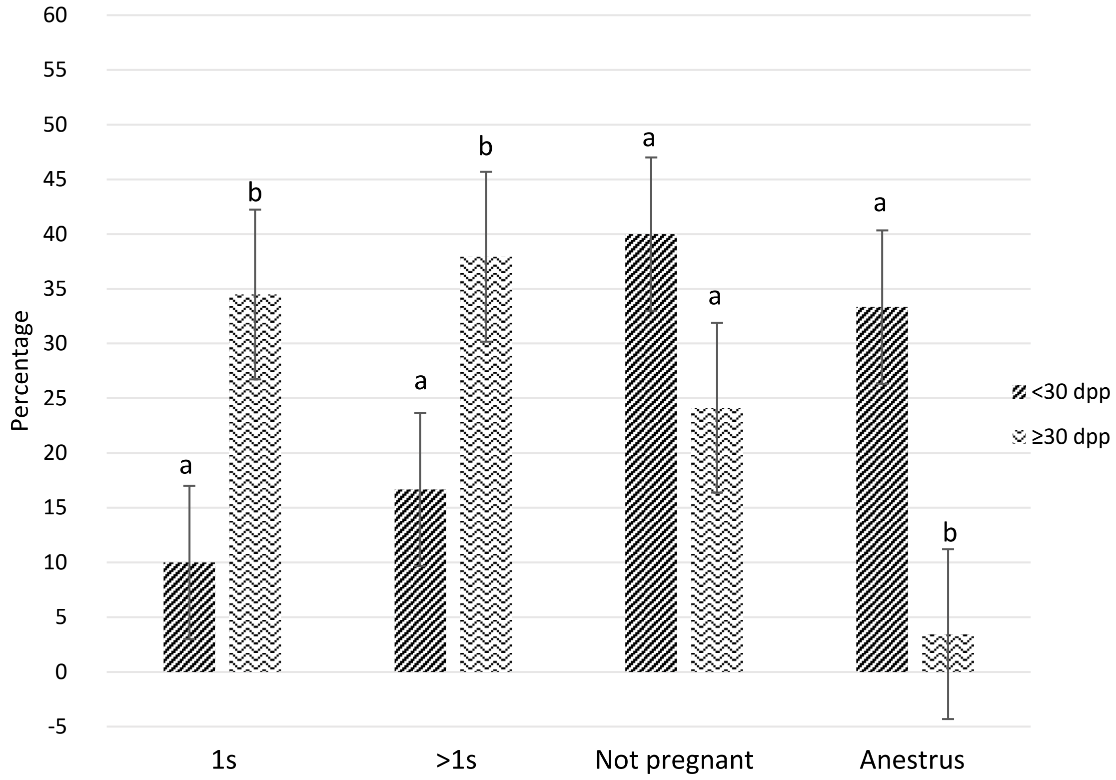


Figure 2: Percentage of animals pregnant at first service (1s), more than one service (>1s), not pregnant and in anestrus in groups <30dpp and ≥30dpp (bars indicate standard error). Different letters within columns indicate significance ($P<0.05$) in groups <30dpp vs. ≥30dpp.

Table 1: Values of back fat for cows 1s, > 1s, not pregnant and in anestrus in the groups <30dpp and ≥ 30dpp.

	Groups	Last trimester of gestation	postpartum	P
< 30dpp	**1s**	0.24 ± 0.01	0.24 ± 0.02	0.958
	>1s	0.26 ± 0.03	0.23 ± 0.03	0.188
	Not pregnant	0.28 ± 0.03	0.24 ± 0.02	0.0001**
	Anestrus	0.3 ± 0.02	0.22 ± 0.02	0.0003**
≥ 30dpp	**1s**	0.26 ± 0.03	0.24 ± 0.02	0.014**
	>1s	0.25 ± 0.02	0.25 ± 0.03	0.884
	Not pregnant	0.27 ± 0.04	0.24 ± 0.01	0.279
	Anestrus*			

* Only one cow remained in anestrus.

** Indicate a highly significant difference in the last trimester of gestation and the early postpartum.

1s: Pregnant in one service.

>1s: Pregnant in more than one service.

P: Levels of significance.

No difference when comparing cows in the groups < 30dpp and ≥ 30dpp (P>0.05).

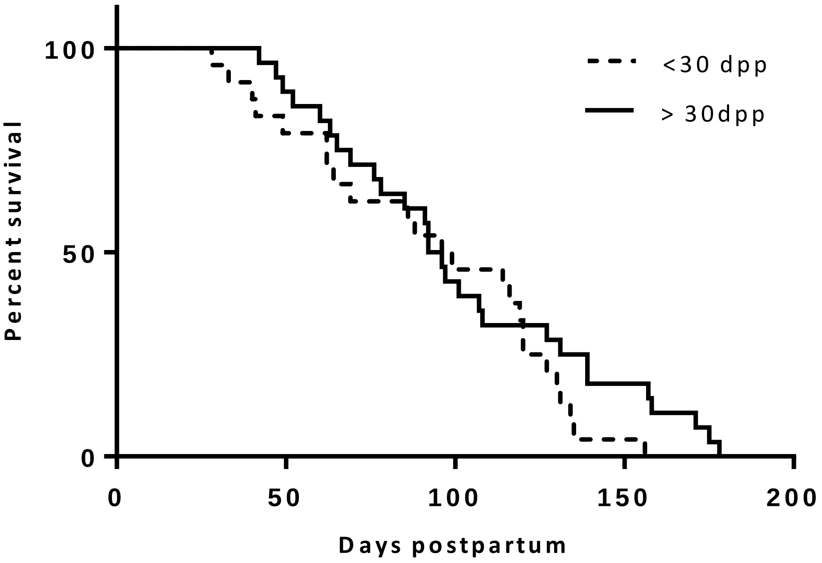


Figure 3: Survival analysis for the time of gestation in the groups <30 dpp and ≥30dpp. Differences were not significant (P>0.05).

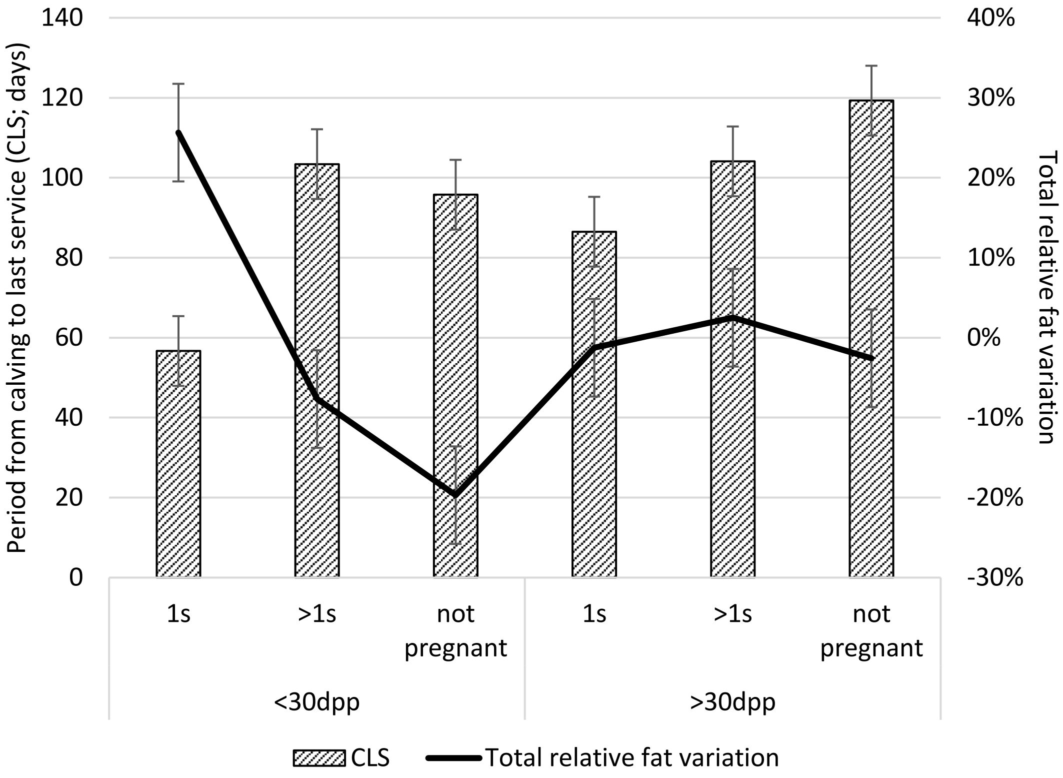


Figure 4: Days postpartum of the two groups studied compared to the percentage of total relative backfat variation between the last third of gestation and the postpartum. Anestrus cows are deleted from the graph.

CAPITULO 4

EXPERIMENTO 3

Influencia del índice de temperatura-humedad y los niveles de grosor de la grasa durante el último trimestre de gestación y posparto temprano sobre la fertilidad de las vacas *Bos indicus* en el trópico.

Artículo enviado para publicación en:

Animals - Open Access Animal Science and Animal Welfare Journal

Article

INFLUENCE OF TEMPERATURE-HUMIDITY INDEX (THI) AND DORSAL FAT THICKNESS DURING THE LAST TRIMESTER OF GESTATION AND EARLY POSTPARTUM ON THE FERTILITY OF *BOS INDICUS* COWS IN THE TROPICS

Ramiro Fernando Díaz [1]*, Carlos Salvador Galina[1], Sandra Estrada[2], Felipe Montiel[3], Gloriana Castillo[2], Juan José Romero[2]

[1] Departamento de Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, México DF, 04510 México
[2] Programa de Investigación en Medicina Poblacional. Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica, Heredia, Costa Rica. Código postal: 304-3000 Heredia.
[3] Departamento de Reproducción, Facultad de Medicina Veterinaria, Universidad Veracruzana Circunvalación S/N Esquina Yáñez, Código Postal 91710, Veracruz, México
* Correspondence: rdiaz@usfq.edu.ec; Tel.: + 52 (1) 5512916066

Simple Summary: The climate in the tropics is a factor that can alter the normal development of animal reproduction. In turn, animals show changes in their energy reserves because of processes that occur before and after calving. This study investigated the effects of high levels of temperature-humidity index and variation of back fat on days at risk to conception, on farms in two dry tropical countries. Animals that showed greater variation in back fat had more difficulty in becoming pregnant. This event showed a strong relation with high levels of temperature-humidity index.

Abstract: In order to measure the influence of THI and the variation of fat thickness on reproductive performance, ninety-two *Bos indicus* cows kept under grazing conditions were used in two farms in Veracruz (Mexico) and Puntarenas (Costa Rica). THI was calculated with the average environmental temperature and relative humidity. Measurements of fat thickness (FAT) were taken two weeks apart from the last trimester of gestation to approximately 80 days postpartum (dpp). Conception by natural mating considered the interval of days at risk to conception (DRC) to be at least 28 dpp. THI was between 65.4 ± 2.9 and 73.2 ± 1.5 in Veracruz and 75.4 ± 0.26 and 76.5 ± 0.55 in Puntarenas. Variations in THI were observed in Puntarenas whereas in Veracruz, THI went from comfort levels to stress alert. In Veracruz, loss of fat during the last trimester of gestation was on average 8.5%, whereas in the postpartum period it was 18.4% (P = 0.042). In Puntarenas, the variation in the last trimester of gestation was on average 18.7% and in the postpartum period, 10.5% (P = 0.012). The relative change in FAT in Veracruz was 36.7%, and into Puntarenas, 29.3%. Overall, 60% of the cows became pregnant. FAT decreased the interval of DRC (R^2=0.06; P=0.033) with a high relationship (R^2=0.76; P< 0.0001) between THI and time to conception. In conclusion, THI levels influence the reproductive performance in early postpartum affecting DRC in tropical areas.

Keywords: temperature-humidity index; fat thickness; Brahman. ovarian activity; time conception.

Introduction

The effect of the environment on productive and reproductive processes in cows has been the subject of much study [1,2]. Stress due to the harsh environmental conditions has several effects on reproductive physiology, such as the presence of overt signs of estrus, ovulation rate, and embryo survival [2,3]. Villa-Mancera *et al.* [4], reported a direct effect of the temperature-humidity index (THI) on conception rate, registering lower levels during the summer months compared to the winter. It is well-known that *Bos indicus* cattle raised under tropical conditions, show prolonged calving intervals [5,6], which are attributed to long periods of postpartum anestrus [7]. Fat thickness (FAT) has been shown to be a reliable indicator of the level of energy reserves in the animal [8]. There is evidence suggesting that the FAT during the last trimester of pregnancy is associated with reproductive capacity [9]. Therefore, early resumption of ovarian activity is closely related to the ability of the dam to recover from the negative energy balance caused by stress during labor [5] and possibly heat stress [10]. Thus, the objective of the present study was to measure the influence of THI and the variation of fat thickness during the last trimester of gestation and early postpartum on the fertility of *Bos indicus* cows in the dry tropics.

Materials and Methods

The Animal Care Internal Committee (CICUA) of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico approved the methods used during the present work in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Location

This study was carried out simultaneously in two farms from February to August 2015, located at 19°10'36.93" N and 96°16'3.20" O in the municipality of Manlio Fabio Altamirano of the state of Veracruz – Mexico with a precipitation and temperature average of 108.4 mm and 27.05 ºC respectively. The other farm at 10°2'39.04" N and 84°54'23.44" O, situated in the province of Puntarenas, Costa Rica with a precipitation and temperature average of 111.87mm and 27.42 ºC respectively. Both farms are located in the zone considered as dry tropics. The THI for each farm was calculated monthly considering the average environmental temperature and relative humidity of the period in which the study was conducted, applying the formula proposed by García-Ispierto *et al*. [11]:

$THI = (0.8 \times T + (RH/100) \times (T - 14.4) + 46.4)$

Where: THI, is the temperature humidity index; T, is the mean temperature expressed in °C; and RH, is the average relative humidity expressed in percentage. Unfortunately, wind speed and solar radiation were not considered, it was assumed that THI was the best indicator of animal heat stress [12-14]. As this was an observational study in private farms, data on climatic factors were taken from weather stations nearby not further apart than 10 km. THI levels over 74 were considered indicators of heat stress.

In addition, the photoperiod (number of daylight hours) in each farm during the study was measured. This measure is known as *maximum duration of insolation* and is estimated as follows [15]:

$N = (24/\pi)\ \omega_s$

Where: N, is the maximum duration of insolation; and ω_s, is the angle of solar radiation at sunset time, calculated with the following equation:

$\omega_s = (\pi/2) - \text{arccos}\ [-\tan(\phi)\ \tan(\delta)]$

Where: ϕ, is the latitude expressed in radians; and δ, is the solar declination expressed in radians, calculated as:

$\delta = 0.409 * \text{sen}\ \{[(2\pi/365)\ J] - 1.39\}$

Where: J, is the number of days in the year between 1 (1º of January) and 365 (31 of December).

Animals

Ninety-two multiparous Brahman cows in their last trimester of gestation were used. Animals were managed under an extensive grazing system. In Veracruz (n= 37), paddocks contained pastures such as Pangola *(Digitaria decumbens),* Guinea *(Panicum maximum),* Estrella *(Cynodon plectostachyus)* and Gramilla *(Cynodon dactylon)*. In Puntarenas (n= 55), pastures were mainly: Braquiaria (*Brachiaria brizantha)* and *Guinea (Panicum maximum).* The animals were not given supplementary feed during this period, although they had access *ad libitum* to water and mineral salts.

Fat thickness measurement

In both farms, all measurements of fat thickness (FAT), expressed in centimeters (cm), were taken two weeks apart from the beginning of the last trimester of gestation to around 80 days postpartum (dpp), using the ALOKA ProSound 2 ultrasound equipment with a convex array transducer 3.5 MHz. The transducer was placed horizontally in the caudal region between the iliac and the ischial tuberosity [8].

Evaluation of time to conception

Natural breeding was used in both farms; the moment when the bull joined the herd was an important factor for evaluation of reproductive performance. As an observational experiment, it did not intervene in the reproductive management of each farm. However, on each farm bull andrological examinations were performed and their fertility tested. Time to conception was calculated based on the interval from days at risk to conception (DRC), corresponding to the number of days that a cow has the higher probability to get pregnant following first exposure to the bull. It was established the DRC starting 28 dpp to the day when the bull joined the herd and was calculated based on the interval from partum to conception (PCI) centered on serial ultrasounds to measure the size of the embryo using the scale proposed by Rosiles *et al.* [16]. Cows were divided in three subgroups in relation to the time when the bull was introduced to the herds:

1. Calved when the bull joined the herd ≥ 28 dpp.
2. Calved when the bull joined the herd < 28 dpp.
3. Still pregnant when the bull joined the herd of cows.

Using this arbitrary division, for animals in subgroup 1 and 2, DRC was calculated by subtracting PCI from calving to the day the bull joined the herd of cows by applying the formula DRC = PCI- calving days until the herd was exposed to the bull.

For the cows that were already pregnant when the bull joined the herd, DRC was calculated subtracting 28 from the PCI (DRC=PCI-28).

The bull was with the herds for 65 days. Pregnancy diagnosis was performed in those cows with more than 28 days pp using an ultrasound ALOKA ProSound 2 equipped with a linear transducer of 7.5 MHz. In addition, the percentage of pregnant cows was measured depending on the length of exposure to the bull. Consequently, an analysis was carried out in those animals that were pregnant within the first 28 days of exposure to the bull, pregnant animals for the period from 28 to 56 days after the entry of the bull and finally, those animals that were pregnant after 65 days from the entrance of the bull.

Statistical analysis

The interval of days at risk to conception for all groups of cows for each farm was calculated using the arithmetic mean and standard deviation. The percentage of cows that were pregnant on different days after the entry of the bull, were compared using a T-Student test for each point of measuring. Changes in back fat thickness (cm) during the last trimester of pregnancy and until the moment of gestation, or until the time when observations finished, were analyzed by descriptive statistics and a T-Student test for each point of measuring. In all cases, in order to establish possible differences, the 95% confidence interval was also calculated. Conversely, total relative change in back fat (ΔFAT= [initial measure - final measure] / initial measure) was determined. This variable was considered in either continuous or categorical form divided into high change (≥ 66th percentile), medium change (between 33th and 66th percentiles) and low change (≤ 33th percentile). Percentiles were established specifically for each farm.

The relationship between THI and daylight hours with the relative changes of FAT and DRC were established by simple lineal regression, and one-way ANOVA to compare the means of FAT relative change, categorized with respect to DRC.

On the other hand, the percentage of pregnant animals was measured depending on the time exposed to the bull. Comparisons of percentages by mean of chi-square test and the 95% confidence interval were performed for each group of cows. Thus, an analysis was carried out in those animals pregnant within the first 28 days of exposure to the bull, those that became pregnant within 56 days after the entry of the male and finally, those animals that became pregnant within 65 days following the entrance of the bull.

Finally, a non-parametric survival analysis was performed using Kaplan-Meier curves to evaluate the effect of total relative BF change on the curves of DRC survival function, using the log-rank as a statistical test for the curves comparison and total relative BF change in a categorical form (high, medium and low change).

In all statistical processes, a significance value of 5% was used as the point of statistical decision. All the analyses were performed using IBM SPSS 22 e Infostat 2016. Statistical analysis were performed between the experimental groups using each of the farms as a reference, without a statistical comparison.

Results

Temperature-humidity index and daylight hours

In Veracruz, THI levels were between 65.4 ± 2.9 and 73.2 ± 1.5; and in Puntarenas between 75.4 ± 0.26 and 76.5 ± 0.55 (Figure 1).

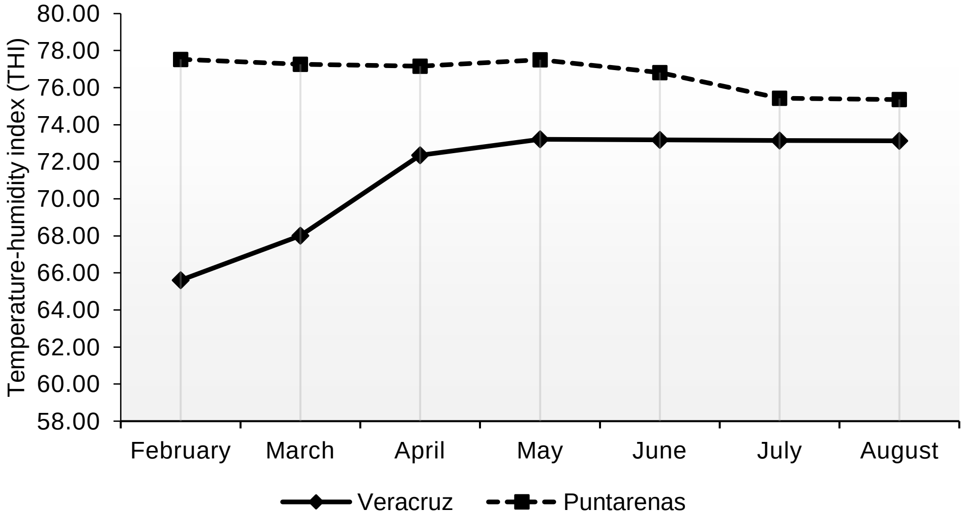


Figure 1. Temperature-humidity index (THI) for Veracruz and Puntarenas from the months of February to August 2015.

Considering their different geographic locations, the farms had diverse daylight changes during the study. In January, Veracruz had less than 11 daylight hours and more than 13 hours in June, while in Puntarenas it was more than 11 hours in January and by June the daylight hours did not reach 13 (Figure 2).

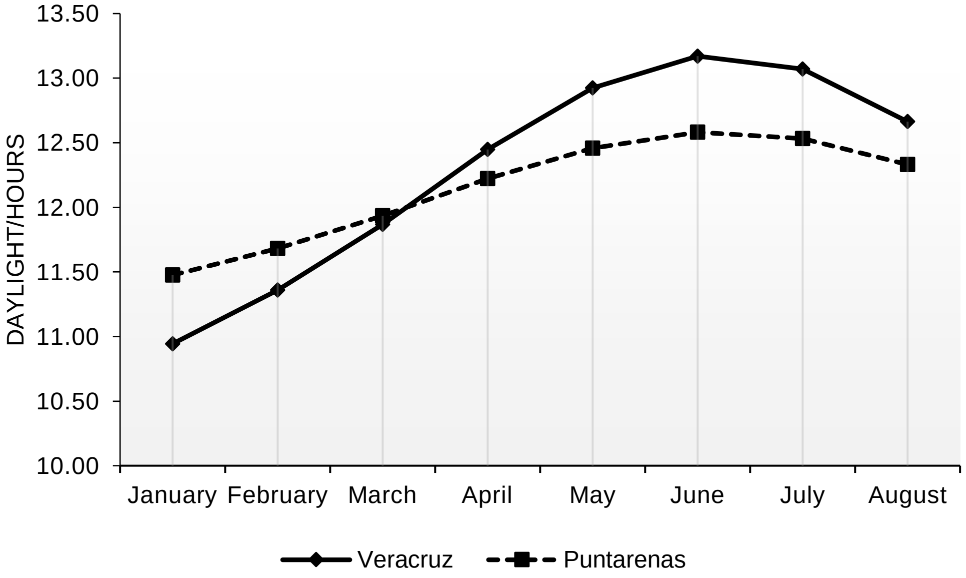


Figure 2. Daylight /hours in Veracruz and Puntarenas from January to August 2015.

Fat thickness (FAT).

In both farms, FAT decreased gradually during all the study. On average, cows initiated with 0.28 cm, reducing to 0.23 cm at calving, and down to 0.20 cm at the end of the observation period. General and specific changes per farm are shown in Figure 3.

Body fat changes showed differences between farms. In Veracruz, loss of FAT during the last trimester of gestation was on average 8.5%, and 18.4% in the postpartum period (P = 0.042). In Puntarenas, the variation in the last trimester of gestation was on average 18.7% and in the postpartum period, 10.5% (P = 0.012). In total, the relative change in BF thickness was greater in Veracruz, 36.7%, than in Puntarenas, 29.3% (Figure 3).

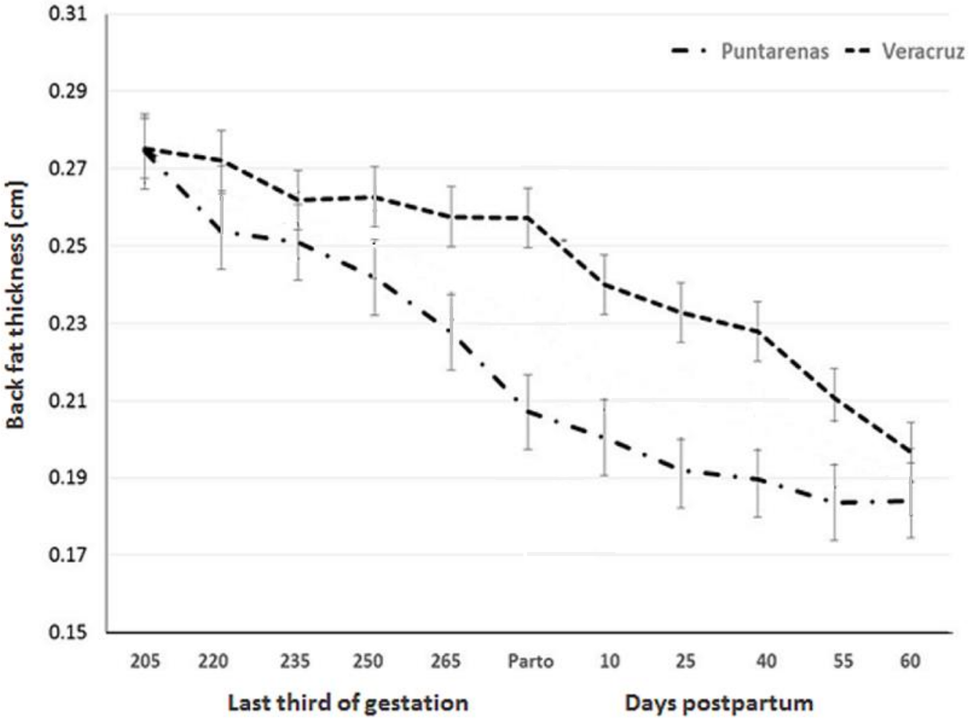


Figure 3. Back fat thickness in all cows, and specifically in Veracruz and Puntarenas cows (error bars are presented).

Time to conception

In Veracruz, the bull joined the herd when 30% of the cows were still pregnant; 46% were less than 28 dpp and 24%, more than 28. In Puntarenas, the bull joined the herd when all cows had calved; 26% were between 15 and 30 dpp; 30% between 31 and 52 dpp; 25% between 53 and 60 dpp and 19% between 61 and 72 dpp. In total, 60% conceived during the observation period, in Veracruz, 49% and 67% in Puntarenas. In Veracruz, none of the non-pregnant animals conceived during the first 28 days after the bull was with the herd. In contrast, at 56 days after bull exposure, 47% had conceived ($P<0.05$). The percentage of pregnancies in Puntarenas for the first 28 days after bull exposure was 47%. Furthermore, by 56 days 48%. had become pregnant. No pregnancies were observed after this period.

When grouping the farms according to the time postpartum when at risk to becoming pregnant, conception rates were 39% for the first 28 days, 28% for the period from 29 to 56 days and 31% for the cows becoming pregnant after this date.

Global average of PCI was 78.5 ± 19.0 dpp. Specifically, the average of PCI per farm was 91.6 ± 14.8 and 72.2 ± 17.6 for Veracruz and Puntarenas, respectively.

Relationship between temperature-humidity index, changes in fat thickness and time to conception

The loss of FAT decreased the interval of DRC (R^2 =0.06; P=0.033). This same tendency was observed within farms, although without statistical significance (P= 0.7931 and P=0.4207 for Puntarenas and Veracruz, respectively). There was a strong relationship (R^2 = 0.76; $P< 0.0001$) between THI during postpartum and time to conception.

Significant differences were not found ($P > 0.05$) between the averages of DRC analyzed by linear regression comparing high, medium and low FAT levels. This was confirmed by the Kaplan-Meier test, where cows with greater FAT loss had a more prolonged survival function for PCI, whereas the cows that showed medium and low FAT loss became pregnant faster (Chi2 log rank test = 1.786, P =0.41). The mean survival (DRC) was 34 days for cows with high FAT, whilst for medium and low 26 and 32 days, respectively. (P = 0.17; Figure 4).

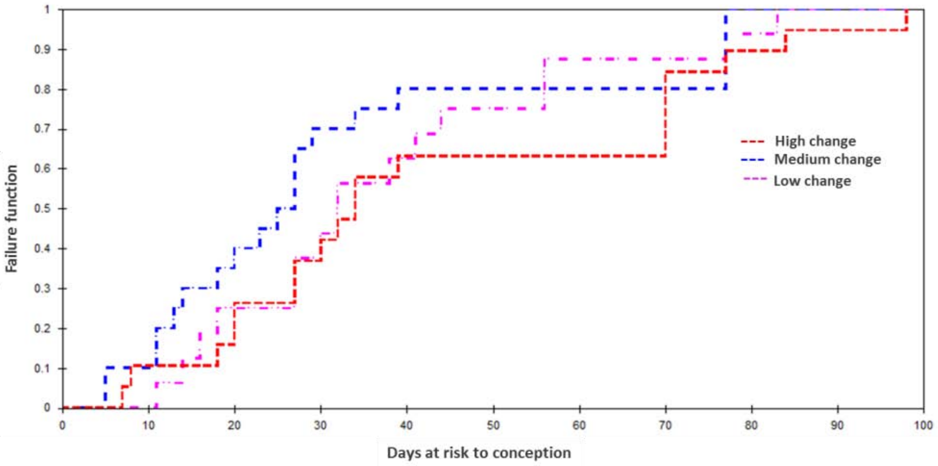


Figure 4. Kaplan-Meier`s failure function curve for time to conception in *Bos indicus* cows from two farms located in the dry tropical climate of Mexico and Costa Rica. No major differences were observed in the medium and low FAT changes whereas the cows with high changes were statistically different ($P<0.05$).

Discussion

Animals from both farms lost back-fat thickness (FAT) during the last trimester of gestation, as well as in the postpartum period. The change in their metabolic status may be associated with specific events taking place towards the end of gestation and the beginning of lactation. Bovines, as do other mammals, exhibit maximum fetal growth in the last trimester of gestation [17,18]. Consequently, the requirements of the fetus are greatest at this time [17]. On the other hand, during the early postpartum period, the animals suffer an imbalance in energy status, due to the beginning of the lactation period, coupled with intensive suckling. This causes the dam to mobilize body energy reserves, which triggers negative energy balance [19]. This was confirmed in the present study, since the animals from the farm in Veracruz had greater back fat thickness loss during the postpartum period as opposed to the prepartum (P = 0.042). In earlier research, a poor correlation has been found between body condition and fat thickness, and several authors have confirmed that dorsal fat thickness is an indicator of nutritional status far more reliable than body condition [8,20].

However, the cows in the farm at Puntarenas lost less fat in the postpartum period, compared to prepartum (19.2% *vs* 11.3%, respectively; P = 0.012). This change in the variation of fat loss may be associated with differences in daylight duration. Veracruz had greater daylight hour variation than Puntarenas during the experiment. Light increases water-soluble carbohydrate levels and decreases the concentration of lignin in the tropical pastures causing an increase in digestibility [21]. The last trimester of gestation in Veracruz occurred in the majority of animals; during the period of shorter daylight hours suggesting that the digestibility of the pastures could have not have been optimal during this period. Unfortunately, no measurements of grass quality were undertaken, however, there is ample evidence [21-27] demonstrating the sensitivity of pastures to the number of light hours. On the contrary, the time of year with more daylight hours, coincided in most of the animals with the postpartum period. Thus, an increase in daylight hours would likely have improved pasture digestibility.

Then again, for the last four decades, the temperature-humidity index has become a standard in cattle management practices [14,28,29] to assess livestock comfort. According to climatological data for the year in which this study was conducted [30], cows in Veracruz were not subjected to heat stress (state of comfort < 74 THI) only during last trimester of gestation. Once calving started, THI increased towards the alert area on the THI scale (between 74 and 78 THI) [30], probably causing stress in the animals and affecting their feed intake. Instead, animals in Puntarenas were constantly in the alert zone and without drastic changes in THI, suggesting a state of physiological adaptation to the stable environmental conditions prevailing during most of the year. The present investigation was carried out in animals at pasture, which makes it difficult to measure items such as respiratory rate and rectal temperature. The division of animals in comfort, alert, damage and emergency were used as in indirect measure of heat stress [30] which has been documented before [1].

Conception rates were 49% in Veracruz and 67% in Puntarenas giving a mean of 60%, in agreement with the 41 to 70% for *Bos indicus* cows previously reported [31-33]. The PCI average of both farms was 78.5 ± 19.0 days, similar to that observed by Schramm *et al*. [34], who reported a calving-conception interval in *Bos indicus* of 81 ± 9 and 79 ± 8 days among their experimental groups. However, PCI was different for the two farms, longer in Veracruz than in Puntarenas (91.6 and 72.2, respectively). Likewise, DRC was greater in Veracruz than in Puntarenas (68.9 and 22.5, respectively). The time postpartum when the animals entered the reproductive program may have a direct effect on fertility. Whereas in Veracruz, 75% of cows had less than 28 dpp or were pregnant when the bull joined the herd; 100% of the cows in Puntarenas had already calved. Consequently, 85% of the animals in Puntarenas were already at risk of conception in the first 28 days after the bull joined the herd, in comparison to only 24% in Veracruz. Previous reports indicate that the time required for cows to become pregnant relates to the average number of days postpartum when the bull joins the herd [35,36].

THI may have a direct effect on fertility [4,11] explaining in part the differences in time to conception between the two farms. In Veracruz, there were drastic changes in THI during the study, from 65.4 in the months of February and March to an average of 73.2 in late June, July and August with conditions varying from comfort to alert [30]. In Puntarenas, in contrast, THI remained stable with an average of 76 for the duration of the study. One shortcoming of the present study is the variation in the numbers of animals at risk of becoming pregnant when the bull joined the herd. However, even with this limitation, results suggest it is important for future studies to specify not only the exact location but provide precise information about daylight hours and THI.

Animals with greater FAT variation tended to delay pregnancy longer compared to those with medium or low variation. Samadi *et al*. [37] reported that animals consuming better quality pastures between the 6th and 7th month of gestation, resumed ovulation earlier. Therefore, it is reasonable to assume that animals with superior energy reserves during the last trimester of gestation and in early postpartum, are likely to conceive in a shorter time after calving than animals mobilizing their fat reserves, regardless of the number of days between parturition and the start of the reproductive program. Insufficient intake of calories during the last stages of gestation, affects fertility even when energy intake is adequate during lactation [9,38-40]. Hess *et al.* [41], in an extensive review, reported that the level of feed intake during prepartum has a greater effect on postpartum anestrus than does feed intake after calving. This indicates that FAT variation during the last trimester of gestation directly influences postpartum reproductive development. High fat loss will negatively affect the capacity to resume reproductive activity and time of conception.

On the other hand, THI has been shown to relate strongly with fertility, particularly during the postpartum period ($R^2 = 0.76$; $P > 0.0001$). These results are similar to those previously reporting a close relationship of climatic factors with conception rate, progesterone levels, duration and intensity of estrus [4,11,42,43]. The change to alert levels of THI in Veracruz during the summer months (between 74 – 78) could help to explain the lower fertility encountered. These results also agree with studies conducted in European cattle, for instance, Petersson *et al.* [43], found that the interval between parturition and first luteal activity is greater when calving takes place during winter and the return to the reproductive program occurs in the summer. In contrast, when calving takes place during summer and the return to the reproductive program occurs in the winter. García-Ispierto *et al* [11], observed that as THI increases around 3 days after artificial insemination, conception rate decreases. Some reports also indicated an effect on conception rate [44], and days open [10] when fluctuations in THI occur in the locality. Moreover, Alves *et al.* [45], found that metabolic changes due to variations in THI could compromise the follicular milieu producing morphological changes in the oocytes, increases in body temperature [46], and in food consumption [47]. These malfunctions affect body condition thus delaying the resumption of ovarian activity postpartum [48]. In Veracruz, increasing levels of THI of more than 7 points, occurred when 70% of cows were at risk of pregnancy. However, in Puntarenas, THI showed only a 1.1 variation during the study suggesting that animals probably did not have to adapt to drastic changes in this important parameter [14,49].

Finally, it is worth pointing out the differences in the reproductive status of cows when the bull enters the breeding program. It is common management practice to employ animals regardless of whether they are likely to conceive or not which was the case of the farm in Veracruz. This policy needs revision.

Conclusions

Temperature-humidity index levels in tropical areas could influence the reproductive development of animals in early postpartum affecting conception rate. The loss of fat either in the prepartum or postpartum periods affects time to conception being shorter which in those animals losing less FAT during the same periods. However, further research is in demand before any conclusions can be safely drawn.

Acknowledgments: The authors wish to sincerely thank Jane Russell Ph.D. for her collaboration in the grammatical review of this article. This study was supported by the Ministry of Higher Education, Science, Technology and Innovation (SENESCYT) of Ecuador with in the program of scholarships for higher education.

Author Contributions: RDB: designed and conducted the research, analyzed the data and wrote the manuscript. CSG: designed the research and edited the manuscript. SE: conducted the research. FM: conducted the research. GC: conducted the research. JJR: analyzed the data.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Azzam, S.M.; Kinder, J.E.; Nielsen, M.K.; Werth, L.A.; Gregory, K.E.; Cundiff, L.V.; Koch, R.M. Environmental effects on neonatal mortality of beef calves. *J Anim Sci*. **1993**, 71, :282-290.
2. Silva, C.F.; Sartorelli, E.S.; Castilho, AC, Satrapa RA, Puelker RZ, Razza EM, Ticianelli JS, Eduardo HP, Loureiro B, Barros CM. Effects of heat stress on development, quality and survival of *Bos indicus* and *Bos taurus* embryos produced in vitro. *Theriogenology.* **2013**, 79, 351-357.
3. Hansen, P.J. Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. *Theriogenology*. **2007**, 68, Suppl.1, S242-S249.
4. Villa-Mancera, A.; Méndez-Mendoza, M.; Huerta-Crispín, R. Vázquez-Flores, F.; Córdova-Izquierdo, A. Effect of climate factors on conception rate of lactating dairy cows in Mexico. *Trop Anim Health Prod*. **2011**, 43, 597-601.
5. Montiel, F.; Ahuja, C. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. *Anim Reprod Sci*. **2005**, 85, 1-26.
6. Escrivão, R.J.A.; Webb, E.C.; Garcês, A.P.J.T. Effects of 12-hour calf withdrawal on conception rate and calf performance of Bos indicus cattle under extensive conditions. *Trop Anim Health Prod*. **2009**, 41, 135–139.
7. Baruselli, P.; Reis, E. Marques, M.; Nasser, L. Bó G. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Anim Reprod Sci*. **2004,** 82-83, 479-486.

8. Schröder, U.J.; Staufenbiel, R. Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. *J Dairy Sci.* **2006**, 89, 1-14.
9. Wettemann, R.P.; Lents, C.A.; Ciccioli, N.H.; White, F.J.; Rubio, I. Nutritional- and suckling-mediated anovulation in beef cows. *J Anim Sci*. **2003**, 81(E. Suppl. 2), E48-E59.
10. Boni, R.; Perrone, L.L.; Cecchini, S. Heat stress affects reproductive performance of high producing dairy cows bred in an area of southern Apennines. *Livest Sci.* **2014,** 160, 172–177.
11. García-Ispierto, I.; López-Gatius, F.; Bech-Sabat, G.; Santolaria, P.; Yániz, J.L.; Nogareda, C.; De Rensis, F.; López-Béjar, M. Climate factors affecting conception rate of high producing dairy cows in northeastern Spain. *Theriogenology*. **2007**, 67, 1379-1385.
12. Dikmen, S.; Hansen, P.J. Is the temperature-humidity index the best indicator of heast stress in lactating dairy cows in a subtropical environment? *J Dairy Sci*. **2009**, 92, 109-116.
13. Schüller, L.K.; Burfeind, O.; Heuwieser, W. Short communication: Comparison of ambient temperature, relative humidity, and temperature-humidity index between on-farm measurements and official meteorological data. *J Dairy Sci.* 2013, 96, 7731-7738.
14. Berman, A.; Horovitz, T.; Kaim, M.; Gacitua, H. A comparison of THI indices leads to a sensible heat-based heat stress index for shaded cattle that aligns temperature and humidity stress. *Int J Biometeorol.* **2016**, 60, 1453-1462.
15. Food and Agriculture Organization of the United Nations [FAO]. Evapotranspiración del cultivo: Guías para la determinación de los requerimientos de agua de los cultivos. Rome, Italy http://www.fao.org/docrep/009/x0490s/x0490s00.htm 2006. Accessed: 10 May 2017.
16. Rosiles, V.A.; Galina, C.S.; Maquivar, M.; Molina, R.; Estrada, S. Ultrasonographic screening of embryo development in cattle (Bos indicus) between days 20 and 40 of pregnancy. *Anim Reprod Sci*. **2005**, 90, 31-37.
17. Bell. A.W. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J Anim Sci*. **1995**, 73, 2804-2819.
18. Guedon, L.; Saumande, J.; Desbals, B. Relationships between calf birth weight, prepartum concentrations of plasma energy metabolites and resumption of ovulation postpartum in limousine suckled beef cows. *Theriogenology.* **1999**, 52, 779-789.
19. Grummer, R.R.; Mashek, D.G.; Hayirli, A. Dry matter intake and energy balance in the transition period. *Vet Clin North Am Food Anim Pract.* **2004**, 20, 447-470.
20. Galindo, J.; Galina, C.S.; Estrada, S.; Romero, J.J.; Alarcón, M.; Maquivar, M. Effect of Changes in Body Weight, Body Condition and Back Fat during Last Month of Pregnancy on the Reproductive Efficiency of Bos indicus Cows in the Tropics of Costa Rica. *Open Journal of Veterinary Medicine*. **2013**, 3, 22-28.
21. Van Soest, P.J.; Mertens, D.R.; Deinum, B. Preharvest Factors Influencing Quality of Conserved Forage. *J Anim Sci*. **1978**, 47, 712-720.
22. Carlson, G.E. Photoperiodic control of adventitious stem initiation on roots. Crop Sci. 1965;5:248-250.
23. Heinrichs DH, Nielsen KF. Growth response of alfalfa varieties of diverse genetic origin to different root zone temperatures. *Can. J. Plant Sci*. **1966**, 46, 291-298.

24. Robertson, G.W. The Light Composition of Solar and Sky Spectra Available to Plants. *Ecology*. **1966**, 47, 640-643.

25. Sato, K. Growth and Development of Alfalfa Plant under Controlled Environment: I. The effects of daylength and temperature on the growth and chemical composition. *Proc. Crop Sci Soc. Jpn*. **1971**, 40, 120-126.

26. Sato, K. Growth and Development of Alfalfa Plant under Controlled Environment: III. The effects of photoperiod and temperature on the growth and anatomical features of photosynthetic tissues. *Proc. Crop Sci Soc. Jpn*. **1974**, 43, 59-67.

27. McCarthy, B.; Delaby, L.; Pierce, K.M.; McCarthy, J.; Fleming, C.; Brennan, A.; Horan, B. The multi-year cumulative effects of alternative stocking rate and grazing management practices on pasture productivity and utilization efficiency. *J Dairy Sci.* **2016**, 99, 3784-3797.

28. Khalifa, H.H. Bioclimatology and adaptation of farm animals in a changing climate. In: Interactions between climate and animal production. Proceedings of the Symposium, EAAP Technical series N° 7, 2003, 15-29.

29. Gaughan, J.B.; Mader, T.L.; Holt, S.M.; Lisle A. A new heat load index for feedlot cattle. *J Anim Sci.* **2008**, 86, 226-234.

30. Arias, R.A.; Mader, T.L.; Escobar, P.C. Factores climáticos que afectan el desempeño productivo del ganado bovino de carne y leche. *Arch Med Vet*. **2008,** 40, 7-22.

31. Rekwot, P.I.; Oyedipe, E.O.; Mukasa-Mugerwa, E.; Sekoni, V.O.; Akinpelumi, O.P.; Anyam, A.A. Fertility in zebu cattle (Bos indicus) after prostaglandin administration and artificial insemination. *Vet J.* **1999**, 158, 53–58.

32. Rekwot, P.; Ogwu, D; Sekoni, V.; Oyedipe, E. Serum progesterone profiles of zebu cattle (*Bos indicus*) in relationship to conception and repeat breeding after artificial insemination. *Anim Reprod Sci.* **2000**, 63, 41–51.

33. Sales, J.N.; Carvalho, J.B.; Crepaldi, G.A.; Soares, J.G.; Girotto, R.W.; Maio, J.R.; Souza, J.C.; Baruselli, P.S. Effect of circulating progesterone concentration during synchronization for fixed-time artificial insemination on ovulation and fertility in *Bos indicus* (Nelore) beef cows. *Theriogenology.* **2015**, 83, 1093-1100.

34. Schramm, R.D.; Roberge, S.; Reeves, J.J. Enclomiphene does not alter the postpartum interval of suckled beef cows. *J Anim Sci.* **1991**, 69, 4112-4116.

35. Molina, R.; Galina, C.S.; Maquivar, M.; Estrada, S.; Chávez, A. Díaz, G.S. Pregnancy rate in zebu cows with two different postpartum intervals exposed to a two-bull rotational system. *Vet Res Commun*. **2003**, 27, 671-680.

36. Berardinelli, J.G.; Joshi, P.S. Introduction of bulls at different days postpartum on resumption of ovarian cycling activity in primiparous beef cows. *J Anim Sci*. **2005**, 83, 2106-2110.

37. Samadi, F.; Phillips, N.J.; Blache, D.; Martin, G.B.; D'Occhio, M.J. Interrelationships of nutrition, metabolic hormones and resumption of ovulation in multiparous suckled beef cows on subtropical pastures. *Anim Reprod Sci*. **2013**, 137, 137-144.

38. Randel, R.D. Nutrition and postpartum rebreeding in cattle. *J Anim Sci*. **1990**, 68, 853–862.

39. Short, R.E.; Bellows, R.A.; Staigmiller, R.B.; Berardinelli, J.G.; Custer, E.E. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *J Anim Sci.* **1990**, 68, 799–816.

40. Dunn, T.G.; Moss, G.E. Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock. *J Anim Sci.* **1992**, 70, 1580–1593.

41. Hess, B.W.; Lake, S.L.; Scholljegerdes, E.J.; Weston, T.R.; Nayigihugu, V.; Molle, J.D.C.; Moss, G.E. Nutritional controls of beef cow reproduction. *J Anim Sci*. **2005**, 83(E.Suppl.), E90–E106.

42. Gwazdauskas, F.C.; Thatcher, W.W.; Kiddy, C.A.; Pape, M.J.; Wilcox, C.J. Hormonal pattern during heat stress following PGF2alpha-tham salt induced luteal regression in heifers. *Theriogenology*. **1981**, 16, 271–285.

43. Petersson, K.J.; Strandberg, E.; Gustafsson, H.; Berglund, B. Environmental effects on progesterone profile measures of dairy cow fertility. *Anim Reprod Sci.* **2006**, 91, 201-214.

44. Mellado, M.; Sepulveda, A.; Meza-Herrera, C.; Veliz, F.G.; Arevalo, J.R.; Mellado, J.; De Santiago, A. Effects of heat stress on reproductive efficiency in high yielding Holstein cows in a hot arid environment. *Rev Colom Cienc Pecua.* **2013**, 26, 193-200.

45. Alves, B.G.; Alves, K.A.; Lúcio, A.C.; Martins, M.C.; Silva, T.H.; Alves, B.G.; Braga, L.S.; Silva, T.V.; Viu, M.A.; Beletti, M.E.; Jacomini, J.O.; Santos, R.M.; Gambarini, M.L. Ovarian activity and oocyte quality associated with the biochemical profile of serum and follicular fluid from Girolando dairy cows postpartum. *Anim Reprod Sci*. **2014**, 146, 117-125.

46. Nabenishi, H.; Ohta, H.; Nishimoto, T.; Morita, T.; Ashizawa, K.; Tsuzuki, Y. Effect of the temperature-humidity index on body temperature and conception rate of lactating dairy cows in southwestern Japan. *J Reprod Dev*. **2011**, 57, 450-456.

47. Oikawa, T. Effect of heat stress on age at first calving of Japanese Black cows in Okinawa. *Anim Sci J*. **2017**, 88, 439–444.

48. Kornmatitsuk, B.; Chantaraprateep, P.; Kornmatitsuk, S.; Kindahl, H. Different types of postpartum luteal activity affected by the exposure of heat stress and subsequent reproductive performance in Holstein lactating cows. *Reprod Dom Anim*. **2008**, 43, 515-519.

49. Kumar, A.; Waiz, S.A.; Sridhar Goud, T.; Tonk, R.K.; Grewal, A.; Singh, S.V.; Yadav, B.R.; Upadhyay, R.C. Assessment of adaptability of zebu cattle (*Bos indicus*) breeds in two different climatic conditions: using cytogenetic techniques on genome integrity. *Int J Biometeorol*. **2016**, 60, 873-882.

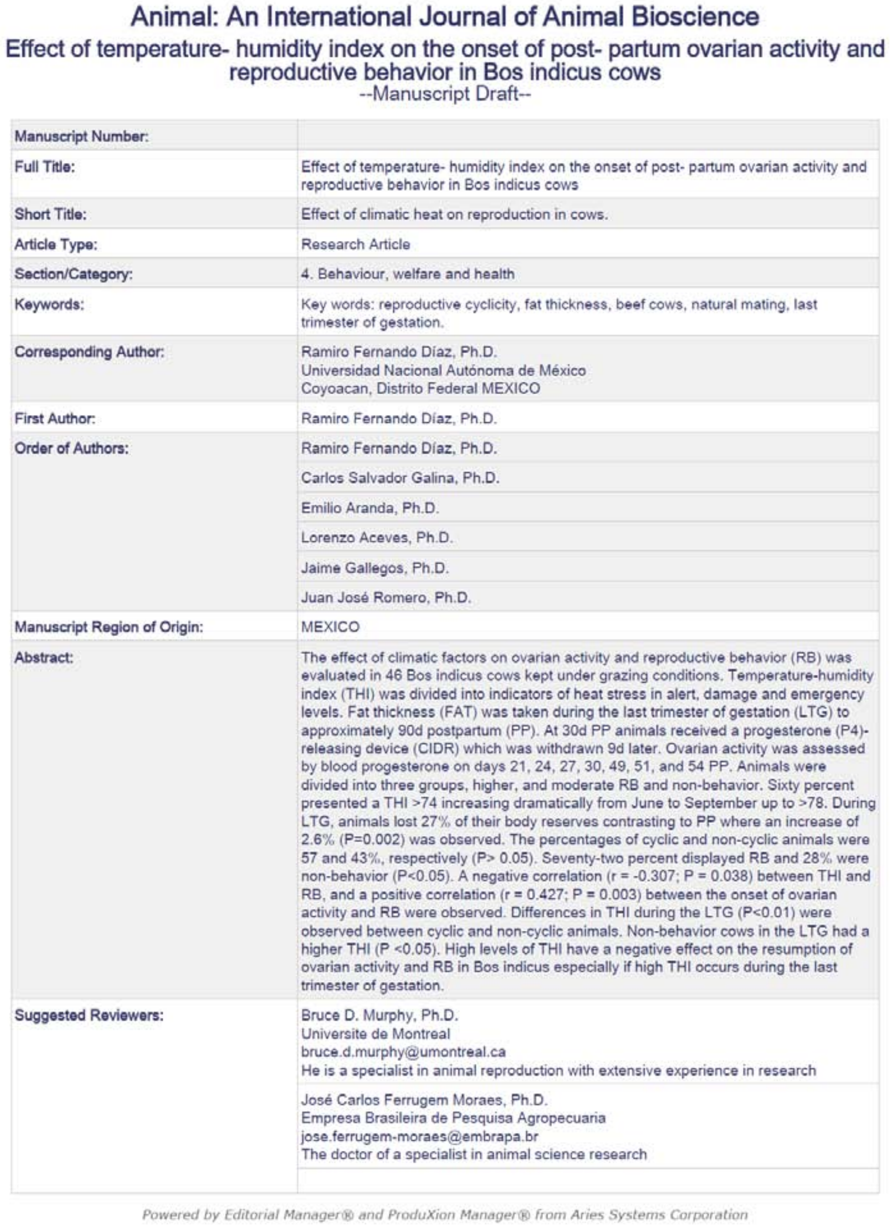
CAPITULO 5

EXPERMENTO 4

Efecto del índice de temperatura-humedad sobre el inicio de la actividad ovárica posparto y el comportamiento reproductivo en vacas *Bos indicus.*

Artículo enviado para publicación en:

Animal: an international journal of animal bioscience.



Animal: An International Journal of Animal Bioscience

Effect of temperature- humidity index on the onset of post- partum ovarian activity and reproductive behavior in Bos indicus cows

--Manuscript Draft--

Manuscript Number:	
Full Title:	Effect of temperature- humidity index on the onset of post- partum ovarian activity and reproductive behavior in Bos indicus cows
Short Title:	Effect of climatic heat on reproduction in cows.
Article Type:	Research Article
Section/Category:	4. Behaviour, welfare and health
Keywords:	Key words: reproductive cyclicity, fat thickness, beef cows, natural mating, last trimester of gestation.
Corresponding Author:	Ramiro Fernando Díaz, Ph.D. Universidad Nacional Autónoma de México Coyoacan, Distrito Federal MEXICO
First Author:	Ramiro Fernando Díaz, Ph.D.
Order of Authors:	Ramiro Fernando Díaz, Ph.D.
	Carlos Salvador Galina, Ph.D.
	Emilio Aranda, Ph.D.
	Lorenzo Aceves, Ph.D.
	Jaime Gallegos, Ph.D.
	Juan José Romero, Ph.D.
Manuscript Region of Origin:	MEXICO
Abstract:	The effect of climatic factors on ovarian activity and reproductive behavior (RB) was evaluated in 46 Bos indicus cows kept under grazing conditions. Temperature-humidity index (THI) was divided into indicators of heat stress in alert, damage and emergency levels. Fat thickness (FAT) was taken during the last trimester of gestation (LTG) to approximately 90d postpartum (PP). At 30d PP animals received a progesterone (P4)-releasing device (CIDR) which was withdrawn 9d later. Ovarian activity was assessed by blood progesterone on days 21, 24, 27, 30, 49, 51, and 54 PP. Animals were divided into three groups, higher, and moderate RB and non-behavior. Sixty percent presented a THI >74 increasing dramatically from June to September up to >78. During LTG, animals lost 27% of their body reserves contrasting to PP where an increase of 2.6% (P=0.002) was observed. The percentages of cyclic and non-cyclic animals were 57 and 43%, respectively (P> 0.05). Seventy-two percent displayed RB and 28% were non-behavior (P<0.05). A negative correlation (r = -0.307; P = 0.038) between THI and RB, and a positive correlation (r = 0.427; P = 0.003) between the onset of ovarian activity and RB were observed. Differences in THI during the LTG (P<0.01) were observed between cyclic and non-cyclic animals. Non-behavior cows in the LTG had a higher THI (P <0.05). High levels of THI have a negative effect on the resumption of ovarian activity and RB in Bos indicus especially if high THI occurs during the last trimester of gestation.
Suggested Reviewers:	Bruce D. Murphy, Ph.D. Universite de Montreal bruce.d.murphy@umontreal.ca He is a specialist in animal reproduction with extensive experience in research
	José Carlos Ferrugem Moraes, Ph.D. Empresa Brasileira de Pesquisa Agropecuaria jose.ferrugem-moraes@embrapa.br The doctor of a specialist in animal science research

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

Effect of temperature- humidity index on the onset of post- partum ovarian activity and reproductive behavior in *Bos indicus* cows

R. F. Díaz [1], C. S. Galina [1], E. Aranda[2], L. Aceves[2], J. Gallegos[3], J. J. Romero[4]

[1] *Departamento de Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. México.*

[2] *Campus Tabasco, Colegio de Postgraduados. México.*

[3] *Campus Montecillo, Colegio de Postgraduados. México.*

[4] *Programa de Investigación en Medicina Poblacional. Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica. Costa Rica.*

Corresponding autor: Ramiro F. Díaz. Email: rdiaz@usfq.edu.ec

Short title: Effect of climatic heat on reproduction in *Bos Indicus.*

ABSTRACT

The effect of climatic factors on ovarian activity and reproductive behavior (RB) was evaluated in 46 *Bos indicus* cows kept under grazing conditions. Temperature-humidity index (THI) was divided into indicators of heat stress in alert, damage and emergency levels. Fat thickness (FAT) was taken during the last trimester of gestation (LTG) to approximately 90d postpartum (PP). At 30d PP animals received a progesterone (P_4)-releasing device (CIDR) which was withdrawn 9d later. Ovarian activity was assessed by blood progesterone on days 21, 24, 27, 30, 49, 51, and 54 PP. Animals were divided into three groups, higher, and moderate RB and non-behavior. Sixty percent presented a THI >74 increasing dramatically from June to September up to >78. During LTG, animals lost 27% of their body reserves contrasting to PP where an increase of 2.6% (P=0.002) was observed. The percentages of cyclic and non-cyclic animals were 57 and 43%, respectively (P> 0.05). Seventy-two percent displayed RB and 28% were non-behavior (P<0.05). A negative correlation (r = -0.307; P = 0.038) between THI and RB, and a positive correlation (r = 0.427; P = 0.003) between the onset of ovarian activity and RB were observed. Differences in THI during the LTG (P<0.01) were observed between cyclic and non-cyclic animals. Non-behavior cows in the LTG had a higher THI (P <0.05). High levels of THI have a negative effect on the resumption of ovarian activity and RB in *Bos indicus* especially if high THI occurs during the last trimester of gestation.

Key words: reproductive cyclicity, fat thickness, beef cows, natural mating, last trimester of gestation.

Implications

Climatic change affect productive parameters in livestock and reproductive efficiency is one of them. Thus, the increase in THI during the last trimester of gestation could have an impact on the cyclicity of the animals. Also, high THI levels during the last trimester of gestation could affect reproductive behavior in the postpartum. Cyclic animals must have enough reserves of fat thickness to be able to mobilize them to commence ovarian activity.

Introduction

The direct effect of climate on livestock production in the tropics has been studied for a long time (Gwazdauskas, 1985; Hansen, 2007). The detrimental effects of high environmental temperature include poor oocyte and embryo quality, reduced dominance and steroidogenic capacity of the selected follicle, decreased uterine blood flow and endometrial dysfunction (Roth, 2008). The temperature-humidity index (THI) which has been widely used as an indicator of thermal stress in livestock (Hansen, 2007), forms the basis of the Livestock Weather Safety Index (Livestock Conservation Incorporated, 1970). THI above 74 is taken as an indicator of climatic stress for cattle (Arias *et al.,* 2008). There is also evidence for a negative effect of a high THI on conception rate, in the latter, lower levels have been recorded during the summer compared to the winter (Villa-Mancera *et al.,* 2011).

High environmental temperatures bring about a decrease in feed intake and play a decisive role in the management of cattle (Renaudeau *et al.,* 2012). The dichotomy between periods of dry weather with scarce forage and the rainy season where fodder is more plentiful complicates the handling and maintenance of cattle (Grings *et al.*, 2005). This situation has been made worse recently by climate change (Myers *et al.,* 2017) as the variability of the rainy season coupled with overgrazing, jeopardize husbandry procedures particularly for animals in the tropics (Raiten and Aimone, 2016).

Fat thickness (FAT) has been shown to be a reliable indicator of the level of energy reserves in the animal (Schröder and Staufenbiel, 2006). There is evidence to suggest that FAT during the last trimester of gestation, is related to reproductive ability (Wettemann *et al.,* 2003). Thus, early resumption of ovarian activity is closely linked to the capacity of the dam to recover from negative energy balance caused by stress during parturition (Montiel and Ahuja, 2005) and possibly from heat stress (Alves *et al.,* 2014).

The objective of the present study is, therefore, to evaluate the effect of THI on resumption of ovarian activity and reproductive behavior of *Bos indicus* cows.

Material and Methods

Ethical Statement

The Animal Care Internal Committee (CICUA) of the Faculty of Veterinary Medicine and Zootechnics of the National Autonomous University of Mexico approved the methods used during the present work in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Location

This study was carried out in the research station belonging to the Colegio de Postgraduados (COLPOS), located in the State of Tabasco, Mexico, at 93° 35' 1.31" W and 17° 59' 12.58" N. The climate is tropical and humid in the absence of a defined dry season (Ruiz-Alvarez *et al.,* 2012).

The temperature-humidity index (THI) was calculated taking the average environmental temperature and relative humidity of the period in which the study was conducted, according to the formula proposed by García-Ispierto *et al*., (2007):

$$THI = (0.8 \times T + (RH/100) \times (T - 14.4) + 46.4)$$

Where: *THI,* is the temperature-humidity index; *T*, is the mean temperature expressed in °C; and *RH*, is the average relative humidity expressed in percentage. Thus, the maximum and minimum ranges of THI levels were calculated. In addition, the number of hours over 74, 78 and 80 of THI for each day between the months of January and September of 2016, were considered as indicators of heat stress in alert, damage, and emergency levels, respectively (Arias *et al.,* 2008). In turn, the THI of each animal was measured 56 days before calving and 56 days postpartum, day zero was the calving date. In addition, three days after the summer solstice (June 24), rectal temperature was measured in a subsample of 14 animals chosen at random. These measurements were taken every 10 minutes from 10:00 until 14:00 hrs.

Animals

Forty-six *Bos indicus* crossbred animals in their last trimester of gestation and raised under an intensive grazing system, were used. Pastures were mainly a combination of *Cynodon nlemfuensis*, *Paspalum virgatum l.*, *Paspalum fasciculatum willd*, *Paspalum conjugatum*, *Desmodium adscendens.* No supplementary feeding was given during this period, although they had access *ad libitum* to water and mineral salts. Due to the nature of the study, it was not feasible to take samples of either forage or consumption.

Fat thickness and body condition score

All measurements of fat thickness (FAT), expressed in centimeters (cm), were taken two weeks apart, from the beginning of the last trimester of gestation to approximately 90d PP, using the ALOKA ProSound 2 ultrasound equipment with a convexed array transducer 3.5 MHz. The FAT was measured in the thurl area located midway between the tuber coxae (hooks) and the tuber ischiae (pins), 2 to 3 cm above the greater trochanter of the femur (Schröder and Staufenbiel, 2006). Body condition score was evaluated at the onset of the study by observation using the scale 1 to 9 (1= extremely thin, 9= very fat) as indicated by Wagner *et al.,* (1988).

Resumption of postpartum ovarian activity and reproductive behavior.

All animals with a confidence interval of 90% around a mean of 30 days postpartum, received a progesterone-releasing device (Eazy-breed CIDR™, 1.9 g of natural progesterone in silicone, Zoetis®, México) which was withdrawn 9 days later followed by an injection of 25mg of Prostaglandin F2α (Dinoprost, Lutalyse, Zoetis®, México). At the time of CIDR withdrawal, animals were exposed to two bulls with proven fertility. Onset of ovarian activity was assessed by blood levels of progesterone (Figure 1). Progesterone concentrations were determined by a solid phase radioimmunoassay in 100 µl of serum using commercial kits (Pharmaceuticals, Diagnostic Division). The intra and inter-assay coefficients of variation were 7.4% and 6.9%, respectively. A progesterone concentration above 1 ng/ml in two or more successive samples was indicative of luteal ovarian activity (Pulido *et al.,* 1991). The cows were then divided in two groups: cyclic and non-cyclic.

All animals were continuously observed for 12 hours (6:00 to 18:00) for 3 days after CIDR withdrawal to characterize behavioral changes of the females in the presence of the bull (Molina *et al*., 2002). ESTROTECT patches (Rockway Inc., Spring Valley, WI, USA) were placed on all cows to identify those that were in estrus after 18:00 h. Changes in the color of the patches were registered every morning at 07:00h. Signs of reproductive behavior (RB) were classified as: sniffing and licking, a score of 1; flehmen, 2; attempt to mount, 3; and mounts or allowed mounting, 4 (Orihuela *et al.,* 1983). Subsequently, animals were divided in three groups: with higher reproductive behavior, which had a score of ≥ 11 in the sum of the RB classification; moderate reproductive behavior with a score between 1 - 10; and those animals not displaying reproductive behavior during the experiment (non-behavior). Pregnancy diagnosis was carried in those cows with more than 28 days of exposure to the bull on a weekly basis up to an average 96d PP, using an ultrasound ALOKA ProSound 2 equipped with linear transducer of 7.5 MHz.

Statistical Analyses.

Possible differences in FAT during the last trimester of gestation and the postpartum were analyzed using Student “t” test. In turn, total relative change in back fat (ΔFAT= [initial measure - final measure] / initial measure) was determined. To analyse the changes in the measurements of rectal temperature at various times of the day, a one-way ANOVA was performed. Variations of FAT between groups were evaluated by ANOVA for repeated measures followed by Bonferroni adjustments for multiple comparisons. The proportions of the ovarian activity and reproductive behavior were evaluated with a 95% confidence interval. A correlation coefficient was used to determine the relationship between THI, onset of ovarian activity and reproductive behavior. All statistical analyses were performed on IBM SPSS 22 and JMP 6.0 statistical packages. P-values <0.05 were statistically different.

Results

Temperature-humidity index (THI)

For the whole duration of the experimental period, 60% presented a THI> 74, that is, levels related to a stressful environment and confirmed by calculating the number of hours presented with a THI> 74,> 78 and > 80 in the same period (table 1). For example, during the month of January, the range of hours >74 was 0-13 h, with up to 7h with values >78 and up to 4h with a THI above 80. In relation to the subsample of 14 animals whose rectal temperature was taken intensively, during mid-morning, the animals presented an average temperature of 38.73 ^{o}C with a range between 37.5 and 39.9. At noon, as expected, the temperature rose to 40.04 ^{o}C (P=0.07) with a range of 38.3 to 45 degrees. At 14:00 hrs, the average temperature was 41.14 (P = 0.001 for the first and P = 0.126 for the second measurement) with a range of 38.5 to 46 degrees.

Fat Thickness (FAT)

The body condition score at the start of the experiment was on average 3.8 ± 1.33 while animals at the start and the end of the last trimester of gestation had an average FAT of 0.197 ± 0.06 and 0.198 ± 0.07 cm, respectively. In this period, the relative change in fat indicates that the animals lost 27% of their reserves. In the postpartum, animals started with an average of 0.21 ± 0.05 and ended with 0.22 ± 0.04 cm. The relative change of fat in this period indicates an increase of 2.6%. A significant difference was found between the relative variations of dorsal fat during the last trimester of gestation and the postpartum period ($P = 0.002$).

Resumption of postpartum ovarian activity and reproductive behavior (RB)

The percentages of cyclic and non-cyclic animals during the experiment were 57% (26/46) and 43% (20/46) respectively ($P > 0.05$). Thus, 27% (7/26) were cycling at 21d PP; 27% (7/26) had ovarian activity between 21 and 30d PP; 27% (7/26) between 30 and 40d PP; and 19% (5/26) cycled after 40d PP ($P > 0.05$).

On the other hand, 72% (33/46) of the animals displayed some reproductive behavior and 28% (13/46) were non-behavior ($P < 0.05$). Of the animals expressing reproductive behavior, 40% (13/33) had a higher RB, whereas the remaining 60% (20/33) showed moderate RB ($P > 0.05$). Figure 2 shows the percentage of different marks in the patches used in observing RB. Animals with higher RB have the highest proportion of skin patches; conversely, non-behavior animals have the highest proportion of intact patches.

Thirty percent of the animals observed with reproductive behavior, expressed mounting activity, of these, 60% had a skin patch, 30% and 10%, dubious and intact patches, respectively. Ultimately, the fertility, in the present study, was 22% (10/46). By calculating only the number of cows at risk of becoming pregnant, the conception rate was 38% (10/26). Of all the pregnant animals displaying mounting behavior and associated signs of estrus, 70% and 30% presented higher and moderate RB, respectively ($P < 0.05$). Moreover, all initiated their ovarian activity as evidenced by high progesterone levels.

Relationship between temperature-humidity index, onset of ovarian activity, reproductive behavior and changes in FAT.

A low negative correlation ($r = -0.307$; $P = 0.038$) was found between the mean THI and reproductive behavior, and a moderate positive correlation ($r = 0.427$; $P = 0.003$) between the onset of ovarian activity and reproductive behavior. Conversely, the THI and the onset of cyclicity had a very low and non-significant correlation (r = -0.058; P = 0.702). Figure 3 depicts the levels of THI during the last trimester of gestation and the postpartum period in cyclic and non-cyclic animals. A significant difference was observed during the last trimester of gestation (P<0.01) and seemingly, the proportion of animals starting their cyclicity decreased as THI increased. In addition, although not significant, the period postpartum of the cows in the experiment, appears to be affected by an increase of THI.

At the onset of the last trimester of gestation, the cyclic and non-cyclic animals had FAT values of 0.21 ± 0.07 vs. 0.19 ± 0.04 cm. FAT in cyclic animals decreased in the last trimester of gestation with a variation of 37%, but increased in the postpartum with a variation of 5% (P=0.002). On the other hand, in the non-cyclic animals, FAT decreased both in the last trimester of gestation and in the postpartum period, (P = 0.315, Figure 4).

Likewise, during the last trimester of gestation, animals that did not display reproductive behavior (non-behavior) had a higher THI (P <0.05) compared to animals with a higher or moderate RB (Figure 5).

Discussion

The temperature-humidity index was > 74 for 60% of the time indicating that stressful levels prevailed for almost 2/3 of the duration of the present investigation. However, between the months of May to September, at no time was THI <74 and at a maximum in the months of March until May (Table 1). This was reflected in the subsample of 14 animals whose temperature on average rose almost three degrees. These observations are similar to those reported in other studies indicating that the state of Tabasco has a climate where high temperature and humidity levels predominate throughout the year (Angulo-Córdova *et al.,* 2004). Thus, a THI above the limit of comfort (> 74) can affect the reproductive ability of the animals (Amundson *et al.,* 2006) causing a decrease in the normal development of cattle (da Costa *et al.,* 2015). Moreover, a high THI can affect feed intake in animals (Renaudeau *et al.,* 2012) which would explain the poor body condition at the start of the experiment (3.8 ± 1.33) and reflected in back fat loss during the last trimester of gestation. A small gain of body fat was observed during the postpartum (2.6%). Possibly, adaptive mechanisms such as are required for recovery from the physiological process of calving, could present a major hurdle to re-establishing normal homeostasis. Indeed, physiological adaptation processes exist as mechanisms of thermoregulation (e.g. increased respiratory rate) allowing animals under conditions of stressful THI levels to feed and recover their energy reserves (Renaudeau *et al.,* 2012).

The percentage of animals starting their cyclicity was 57%. This proportion is low compared to previous reports where values fluctuated between 70 to 92% (Pérez-Torres *et al.,* 2015; Mondragón *et al.,* 2016). On the other hand, 43% of animals did not ovulate. Nonetheless, this percentage is higher than that reported in previous studies where this figure does not exceed 29% (Pérez- Torres *et al.,* 2015). The discrepancies between values could be the result of differences in the high levels of THI that animals were able to tolerate. Unfortunately, having the animals at pasture did not allow us to consider taking accurate measurements of stress, such as respiratory rate and body temperature. Decreased steroidogenic capacity of the dominant follicle is one of the characteristics of the negative effects of heat stress (Roth, 2008). This may decrease their ability to generate a pre-ovulatory peak of luteinizing hormone (LH), ovulate and form a corpus luteum. At the same time, animals without ovarian activity tolerated higher THI levels during the last trimester of gestation compared to those cycling ($P < 0.01$, Figure 3). Possibly the effect of even higher THI in the last trimester of gestation, may have affected the resumption of ovarian activity. Some authors suggest that heat stress alters follicular development by reducing size in both the first and the second wave (Badinga *et al.,* 1993;). At the same time, it attenuates the dominance by increasing the number of large follicles (Wolfenson *et al.,* 1995). In the present experiment, the follicular diameter of the animals was not evaluated, but it could be speculated that the effects of a higher THI during the last trimester of gestation annulled the resumption of ovarian activity (Hansen, 2007). This could be confirmed by observing if the proportion of animals starting their cyclicity remains constant during postpartum when THI is also unchanging. More research is needed on this topic.

Cows that cycled before 21d PP appear to be a constant feature in Zebu type cattle, in effect previous studies have shown a similar trend (Mondragón *et al.,* 2016; Pérez-Torres *et al.,* 2015). The first dominant follicle in *Bos indicus* appears from 10 to 15d PP (Murphy *et al.,* 1990; Crowe *et al.,* 2014) but ovulation does not occur until approximately 30d PP (Murphy *et al.,* 1990). A similar trend in ovulation was found in the present study.

On the other hand, the group displaying ovarian activity showed greater variation in body fat compared to non-cyclic cows (Figure 4). Whilst in the cyclic group, variations occurred both during the last trimester of gestation (down 37%) and in the postpartum (increase of 5%; $P = 0.002$), in the non-cyclic group, body fat reserves were not statistically modified ($P = 0.315$). In a previous study, non-cyclic cows showed a decrease in dorsal fat from the last trimester of gestation to the postpartum period, while animals with luteal activity maintained their energy reserves over these two periods (Díaz *et al.,* 2017). This apparent contradiction between the results of the two studies is possibly due to the level of FAT reserves in the animals. While in the present investigation the FAT levels did not exceed 0.21 cm, in other studies, animals had variations of body fat up to 0.40 cm. This means that the animals in this experiment had very low levels of fat and possibly only have what is needed to maintain basic vital processes. Only those able to mobilize their energy reserves could trigger their ovarian activity.

In the present experiment, 72% of animals showed some reproductive behavior (RB). This percentage is similar to that observed in previous studies where animals in estrus amounted to about 75% (Maquivar *et al.,* 2002; Acevedo *et al.,* 2007). Interestingly, these results are similar to those observed by other authors despite the fact that the animals in the present experiment were exposed to almost constant heat stress. Perhaps the explanation is that reproductive behavior in the present study was evaluated based on both suggestive (sniffing, licking, flehmen, etc.) and the definitive (mounts or allowed to be mounted) signs of estrus. When analyzing all signs, 40% of the animals showed a higher RB and 60%, a moderate RB ($P > 0.05$). In contrast to our experiment, other studies that focused on the final signs of estrus and on cows that were not experiencing high THI, a higher percentage of animals in estrus was observed (Maquivar *et al.,*2002; Acevedo *et al.,* 2007).

Conception rate was 38% and low compared to other studies in the tropics with rates over 70% (Escrivão *et al.,* 2012). However, animals in the previous studies, probably did not experience the harsh environmental conditions of the present experiment. Interestingly, some animals with moderate reproductive behavior became pregnant but to a lesser extent than animals with high RB. The expression of estrous behavior is characteristic of animals that have started cyclicity and therefore, it is understandable that they are more likely to become pregnant (for review see Galina and Orihuela, 2007).

Furthermore, 28% of all animals did not present RB (non-behavior). During the last trimester of gestation, these animals were under higher THI levels than cows with higher and moderate RB ($P < 0.05$). A reduction in steroidogenic capacity of follicles under thermal stress is characterized by less aromatase activity of granulosa cells and decreased estradiol concentration in the fluid of the dominant follicle (Badinga *et al.,* 1993). Among the potentially adverse effects of heat stress associated with low estradiol levels, are reduced plasma estradiol concentration (Badinga *et al.,* 1993; Wolfenson *et al*., 1995) impairing estrus duration and intensity, thus reducing the number of mounts (Gwazdauskas *et al*., 1981) as observed in the present investigation.

In conclusion, high levels of THI have a negative effect on the resumption of ovarian activity postpartum and expression of reproductive behavior in *Bos indicus* cows, especially when high THI occurs during the last trimester of gestation.

ACKNOWLEDGEMENTS

The authors wish to sincerely thank Jane Russell Ph.D. for her collaboration in the grammatical review of this article. Also, the authors would like to thank MVZ. Karen Córdova Hernández for their help in data collection. This study was supported by the Ministry of Higher Education, Science, Technology and Innovation (SENESCYT) of Ecuador with the program of scholarships for higher education.

References

Acevedo N, Galina CS, Pulido A and Orihuela A. 2007. Dynamics in sexually active groups of Zebu cattle (*Bos indicus*) comparing two procedures for estrus induction Journal Veterinary Behavior Clinical Applications and Research 2, 5–9.

Alves BG, Alves KA, Lúcio AC, Martins MC, Silva TH, Alves BG, Braga LS, Silva TV, Viu MA, Beletti ME, Jacomini JO, Santos RM and Gambarini ML. 2014. Ovarian activity and oocyte quality associated with the biochemical profile of serum and follicular fluid from Girolando dairy cows postpartum. Animal Reproduction Science 14, 117-125.

Amundson JL, Mader TL, Rasby RJ and Hu QS. 2006. Environmental effects on pregnancy rate in beef cattle. Journal of Animal Science. 84, 3415-3420.

Angulo-Córdova Q, Muñoz-Orozco A and Barradas-Miranda VL. 2004. Estudio del bio-clima humano en dieciséis localidades del estado de tabasco mediante el índice de confort temperatura efectiva. Universidad y Ciencia 20, 69-80.

Arias RA, Mader TL and Escobar PC. 2008. Factores climáticos que afectan el desempeño productivo del ganado bovino de carne y leche. Archivos de Medicina Veterinaria 40, 7-22.

Badinga L, Thatcher WW, Diaz T, Drost M and Wolfenson D. 1993. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. Theriogenology 39, 797–810.

Crowe MA, Diskin MG and Williams EJ. 2014. Parturition to resumption of ovarian cyclicity: comparative aspects of beef and dairy cows. Animal. 8 Suppl 1, 40-53.

da Costa AN, Feitosa JV, Montezuma PA Jr, de Souza PTand de Araújo AA. 2015. Rectal temperatures, respiratory rates, production, and reproduction performances of crossbred Girolando cows under heat stress in northeastern Brazil. International Journal of Biometeorology. 59, 1647-1653.

Díaz R, Galina CS, Rubio I, Corro M, Pablos JL, Rodríguez A and Orihuela A. 2017. Resumption of ovarian function, the metabolic profile and body condition in Brahman cows (Bos indicus) is not affected by the combination of calf separation and progestogen treatment. Animal Reproduction Science. 185, 181-187.

Escrivão RJ, Webb EC, Garcês AP and Grimbeek RJ. 2012. Effects of 48-hour calf withdrawal on conception rates of Bos indicus cows and calf weaning weights in extensive production systems. Tropical animal health and production. 44, 1779-1782.

Galina CS and Orihuela A. 2007. The detection of estrus in cattle raised under tropical conditions: what we know and what we need to know. Hormones and Behavior. 52, 32-38.

García-Ispierto I, López-Gatius F, Bech-Sabat G, Santolaria P, Yániz JL, Nogareda C, De Rensis F and López-Béjar M. 2007. Climate factors affecting conception rate of high producing dairy cows in northeastern Spain. Theriogenology. 67, 1379–1385.

Grings EE, Short RE, Klement KD, Geary TW, MacNeil MD, Haferkamp MR, and Heitschmidt RK. 2005. Calving system and weaning age effects on cow and preweaning calf performance in the Northern Great Plains. Journal of Animal Science 83, 2671–2683.

Gwazdauskas FC, Thatcher WW, Kiddy CA, Paape MJ and Wilcox CZ. 1981. Hormonal patterns during heat stress following PGF2α-tham salt induced luteal regression in heifers. Theriogenology 16, 271–285

Gwazdauskas FC. 1985. Effects of climate on reproduction in cattle. Journal of Dairy Science 68, 1568-1578.

Hansen PJ. 2007. Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. Theriogenology 68 Suppl 1, S242-249.

Livestock Conservation Incorporated. 1970. Patterns of Transient Losses. Livestock Conservation, Incorporated, Omaha, NE.

Maquivar M, Galina CS and Orihuela A. 2002. Cows treated with Synchromate B may cluster their sexual behavior independent of follicular growth at the time of oestrus. Physiology and Behavior. 76, 199-203.

Mondragón V, Galina CS, Rubio I, Corro M and Salmerón F. 2016. Effect of restricted suckling on the onset of follicular dynamics and body condition score in Brahman cattle raised under tropical conditions. Animal Reproduction Science. 167, 89-95.

Molina R, Galina CS, Camacho J, Maquivar M, Diaz GS, Estrada S and Martínez L. 2002. Effect of alternating bulls as a management tool to improve the reproductive performance of suckled Zebu cows in the humid tropics of Costa Rica. Animal Reproduction Science. 69, 159-173.

Montiel F and Ahuja C. 2005. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. Animal Reproduction Science. 85, 1-26.

Murphy MG, Boland MP and Roche JF. 1990. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. Journal of Reproduction and Fertility. 90, 523–533.

Myers SS, Smith MR, Guth S, Golden CD, Vaitla B, Mueller ND, Dangour AD and Huybers P. 2017. Climate Change and Global Food Systems: Potential Impacts on Food Security and Undernutrition. The Annual Review of Public Health. 38, 259–277.

Orihuela A, Galina CS, Escobar J and Riquelme E. 1983. Estrous behavior following prostaglandin f2α injection in zebu cattle under continuous observation. Theriogenology. 9, 795-809.

Pérez-Torres L, Rubio I, Corro M, Cohen A, Orihuela A, Galina CS and Pablos JL. 2015. A pre-synchronization program at early pospartum might increase the chances of Bos indicus cows cycling prior to 50 days regardless of the length of calf separation. Journal of Reproduction and Development. 61, 199-203.

Pulido AL, Zarco CS, Galina C, Murcia G, Flores and Posadas E. 1991. Progesterone metabolism during storage of blood samples from Gyr cattle: Effects of anticoagulant, time and temperature of incubation. Theriogenology. 35, 965-975.

Raiten DJ and Aimone AM. 2016. The intersection of climate/environment, food, nutrition and health: crisis and opportunity. Current Opinion in Biotechnology, 22, 52-62.

Renaudeau D, Collin A, Yahav S, de Basilio V, Gourdine JL and Collier RJ. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal. 6, 707-728.

Roth Z. 2008. Heat stress, the follicle, and its enclosed oocyte: mechanisms and potential strategies to improve fertility in dairy cows, Reproduction in Domestic Animals. 43, 238–244.

Ruiz-Álvarez O, Arteaga-Ramírez R, Vázquez-Peña MA, Ontiveros Capurata RE and López-López R. 2012. Balance hídrico y clasificación climática del estado de Tabasco, México. Universidad y Ciencia. 28, 1-14.

Schröder UJ and Staufenbiel R. 2006. Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. Journal of Dairy Science. 89, 1-14.

Villa-Mancera A, Méndez-Mendoza M, Huerta-Crispín R, Vázquez-Flores F and Córdova-Izquierdo A. 2011. Effect of climate factors on conception rate of lactating dairy cows in Mexico. Tropical Animal Health and Production. 43, 597-601.

Wagner JJ, Lusby KS, Oltjen JW, Rkestrw J, Wattermann RP and Walters LE. 1988. Carcass composition in mature Hereford cows: estimation and effect on daily metabolizable energy requeriment during winter. Journal of Animal Science. 66, 603-612.

Wettemann RP, Lents CA, Ciccioli NH, White FJ and Rubio I. 2003. Nutritional- and suckling-mediated anovulation in beef cows. Journal of Animal Science. 81 (E. Suppl. 2), E48-E59.

Wolfenson D, Thatcher WW, Badinga L, Savio JD, Meidan R, Lew BJ, Braw-Tal R and Berman A. 1995. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle, Biology of Reproduction. 52, 1106–1113.

Table 1: Number of hours (mean; standard deviation (SD) and range) where the THI was above 74, 78 and 80 indicating animals were exposed to a stressful environment.

Month	***THI > 74***			***THI > 78***			***THI > 80***		
	Mean	**SD**	**range**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
January	3.3	4.6	0-13	1.2	2.2	0-7	0.5	1.2	0-4
February	2.4	3.3	0-12	0.4	1.5	0-7	0.2	1.0	0-5
March	11.1	4.6	1-19	5.7	4.1	0-13	3.7	3.2	0-10
April	13.5	6.6	0-19	7.1	4.5	0-14	4.7	4.0	0-11
May	15.3	4.0	7-22	9.0	4.1	0-15	6.0	3.9	0-12
June	15.2	2.4	7-20	8.1	2.5	1-12	4.7	2.3	0-8
July	14.3	2.1	5-16	7.6	1.7	2-10	4.2	1.7	0-7
August	14.4	2.3	5-19	6.8	2.2	3-10	3.2	2.2	0-7
September	13.5	2.1	5-16	6.1	2.1	3-8	2.7	1.8	0-5

Figure captions

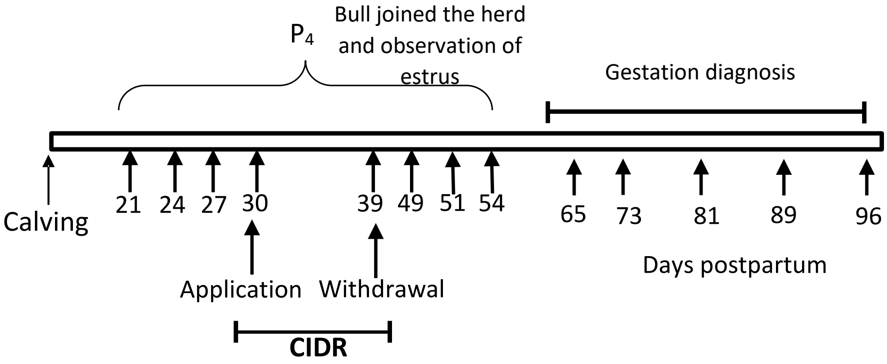


Figure 1. Timeline of specific experimental events with *Bos indicus* crossbred cows. P_4: Day postpartum when the blood sample was taken to measure concentrations of progesterone.

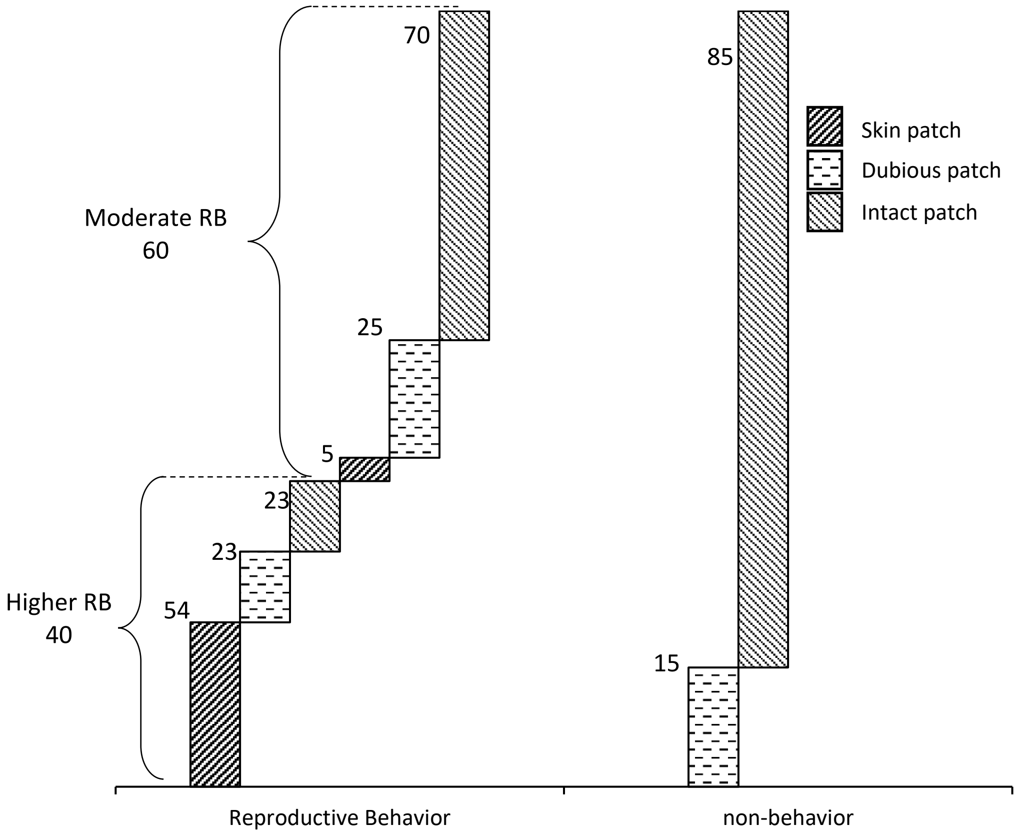


Figure 2: Proportions of different marks in the patches used in cattle *Bos indicus* to indicate their reproductive behavior (RB). Values expressed in percentages.

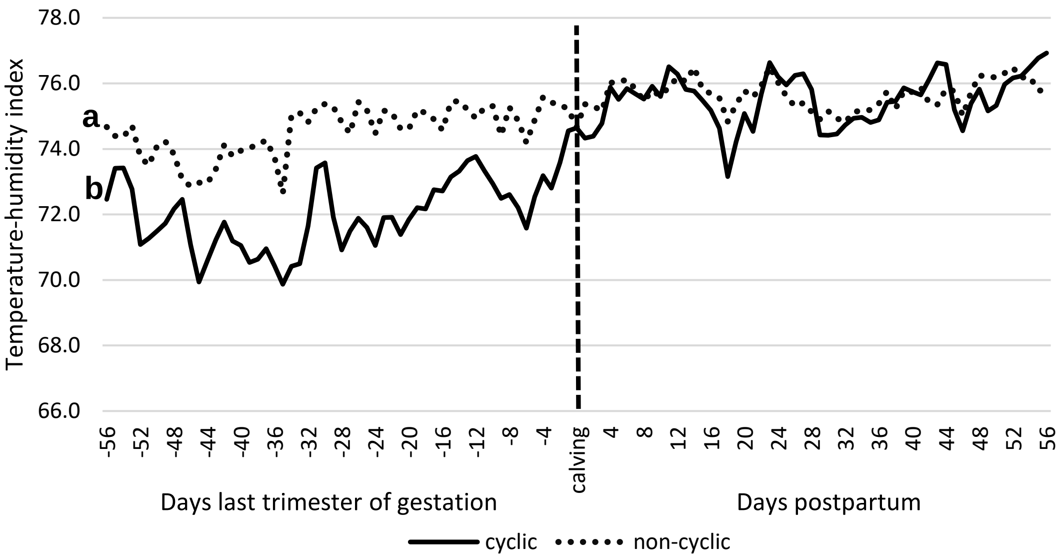


Figure 3: THI levels during the last trimester of gestation and postpartum of animals that cycled and non-cycling between the months of January and September of 2016. a, b: significant difference during the last trimester of gestation (P <0.01). In the postpartum period, there was no significant difference (P>0.05).

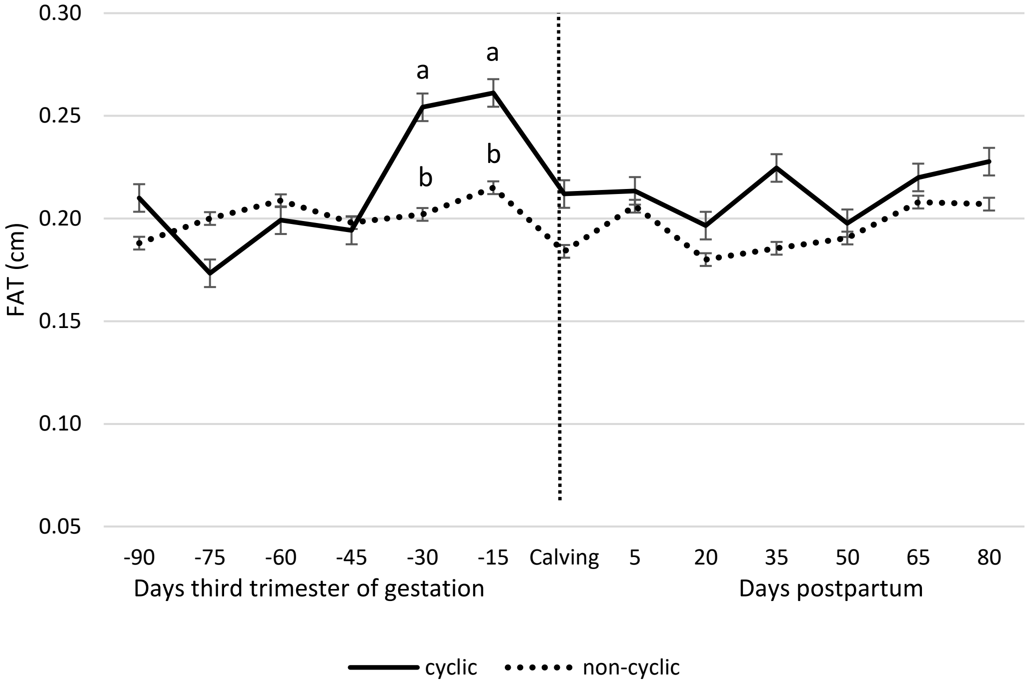


Figure 4: FAT during the last trimester of gestation and postpartum of cyclic and non- cyclic cows. Different letters indicate statistical difference p <0.05) on days 250 and 265 of gestation (P <0.05).

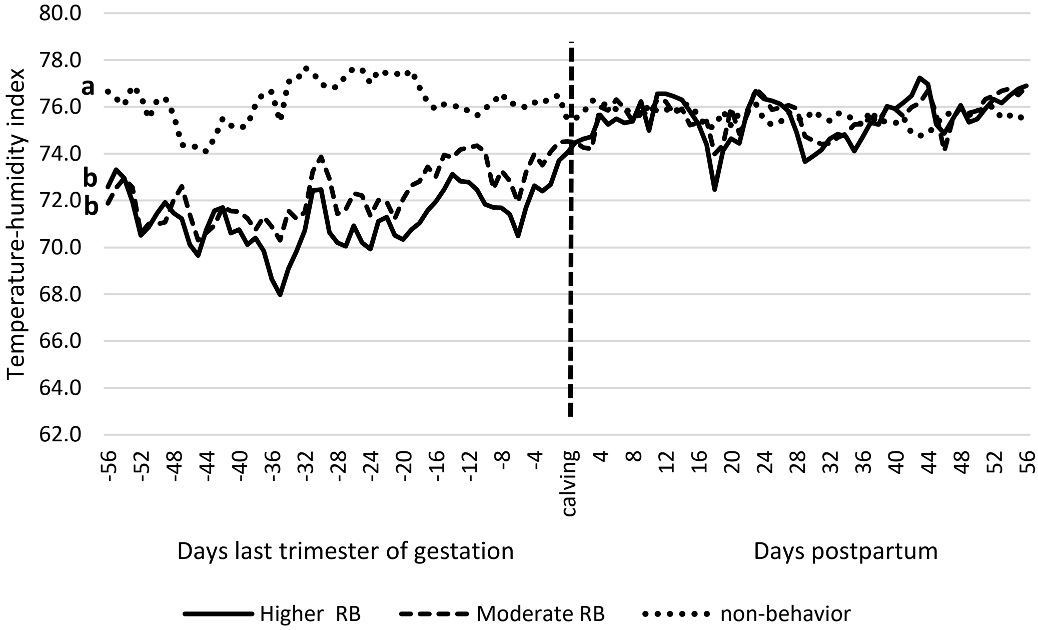


Figure 5: THI levels in animals with higher, moderate and no reproductive behavior between the months of January and September of 2016. Different letters indicate statistical difference (P <0.05) in the last trimester of gestation.

CAPITULO 6

DISCUSIÓN GENERAL

Los estudios realizados durante esta tesis proveen evidencia del efecto que produce el perfil de reservas energéticas y la movilización grasa durante el último tercio de gestación y después del parto sobre el reinicio de la actividad ovárica posparto y la fertilidad. A su vez, también se observó que los niveles del índice de temperatura-humedad (ITH) son un factor importante en el desarrollo reproductivo del animal en el posparto.

En todos los experimentos realizados los animales después del parto pierden grasa dorsal. Este evento se observó anteriormente (Mondragón *et al.,* 2016), reflejando los cambios fisiológicos en el metabolismo energético que ocurren durante y después del parto y es en ese momento cuando el animal moviliza sus reservas de grasas provocando un balance energético negativo (Bell, 1995; Grummer *et al.,* 2004). Estos hallazgos se confirman con lo observado en el **primer experimento** de esta tesis donde se evaluó el perfil metabólico de vacas *Bos indicus* durante el último tercio de la gestación y el posparto y se encontró que los metabolitos sanguíneos como ácidos grasos no esterificados (NEFA), los triglicéridos y la urea, cambian sus niveles entre estos dos períodos. Existe una gran cantidad de trabajos que reportan el mismo patrón de comportamiento en los niveles sanguíneos de estos metabolitos (Richards *et al.,* 1989; Rusche *et al.,* 1993; Bell, 1995; Radunz *et al.,* 2010; Lee *et al.,* 2012; Folnožić *et al.,* 2015), confirmando que existe un drástico cambio metabólico al momento del parto. A su vez, en el mismo experimento se observó que la separación de la cría no influyó en los niveles de estos metabolitos ni tampoco en los de la grasa dorsal (GD). Igualmente, la presencia o ausencia de la cría no afectó el inicio de la actividad ovárica posparto. A este respecto, a pesar de que existe abundante evidencia que indica que el amamantamiento afecta la duración del anestro posparto (Murphy *et al.,* 1990; Crowe *et al.,* 1993; Duffy *et al.,* 2000), en esta investigación no se demostró este efecto. Es posible que la pérdida de energía debida a la lactancia en estos animales no es tan severa como para afectar los resultados. Fröberg *et al.* (2007), informaron que las vacas de doble propósito bajo amamantamiento restringido presentaban niveles menores de grasa en la leche que las vacas sin su cría. Probablemente, los efectos del vaciado de la ubre al momento del amamantamiento junto con el período de espera voluntaria pueden causar un estímulo fisiológico que desencadena el metabolismo de los lípidos.

Por otro lado, en el **segundo experimento**, donde se evaluó la GD en forma similar que en el experimento anterior, se observó que los cambios ligeros de la GD podrían mejorar las oportunidades de que el animal quede gestante sin importar el momento en que inicie un programa reproductivo. Al igual que en el primer experimento, todas las vacas perdieron grasa dorsal entre el último tercio de gestación y el posparto temprano. Sin embargo, aquellas vacas que presentaron variaciones menores de la GD ($P>0.05$) se gestaron aun cuando su programa reproductivo inicie con la aplicación del CIDR antes de los 30dpp. En cambio, aquellos animales que presentaron una disminución significativa de su GD después el parto ($P<0.05$), tuvieron que esperar a reponerse de su desbalance energético para gestarse. Esto podría explicar la presencia de animales gestantes al primer servicio que presentaron variación de su GD después del parto pero que su programa reproductivo inició después de los 30 dpp. Existe evidencia que señala que los animales bien alimentados durante las fases más tardías de su gestación pueden mejorar su fertilidad posparto (Samadi *et al.,* 2013); mientras que en aquellos animales en que los cambios en el metabolismo de su grasa después del parto son significativos al estabilizase durante el posparto puedan también permitir al animal gestarse (Lamb *et al.,* 2001). Ambos grupos, aunque con diferente tasa de gestación, presentaron la misma velocidad de gestación, por lo que los animales con condiciones metabólicas adecuadas tendrían una mayor probabilidad de gestación independientemente del momento posparto cuando comience el programa reproductivo (Shrestha *et al.,* 2005). De manera similar, en el **tercer experimento**, donde la GD se evaluó con el mismo protocolo que los dos experimentos anteriores, esta disminuyó después del parto. En este caso la GD disminuyó tanto durante el último tercio de gestación como durante el posparto temprano. Mientras que en el primer y segundo experimentos los animales pertenecían a un Centro de enseñanza, investigación y extensión, los animales del tercer experimento pertenecían a dos fincas privadas ubicadas en dos países (Veracruz – México vs Puntarenas – Costa Rica) con diferente clima, manejo y nutrición. En este experimento se encontró que la variación de la GD incide directamente en el número de días en que el animal está en riesgo de quedar gestante. Los animales con más variación de su GD tendieron a demorar más tiempo en gestarse en comparación con aquellos que variaron menos su GD. Estos resultados sugieren que los niveles nutricionales durante el último tercio de la gestación son muy importantes ya que la insuficiente ingesta de calorías durante las últimas etapas de la gestación afecta la fertilidad aun cuando el consumo de energía sea el adecuado durante el posparto (Wettemann *et al.,* 2003; Hess *et al.,* 2005).

Por otro lado, en este experimento se evaluó el efecto del índice de temperatura – humedad (ITH) sobre los días a riesgo de gestación y fertilidad. Se observó que los animales sobrellevaron un ambiente con un ITH por sobre 74 (es decir por encima del nivel de confort; Arias *et al.,* 2008) durante aproximadamente el 57 y el 100 % del tiempo del experimento en Veracruz y Puntarenas respectivamente. Esto es un indicador del clima extremo que debieron soportar los animales. En estas condicione se encontró una alta relación entre los niveles de ITH y los días a riesgo de quedar gestantes, lo que permite especular un efecto directo del clima sobre la fertilidad. En el **cuarto experimento**, que se realizó en una estación experimental en el estado de Tabasco, también se evaluaron la GD y el ITH y se detectó que en aproximadamente el 60% del tiempo que duró el experimento el ITH fue >74. Entre los meses de marzo y mayo los animales no experimentaron en ningún momento niveles de ITH <74. Esto quiere decir que, según la clasificación de estrés térmico donde un ITH >74, >78 y >80 son para alerta, daño y emergencia respectivamente (Arias *et al.,* 2008; Renaudeau *et al.,* 2012), estos animales deben adaptarse a un medio de constante estrés calórico. Esto a su vez indica que la adaptación a climas extremos es muy importante en animales en el trópico si se desea que un programa reproductivo tenga éxito. En este experimento se observó que cuando los niveles más altos de ITH se presentan durante el último tercio de gestación se puede retardar la ciclicidad de los animales, haciéndolos caer en anestro. Esto puede deberse al efecto del estrés calórico sobre la capacidad reproductiva de los animales (Amundson *et al*., 2006), causando una disminución en el desarrollo normal del ganado (da Costa *et al.,* 2015). Igualmente, un ITH alto puede afectar la ingesta de alimento en los animales (Renaudeau *et al.,* 2012), lo que explicaría la mala condición corporal de los animales al inicio del cuarto experimento y la pérdida de GD durante el último tercio de gestación.

Las tasas de concepción en la presente tesis fueron de 49, 60 y 38% para el 2do, 3er y 4to experimento respectivamente. Estos porcentajes están acordes con las tasas de concepción reportadas por otros autores, que van de 41 a 70% (Rekwot *et al.,* 1999; Rekwot *et al.,* 2000; Sales *et al.,* 2015). Aunque las condiciones climáticas de los experimentos fueron distintas, los animales dieron muestras de adaptación a su medio. Por ejemplo, en el cuarto experimento todas las vacas gestantes mostraron algún comportamiento reproductivo (olfateo, flehmen, intento de monta o dejarse montar) a pesar de las condiciones climáticas y de nutrición adversas en las que se encontraban. A su vez, en los diferentes experimentos se observó que el reinicio de la actividad ovárica podía ser tan temprano como antes de los 21dpp en una proporción de alrededor del 20%, incrementando más adelante hasta un 80 y 97% como sucedió en el segundo y tercer experimentos respectivamente. Estos porcentajes son similares a los reportados en trabajos anteriores realizados en clima tropical, donde se observaron valores que fluctuaban entre 70 y 92% de animales cíclicos (Pérez-Torres *et al.,* 2015; Mondragón *et al.,* 2016).

La fisiología posparto de los animales productores de carne revela que la actividad ovárica puede iniciar al poco tiempo después del parto, entre los 10 y 15 dpp (Kamimura *et al.,* 1994; Stagg *et al.,* 1995), lo que significa que, aunque muchos animales tienen la posibilidad de gestarse en un tiempo corto después del parto no lo hacen. Las razones por las que esto sucede pueden estar relacionadas al estado nutricional en el que se encuentran los animales al iniciar el programa reproductivo, así como también al tiempo que necesitan para reponerse del desbalance energético posparto. Un pobre nivel nutricional tiene un efecto negativo sobre algunos procesos reproductivos. Los animales con baja condición corporal pueden presentar niveles de LH disminuidos (Tatman *et al.,* 1990; Kile *et al.,* 1991) aun presentándose niveles de FSH normales (Rhodes *et al.,* 1996; Mackey *et al.,* 2000), lo que permite el desarrollo folicular pero no la ovulación (Savio *et al.,* 1990). Se ha observado que, en bovinos productores de carne con buena condición corporal, el primer folículo capaz de ovular se presenta alrededor de los 30 dpp perteneciendo a la oleada 3.2 ± 0.2 (Murphy *et al.,* 1990). En cambio, en aquellos animales con baja condición corporal, su primera ovulación ocurre entre los 70 y los 100 dpp, después de 10.6 ± 1.2 oleadas de desarrollo folicular (Stagg *et al.,* 1995). En los presentes trabajos de investigación se realizó un seguimiento hasta los 90 a 100 dpp en promedio, por lo que se debe analizar el tiempo posparto en el que los animales deben recuperarse del desbalance energético que sufren después del parto. Durante el balance energético negativo posparto, una vaca debe utilizar sus propias reservas corporales en un esfuerzo para revertir el déficit energético. Esto da como resultado efectos negativos a nivel hipotálamo-hipofisiario (Scaramuzzi *et al.,* 2006). Las acciones de la FSH y de la LH son dependientes de algunas vías metabólicas controladas por hormonas como insulina (Richards *et al.,* 2002) y leptina (Ryan *et al*., 2002; Hamm *et al.,* 2004) que tienen un efecto, a su vez, sobre la secreción de GnRH. Estas señales varían dependiendo del estado nutricional de los animales afectando el desarrollo reproductivo, retomando sus niveles óptimos desde el cuarto mes posparto cuando el balance energético se ha restablecido (Corro *et al.,* 1999; Grummer *et al.,* 2004). Por consiguiente, muchos de los animales que inician su actividad ovárica posparto deberán esperar el tiempo suficiente hasta que se presenten las condiciones adecuadas para mantener una gestación.

Por otro lado, también se observaron algunos parámetros reproductivos distintos a lo reportado por otros autores. Por ejemplo, en el cuarto experimento el porcentaje de animales cíclicos durante el tiempo que duró la investigación fue del 57% y el número de hembras no cíclicas fue del 43%. Esta diferencia en las proporciones de animales cíclicos con los demás experimentos podría deberse a las diferencias climáticas y nutricionales entre estos. Mientras que en el primer segundo experimento los animales ganaron GD durante el último tercio de gestación y en el tercer experimento iniciaron la investigación con una GD de 0.28 cm, en el cuarto experimento los animales presentaron un promedio de GD que no sobrepasaba los 0.2 cm y en general este promedio se mantuvo durante todo el experimento. A su vez, los niveles de ITH fueron los más altos de todos los estudios, donde se observó en el mes de mayo hasta 12 horas al día con un ITH >80, lo que significa que los animales se encontraban en un estado de emergencia (Arias *et al.,* 2008). Desafortunadamente, tener a los animales en pastoreo no permitió considerar la toma de medidas precisas del estrés calórico, como la frecuencia respiratoria y la temperatura corporal. De todas maneras, existen investigaciones que indican que la disminución de la capacidad esteroidogénica del folículo dominante es una de las características de los efectos negativos del estrés por calor (Hansen, 2007; Roth, 2008). Esto puede disminuir su capacidad para generar un pico preovulatorio de la hormona luteinizante (LH), ovular y formar un cuerpo lúteo. Al mismo tiempo, en este cuarto experimento los animales sin actividad ovárica toleraron niveles más altos de ITH durante el último trimestre de gestación en comparación con las hembras cíclicas (P <0.01). Es posible que el efecto de un ITH aún mayor en el último trimestre de la gestación haya afectado la reanudación de la actividad ovárica. Algunos autores sugieren que el estrés por calor altera el desarrollo folicular al reducir el tamaño folicular tanto en la primera como en la segunda oleada (Badinga *et al.,* 1993;). Al mismo tiempo, atenúa el establecimiento de dominancia al aumentar la cantidad de folículos grandes (Wolfenson *et al.,* 1995). En este experimento, no se evaluó el diámetro folicular de los animales, pero se puede especular que los efectos de un ITH más elevado durante el último tercio de la gestación deroga la reanudación de la actividad ovárica (Hansen, 2007) y en consecuencia afecta el comportamiento reproductivo.

En ninguno de los experimentos se controló la ingesta de alimentos tanto durante el último tercio de gestación como en el posparto temprano. Esto se hizo con la visión de no interferir con el manejo normal de los animales en su finca. Sin embargo, existe amplia información que indica el efecto del manejo nutricional sobre los procesos reproductivos en los bovinos (Rhodes *et al.,* 1996; Boland *et al.,* 2001; Armstrong *et al.,* 2001, 2003; Da Silva *et al.,* 2002). Es posible que la movilización de las reservas de grasa se pueda controlar con el manejo adecuado de la nutrición de los animales en fases tardías de la gestación, evitando así la pérdida significativa de grasa durante el último tercio de gestación.

En conclusión, los estudios realizados demuestran la importancia que tiene preservar las reservas energéticas durante el último tercio de gestación, ya que la movilización de grasas será necesaria para poder enfrentar el desbalance energético posparto. Si esta variación no es muy drástica podría no ser necesario realizar un destete temprano de los animales para iniciar la actividad ovárica, pero si debe ser lo suficiente para estimular este reinicio de la ciclicidad. Así, las vacas con menores variaciones en sus reservas de grasa durante el último tercio de la gestación y el posparto temprano podrían tienen mejores oportunidades de gestarse independientemente del momento del posparto en que inicien el programa reproductivo. A su vez, se debe tomar en cuenta que un clima extremo durante el último tercio de gestación puede tener un efecto para retrasar el reinicio de la actividad ovárica posparto, afectando la expresión del comportamiento reproductivo del ganado y la tasa de concepción.

REFERENCIAS

Abecia JA, Rhind SM, Bramley TA, McMillen SR. Steroid production and LH receptor concentrations of ovarian follicles and corpora lutea and associated rates of ova wastage in ewes given high and low levels of food intake before and after mating. J. Anim. Sci. 1995;60:57–62.

Abecia JA, Lozano JM, Forcada F, Zarazaga L. Effect of level of dietary energy and protein on embryo survival and progesterone production on day eight of pregnancy in Rasa Aragonesa ewes. Anim. Reprod. Sci. 1997;48:209-218.

Abecia JA, Sosa C, Forcada F, Meikle A. The effect of undernutrition on the establishment of pregnancy in the ewe. Reprod. Nutr. Dev. 2006;46:367-378.

Adams NR, Abordi JA, Briegel JR, Sanders MR. Effect of diet on the clearance of estradiol-17-beta in the ewe. Biol. Reprod. 1994;51:668-674.

Adams NR, Briege JR, Sanders MR, Blackberry MA, Martin GB. Level of nutrition modulates the dynamics of oestradiol feedback on plasma FSH in ovariectomized ewes. Anim. Reprod. Sci. 1997;47:59-70.

Ahmadzadeh A, Barnes MA, Pearson RE. Effect of naloxone on serum luteinizing hormone concentration in annovulatory Holstein cows during the early postpartum period. Domest. Anim. Endocrinol. 1998;15:177-181.

Al-Katanani YM, Paula-Lopes FF, Hansen PJ. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. J. Dairy Sci. 2002;85:390–396.

Amundson JL, Mader TL, Rasby RJ and Hu QS. Environmental effects on pregnancy rate in beef cattle. J. Anim. Sci. 2006;84:3415-3420.

Aréchiga CF, Staples CR, McDowell LR, Hansen PJ. Effects of timed insemination and supplemental beta-carotene on reproduction and milk yield of dairy cows under heat stress. J. Dairy Sci. 1998;81:390–402

Arias RA, Mader TL, Escobar PC. Factores climáticos que afectan el desempeño productivo del ganado bovino de carne y leche. Arch. Med. Vet. 2008;40:7-22.

Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. Developmental programming of metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? The Journal of Physiology. 2004;561:355–377.

Armstrong DG, McEvoy TG, Baxter G, Robinson JJ, Hogg CC, Woad KJ, kakar R, Sinclair KD. Effect of dietary energy and protein on bovine follicular dynamics and embryo production *in vitro*: associations with the ovarian insulin-like growth factor system. Biol. Reprod. 2001;64:1624-1632.

Armstrong DG, Gong JG, Webb R. Interaction between nutrition and ovarian activity in cattle: physiological, cellular, and molecular mechanisms. Reprod. Suppl. 2003;61:403- 414.

Austin EJ, Mihm M, Evans ACO, Knight PG, Ireland JLH, Ireland JJ, Roche JF. Alterations in intrafollicular regulatory factors and apoptosis during selection of follicles in the first follicular wave of the bovine oestrous cycle. Biol. Reprod. 2001;64:839–848.

Badinga L, Thatcher WW, Diaz T, Drost M, Wolfenson D. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. Theriogenology 1993;39:797–810

Barb CR, Kraeling RR, Rampacek GB. Opioid modulation of gonadotropin and prolactin secretion in domestic farm animals. Domest. Anim. Endocrinol. 1991;8:15–27.

Barker DJP. Maternal Nutrition, Fetal Nutrition, and Disease in Later Life. Nutrition. 1997;13:807-813.

Bell AW. Regulation of organic nutrient late pregnancy metabolism during transition from to early lactation. J. Anim. Sci. 1995;73:2804–2819.

Bernard DJ, Fortin J, Wang Y, Lamba P. Mechanisms of FSH synthesis: what we know, what we don't, and why you should care. Fertil. Steril. 2010;93:2465-2485.

Bilby TR, Block J, do Amaral BC, Sa Filho O, Silvestre FT, Hansen PJ, Staples CR, Thatcher WW. Effects of dietary unsaturated fatty acids on oocyte quality and follicular development in lactating dairy cows in summer. J. Dairy Sci. 2006.;89:3891–3903.

Blecha F, Boyles SL, Riley JG. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman x Angus feeder calves. J. Anim. Sci. 1984;59:576-583.

Boland MP, Lonergon P, O'Callaghan D. Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology. 2001;55:1323-40.

Borowczyk E, Caton JS, Redmer DA, Bilski JJ, Weigl RM, Vonnahme KA, Borowicz PP, Kirsch JD, Kraft KC, Reynolds LP, Grazul-Bilska AT. Effects of plane of nutrition on *in vitro* fertilization and early embryonic development in sheep. J. Anim. Sci. 2006;84:1593-1599.

Borradaile NM, Han X, Harp JD, Gale SE, Ory DS, Schaffer JE. Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. J. Lipid. Res. 2006;47:2726-2737.

Boukhliq R, Goodman RL, Berriman SJ, Adrian B, Lehman MN. A subset of gonadotropin-releasing hormone neurons in the ovine medial basal hypothalamus is activated during increased pulsatile luteinizing hormone secretion. Endocrinology. 1999;140:5929-5936.

Brooks AN, Lamming GE, Haynes NB. Endogenous opioid peptides and the control of gonadotrophin secretion. Res. Vet. Sci. 1986;41:285–299.

Brown CH, Russell JA, Leng G. Opioid modulation of magnocellular neurosecretory cell activity. Neurosci. Res. 2000;36:97–120.

Canty MJ, Boland MP, Evans ACO and Crowe MA Alterations in follicular IGFBP-2, -3 and -4 mRNA expression and intrafollicular IGFBP concentrations during the first follicle wave in beef heifers. Anim. Reprod. Sci. 2006;93:199–217.

Castaneda CA, Kaye P, Pantaleon M, Phillips N, Fry R, D'Occhio MJ. Circulating concentrations of leptin, ovarian follicle number, and oocyte lipid content and active mitochondria, in Zebu crossbred cows maintained on standard or improved nutrition. Anim. Reprod. Sci. 2013;140:7-13.

Chan KA, Bernal AB, Vickers MH, Gohir W, Petrik JJ, Sloboda DM. Early life exposure to undernutrition induces ER stress, apoptosis, and reduced vascularization in ovaries of adult rat offspring. Biol. Reprod. 2015;92:110.1-14.

Connor HC, Houghton PL, Lemenager RP, Malven PV, Parfet JR, Moss GE. Effect of dietary energy, body condition and calf removal on pituitary gonadotropins, gonadotroping-releasing hormone (GnRH) and hypothalamic opioids in beef cows. Domest. Anim. Endocrinol. 1990;7:403-411.

Corro M, Rubio I, Castillo E, Galindo L, Aluja A, Galina CS, Murcia C. Effect of metabolites, body condition and pasture management on milk yield and postpartum intervals in dual-purpose cattle farms in the tropics of the State of Veracruz, México. Preventive Veterinary Medicine. 1999;38:101-117.

Cosgrove JR, de Rensis F, Foxcroft GR. Opioidergic pathways in animal reproduction: their role and effects of their pharmacological control. Anim. Reprod. Sci. 1993;33:373–392.

Crowe MA, Goulding D, Baguisi A, Boland MP, Roche JF Induced ovulation of the first postpartum dominant follicle in beef suckler cows using a GnRH analogue. J. Reprod. Fertil. 1993;99:551–555.

Crowe MA, Padmanabhan V, Mihm M, Beitins IZ, Roche JF. Resumption of follicular waves in beef cows is not associated with periparturient changes in follicle-stimulating hormone heterogeneity despite major changes in steroid and luteinizing hormone concentrations. Biol. Reprod. 1998;58:1445–1450.

da Costa AN, Feitosa JV, Montezuma PA Jr, de Souza PT, de Araújo AA. Rectal temperatures, respiratory rates, production, and reproduction performances of crossbred Girolando cows under heat stress in northeastern Brazil. Int. J. Biometeorol. 2015;59:1647-1653.

Da Silva P, Aitken RP, Rhind SM, Racey PA, Wallace JM. Impact of maternal nutrition during pregnancy on pituitary gonadotrophin gene expression and ovarian development in growth-restricted and normally grown late gestation sheep fetuses. Reprod. 2002;123:769-77.

Das SM, Redbo II, Wiktorsson H. Effect of age of calf on suckling behaviour and other behavioural activities of Zebu and crossbred calves during restricted suckling periods. Appl. Anim. Behav. Sci. 2000;67:47–57.

de S. Torres-Júnior R, de FA Pires M, de Sá WF, de M Ferreira A, Viana JHM, Camargo LSA, Ramos AA, Folhadella IM, Polisseni J, de Freitas C, Clemente CAA, de Sá Filho MF, Paula-Lopes FF, Baruselli PS. Effect of maternal heat-stress on follicular growth and oocyte competence in *Bos indicus* cattle. Theriogenology. 2008;69:155–166.

Diakogiannaki E, Welters HJ, Morgan NG. Differential regulation of the endoplasmic reticulum stress response in pancreatic β-cells exposed to long-chain saturated and monounsaturated fatty acids. J. Endocrinol. 2008;197:553-563.

Díaz R, Galina CS, Rubio I, Corro M, Pablos JL, Rodríguez A, Orihuela A. Resumption of ovarian function, the metabolic profile and body condition in Brahman cows (*Bos indicus*) is not affected by the combination of calf separation and progestogen treatment. Anim. Reprod. Sci. 2017;185:181-187.

Dikmen S, Hansen PJ. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J. Dairy Sci. 2009;92:109-116.

Duffy P, Crowe MA, Boland MP, Roche JF. Effect of exogenous LH pulses on the fate of the first dominant follicle in postpartum beef cows nursing calves. J. Reprod. Fertil. 2000;118:9–17.

Dunning KR, Robker RL. Promoting lipid utilization with l-carnitine to improve oocyte quality. Anim. Reprod. Sci. 2012;134:69-75.

Farnworth PG. Gonadotrophin secretion revisited. How many ways can FSH leave a gonadotroph? J. Endocrinol. 1995;145:387-395.

Ferraretto LF, Gencoglu H, Hackbar KS, Nascimento AB, Dalla Costa F, Bender RW, Guenther JN, Wiltbank MC. Effect of feed restriction on reproductive and metabolic hormones in dairy cows. J. Dairy Sci. 2014;97:754-763.

Ferguson EM, Leese HJ. A potential role for triglyceride as an energy source during bovine oocyte maturation and early embryo development. Mol. Reprod. Dev. 2006;73:1195-1201.

Folnožić I, Turk R, Đuričić D, Vince S, Pleadin J, Flegar-Meštrić Z, Valpotić H, Dobranić T, Gračner D, Samardžija M. Influence of body condition on serum metabolic indicators of lipid mobilization and oxidative stress in dairy cows during the transition period. Reprod. Domest. Anim. 2015;50:910–917.

Fouladi-Nashta AA, Gutierrez CG, Gong JG, Garnsworthy PC, Webb R. Impact of dietary fatty acids on oocyte quality and development in lactating dairy cows. Biol. Reprod. 2007;77:9–17.

Fröberg S, Aspegren-Güldorff A, Olsson I, Marin B, Berg C, Hernández C, Galina CS, Lidfors L. Svennersten-Sjaunja K. Effect of restricted suckling on milk yield, milk composition and udder health in cows and behaviour and weight gain in calves, in dual-purpose cattle in the tropics. Trop. Anim. Health. Prod. 2007;39:71–81.

Fuchs AR, Ivell R, Ganz N, Fields MJ, Gimenez T.Secretion of oxytocin in pregnant and parturient cows: corpus luteum may contribute to plasma oxytocin at term. Biol. Reprod. 2001;65:1135-1141.

Galina CS, Arthur GH. Review of cattle reproduction in the tropics. Part I. Puberty and age at first calving. Animal Breeding Abstracts. 1989;57:583-590.

Galina CS, Rubio I, Basurto H, Orihuela A. Consequences of different suckling systems for reproductive activity and productivity of cattle in tropical conditions. Appl. Anim. Behav. Sci. 2001;72:255-262.

Galindo J, Galina CS, Estrada S, Romero JJ, Alarcón M, Maquivar M. Effect of changes in body weight, body condition and back fat during last month of pregnancy on the reproductive efficiency of *Bos indicus* cows in the tropics of Costa Rica. Open Journal of Veterinary Medicine. 2013;3:22-28.

Garcia-Ispierto I, Abdelfatah A, López-Gatius F. Melatonin treatment at dry-off improves reproductive performance postpartum in high-producing dairy cows under heat stress conditions. Reprod. Domest. Anim. 2013;48:577–583.

García-Winder M, Imakawa J, Day ML, Zalesky DD, Kittock RJ, Kinder JE. Effect of suckling and ovariectomy on the control of luteinizing hormone secretion during the postpartum period in beef cows. Biol. Reprod. 1984;31:771-778.

Gazal OS, Leshin LS, Stanko RL, Thomas MG, Keisler DH, Anderson LL, Williams GL. Gonadotroping-releasing hormone secretion into third-ventricle cerebrospinal fluid of cattle: correspondence with the tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptide Y. Biol. Reprod. 1998;59:676-683.

Gendelman M, Aroyo A, Yavin S, Roth Z. Seasonal effects on gene expression, cleavage timing, and developmental competence of bovine preimplantation embryos. Reproduction 2010;140:73–82.

Gendelman M, Roth Z. *In vivo* vs*. in vitro* models for studying the effects of elevated temperature on the GV-stage oocyte, subsequent developmental competence and gene expression. Anim. Reprod. Sci. 2012;134:125–134.

Gilad E, Meidan R, Berman A, Graber Y, Wolfenson D. Effect of heat stress on tonic and GnRH-induced gonadotrophin secretion in relation to concentration of oestradiol in plasma of cyclic cows. J. Reprod. Fertil. 1993;99:315–321.

Gong JG. Influence of metabolic hormones and nutrition on ovarian follicle development in cattle: practical implications. Domest. Anim. Endocrinol. 2002;23:229-241.

Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. Endocr. Rev. 1996;17:121-155.

Grazul-Bilska AT, Kirsch JD, Bilski JJ, Kraft KC, Windorski EJ, Luther JS, Vonnahme KA, Reynolds LP, Redmer DA. Superovulation in sheep: number and weight of the corpora lutea and serum progesterone. Sheep and Goat Research Journal. 2007;22:26-31.

Grazul-Bilska AT, Borowczyk E, Bilski JJ, Reynolds LP, Redmer DA, Caton JS, Vonnahme KA. Overfeeding and underfeeding have detrimental effects on oocyte quality measured by in vitro fertilization and early embryonic development in sheep. Domest. Anim. Endocrin. 2012;43:289-298.

Griffith MK, Williams GL. Roles of maternal vision and olfaction in suckling-mediated inhibition of luteinizing hormone secretion. Expression of maternal selectivity, and lactational performance of beef cows. Biol. Reprod. 1996;54:761–768.

Grummer RR, Mashek DG, Hayirli A. Dry matter intake and energy balance in the transition period. Vet. Clin. North. Am. Food Anim. Pract. 2004;20:447–470.

Gwazdauskas FC, Thatcher WW, Kiddy CA, Paape MJ, Wilcox CZ. Hormonal patterns during heat stress following $PGF_2\alpha$-tham salt induced luteal regression in heifers. Theriogenology 1981;16:271–285.

Hahn G, Gaughan JB, Mader TL, Eigenberg RA. Thermal indices and their applications for livestock environments. In Livestock energetics and thermal environmental management (ed. JA DeShazer), pp. 113–130. American Society of Agricultural and Biological Engineers, St Joseph, MI, USA. 2009.

Hamm ML, Bhat GK, Thompson WE, Mann DR. Folliculogenesis is impaired and granulosa cell apoptosis is increased in leptin-deficient mice. Biol. Reprod. 2004;71:66-72.

Hamudikuwanda H, Gallo G, Block E, Downey BR. Adipose tissue progesterone concentrations in dairy cows during late pregnancy and early lactation. Anim. Reprod. Sci. 1996;43:5– 23.

Han H, Hansen TR, Berg B, Hess BW, Ford SP. Maternal undernutrition induces differential cardiac gene expression in pulmonary hypertensive steers at high elevation. Am. J. Physiol. Heart. Circ. Physiol. 2008;295:H382-389.

Hansen PJ. Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. Theriogenology. 2007;68 Suppl 1:S242-S249.

Harding JE. The nutritional basis of the fetal origins of adult disease. Int. J. Epidemiol. 2001;30:15-23.

Haynes NB, Lamming CE, Yang KP, Brooks AN, Finnie AD. Endogenous opioid peptides and farm animal reproduction. Oxf. Rev. Reprod. Biol. 1989;11:111–145.

Henao G, Olivera AM, Maldonado EJG. Follicular dynamics during postpartum anestrus and the first estrous cycle in suckled or non-suckled Brahman (*Bos indicus*) cows. Anim. Reprod. Sci. 2000;63:127-136.

Henao G. Reactivación ovárica postparto en bovinos. Revisión. Revista Facultad Nacional de Agronomía, Medellín. 2001;54:1285-1302.

Henao GR, González CV. Relationship of live weight and corporal condition variation with postpartum follicular dynamic in primiparous zebu cows. Revista Facultad Nacional de Agronomía, Medellín. 2008;61:1.

Hess BW, Lake SL, Scholljegerdes EJ, Weston TR, Nayigihugu V, Molle JDC, Moss GE. Nutritional controls of beef cow reproduction. J. Anim. Sci. 2005;83(E.Suppl.):E90–E106.

Hoffman DP, Stevenson JS, Minton JE, Restricting calf presence without suckling compared with weaning prolongs postpartum anovulation in beef cattle. J. Anim. Sci. 1996;74:190-198.

Howell JL, Fuquay JW, Smith AE. Corpus luteum growth and function in lactating Holstein cows during spring and summer. J. Dairy Sci. 1994;77:735–739.

Jenkins GM, Cowart LA, Signorelli P, Pettus BJ, Chalfant CE, Hannun YA. Acute activation of de novo sphingolipid biosynthesis upon heat shock causes an accumulation of ceramide and subsequent dephosphorylation of SR proteins. J. Biol. Chem. 2002;277:42572–42578.

Jonsson NN, McGowan MR, McGuigan K, Davison TM, Hussain AM, Matschoss M. Relationships among calving season, heat load, energy balance and postpartum ovulation of dairy cows in a subtropical environment. Anim. Reprod. Sci. 1997;47:315–326.

Jorritsma R, César ML, Hermans JT, Krintwagen CLJJ, Vos PLAM, Kruip TAM. Effects of non-esterified fatty acids on bovine granulosa cells and developmental potential of oocytes in vitro. Anim. Reprod. Sci. 2004;81:225-35.

Kalo D, Roth Z. Involvement of the sphingolipid ceramide in heat-shock-induced apoptosis of bovine oocytes. Reprod. Fertil. Dev. 2011;23:876–888.

Kamimura S, Sameshima H, Enomoto S, Hamana K. Turnover of ovulatory and non-ovulatory dominant follicles in postpartum japanese black cows. J. Reprod. Dev. 1994;40:171-176.

Kaminski SL, Redmer DA, Bass CS, Keisler DH, Carlson LS, Vonnahme KA, Dorsam ST, Grazul-Bilska AT. The effects of diet and arginine treatment on serum metabolites and selected hormones during the estrous cycle in sheep. Theriogenology. 2015;83:808-816.

Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Gene. Dev. 1999;13:1211-33.

Kile J, Alexander B, Moss G, Hallford D, Nett T. Gonadotropin-releasing hormone overrides the negative effect of reduced dietary energy on gonadotropin synthesis and secretion in ewes. Endocrinology. 1991;128:843-849.

Kiyma Z, Alexander BM, Van Kirk EA, Murdoch WJ, Hallford DM, Moss GE. Effects of feed restriction on reproductive and metabolic hormones in ewes. J. Anim. Sci. 2004;82:2548-2557.

Lamb GC, Stevenson JS, Kesler DJ, Garverick HA, Brown DR, Salfen BE. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 2001;79:2253-2259.

Lassoued N, Rekik M, Mahouachi M, Hamouda B. The effect of nutrition prior to and during mating on ovulation rate, reproductive wastage, and lambing rate in three sheep breeds. Small. Ruminant. Res. 2004;52:117-125.

Lee HH, Katsuya K, Ryotaro M, Hisashi I, Miyamoto A, Kawashima C, Haneda S, Miyake YI, Matsui M. Slow recovery of blood glucose in the insulin tolerance test during the prepartum transition period negatively impacts the nutritional status and reproductive performance postpartum of dairy cows. J. Vet. Med. Sci. 2012;74:457–464.

Leng G, Caquineau C, Sabatier N. Regulation of oxytocin secretion. Vitam. Horm. 2005;71:27-58.

Long NM, Vonnahme KA, Hess BW, Nathanielsz PW, Ford SP. Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine. J. Anim. Sci. 2009;87:1950-1959.

Lozano JM, Lonergan P, Boland MP, O'Callaghan D. Influence of nutrition on the effectiveness of superovulation programmes in ewes: effect on oocyte quality and postfertilization development. Reproduction. 2003;125:543-553.

Lublin A, Wolfenson D. Lactation and pregnancy effects on blood flow to mammary and reproductive systems in HS rabbits. Comp. Biochem. Physiol. A. Physiol.1996;115:277–285.

Mackey DR, Sreenan JM, Roche JF, Diskin MG. Effect of acute nutritional restriction on the incidence of anovulation and periovulatory estradiol and gonadotropin concentrations in beef heifers. Biol. Reprod. 1999;61:1601-1607.

Mackey DR, Wylie ARG, Sreenan JM, Roche JF, Diskin MG. The effect of acute nutritional change on follicle wave turnover, gonadotropin, and steroid concentration in beef heifers. J. Anim. Sci. 2000;78:429-442.

Mann GE, Lamming GE, Robinson RS, Wathes DC. The regulation of interferon-tau production and uterine hormone receptors during early pregnancy. J. Reprod. Fertil. Suppl. 1999;54:317–328.

Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction 2001;121:175–180.

Mann GE, Fray MD, Lamming GE. Effects of time of progesterone supplementation on embryo development and interferon-tau production in the cow. Vet. J. 2006;171:500–503.

Maquivar M, Galina CS. Factors affecting the readiness and preparation of replacement heifers in tropical breeding environments. Reprod. Domest. Anim. 2010;45:937-942.

Marquezini GH, Mercadante VR, Bischoff KM, Black TE, Dilorenzo N, Bird SL, Funnell BJ, Klein SI, Dahlen CR, Larson JE, Lamb GC. Effects of temporary calf removal before fixed-time artificial insemination on pregnancy rates and subsequent calf performance in suckled beef cows. J. Anim. Sci. 2013;91:2414-2425.

Martyn CN, Barker DJ, Osmond C. Mothers' pelvic size, fetal growth, and death from stroke and coronary heart disease in men in the UK. Lancet. 1996;348:1264-1268.

Matsuzuka T, Ozawa M, Hirabayashi M, Ushitani A, Kanai Y. Developmental competence and glutathione content of maternally heat-stressed mouse oocytes and zygotes. Anim. Sci. J. 2004;75:117–124.

Matsuzuka T, Sakamoto N, Ozawa M, Ushitani A, Hirabayashi M, Kanai Y. Alleviation of maternal hyperthermia-induced early embryonic death by administration of melatonin to mice. J. Pineal Res. 2005;39:217–223.

McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Palmer RM, Robertson IS. Dietary induced suppression of pre-ovulatory progesterone concentrations in superovulated ewes impairs the subsequent in vivo and in vitro development of their ova. Anim. Reprod. Sci. 1995;38:89-107.

McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol. Rev. 2005;85:571–633.

McNeilly AS, Brooks J, McNeilly JR, Brown P. Synthesis and release of FSH. J. Reprod. Fert. Abstract Series. 1995;S2:2.

Metwally M, Li TC, Ledger WL. The impact of obesity on female reproductive function. Obes. Rev. 2007;8:515-23.

Mihm M, Baguisi A, Boland MP, Roche JF. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J. Reprod. Fertil. 1994;102:123–130.

Miller HM. The effects of feed intake and body fatness on progesterone metabolism in ovariectomized gilts. J. Anim. Sci. 1999;77:3253-3261.

Minge CE, Bennett BD, Norman RJ, Robker RL. Peroxisome proliferator-activated receptor-γ agonist rosiglitazone reverses the adverse effects of diet-induced obesity on oocyte quality. Endocrinology. 2008;149:2646-2656.

Molina-Sánchez R, Galina CS, Díaz-Sánchez, Galicia-Angeles L, Estrada-Koning S. Evaluación de un empadre rotativo con monta natural: efecto en el rendimiento reproductivo de vacas cebú. Agrociencia. 2003;37:1-10.

Mondragón V, Galina CS, Rubio I, Corro M, Salmerón F. Effect of restricted suckling on the onset of follicular dynamics and body condition score in Brahman cattle raised under tropical conditions. Anim. Reprod. Sci. 2016;167:89–95.

Murphy MG, Boland MP, Roche JF. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. J. Reprod. Fertil. 1990;90:523–533.

Murphy MG, Enngth WJ, Crow MA, McConnel K, Spicer LJ, Boland MP, Roche JE. Effect of dietary intake on pattern of growth of dominant follicles during the oestrous cycle in beef heifers. J. Reprod. Fertil. 1991;92:333-338.

Myers TR, Myers DA, Gregg DW, Moss GE. Endogenous opioid suppression of release of luteinizing hormone during suckling in postpartum anestrous beef cows. Domest. Anim. Endocrinol. 1989;6:183–190.

Nabenishi H, Takagi S, Kamata H, Nishimoto T, Morita T, Ashizawa K, Tsuzuki Y. The role of mitochondrial transition pores on bovine oocyte competence after heat stress, as determined by effects of cyclosporin A. Mol. Reprod. Dev. 2012;79:31–40.

Nakahara T, Uenoyama Y, Iwase A, Oishi S, Nakamura S, Minabe S, Watanabe Y, Deura C, Noguchi T, Fujii N, Kikkawa F, Maeda K, Tsukamura H. Chronic peripheral administration of kappa-opioid receptor antagonist advances pubertyonset associated with acceleration of pulsatile luteinizing hormone secretion in female rats. J. Reprod. Dev. 2013;59:479–484.

Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J. Neurosci. 2009;29:11859–11866.

Negrão JA. Hormone release and behavior during suckling and milking in Gir, Gir x Holstein, and Holstein cows. J. Anim. Sci. 2008;86(13 Suppl):21-26.

Nienaber JA, Hahn GL. Livestock production system management responses to thermal challenges. Int. J. Biometeorol. 2007;52**:**149–157.

Niswender GD, Juengel JL, Silva PJ, Rollyson MK, McIntush EW. Mechanisms controlling the function and life span of the corpus luteum. Physiol. Rev. 2000;80:1-29.

Noguchi J, Yamashita H. Adenosine inhibits voltage-dependent Ca2 currents in rat dissociated supraoptic neurones via A1 receptors. J. Physiol. 2000;526:313–326.

Ojeda NB, Grigore D, Alexander BT. Developmental programming of hypertension: insight from animal models of nutritional manipulation. Hypertension. 2008;52:44–50.

Osawa T, Nakao T, Moriyoshi M, Nakada K. Plasma beta-endorphin around parturition and its relationship to cortisol level and resumption of pituitary and ovarian functions in dairy cows. Anim. Reprod. Sci. 1998;52:27-38.

Ozawa M, Hirabayashi M, Kanai Y. Developmental competence and oxidative state of mouse zygotes heat-stressed maternally or in vitro. Reproduction. 2002;124:683–689.

Palta P, Mondal S, Prakash BS, Madan ML. Peripheral inhibin levels in relation to climatic variations and stage of estrous cycle in buffalo *Bubalus bubalis*. Theriogenology 1997;47:989–995.

Parr RA. Nutrition–progesterone interactions during early pregnancy in sheep. Reprod. Fertil. Dev. 1992;4:297-300.

Payton RR, Romar R, Coy P, Saxton AM, Lawrence JL, Edwards JL. Susceptibility of bovine germinal vesicle stage oocytes from antral follicles to direct effects of heat stress *in vitro*. Biol. Reprod. 2004;71:1303–1308.

Pérez HP, Sanchez del Real C, Gallegos SJ. Anestro postparto y alternativas de manejo del amamantamiento en vacas de doble propósito en trópico. Investigación agraria. Producción y Sanidad Animales. 2001;16:257-270.

Pérez-Torres L, Rubio I, Corro M, Cohen A, Orihuela A, Galina CS, Pablos JL. A pre-synchronization program at early postpartum might increase the chances of *Bos indicus* cows cycling prior to 50 days regardless of the length of calf separation. J. Reprod. Dev. 2015;61:199-203.

Pérez-Torres L, Orihuela A, Corro M, Rubio I, Alonso M, Galina CS. Effects of separation time on behavioral and physiological characteristics of Brahman cows and their calves. Appl. Anim. Behav. Sci. 2016;179:17–22.

Peura TT, Kleemann DO, Rudiger SR, Nattrass GS, McLaughlan CJ, Walker SK. Effect of nutrition of oocyte donor on the outcomes of somatic cell nuclear transfer in the sheep. Biol. Reprod. 2003;68:45-50.

Rabiee AR, Macmillan KL, Schwarzenberger F, Wright PJ. Effects of level of feeding and progesterone dose on plasma and faecal progesterone in ovariectomised cows Anim. Reprod. Sci. 2001;73:185-95.

Radunz AE, Fluharty FL, Day ML, Zerby HN, Loerch SC. Prepartum dietary energy source fed to beef cows: I: Effects on pre- and postpartum cow performance. J. Anim. Sci. 2010;88:2717–2728.

Ravelli AC, Van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. Am. J. Clin. Nutr. 1999;70:811-816.

Rayco-Solon P, Fulford AJ, Prentice AM. Differential effects of seasonality on preterm birth and intrauterine growth restriction in rural Africans. Am. J. Clin. Nutr. 2005;81:134-139.

Rekwot PI, Oyedipe EO, Mukasa-Mugerwa E, Sekoni VO, Akinpelumi OP, Anyam AA. Fertility in zebu cattle (*Bos indicus*) after prostaglandin administration and artificial insemination. Vet J. 1999;158:53–58.

Rekwot P, Ogwu D, Sekoni V, Oyedipe E. Serum progesterone profiles of zebu cattle (*Bos indicus*) in relationship to conception and repeat breeding after artificial insemination. Anim. Reprod. Sci. 2000;63:41–51.

Renaudeau D, Anais C, Tel L, Gourdine JL. Effect of temperature on thermal acclimation in growing pigs estimated using a nonlinear function. J. Anim. Sci. 2010;88:3715–3724.

Renaudeau D, Collin A, Yahav S, de Basilio V, Gourdine JL, Collier RJ. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal. 2012;6:707-728.

Rhodes FM, Entwistle KW, Kinder JE. Changes in ovarian function and gonadotrophin secretion preceding the onset of nutritionally induced anoestrus in Bos indicus heifers. Biol. Reprod. 1996;55:1437-1443.

Richards MW, Wettemann RP, Schoenemann HM. Nutritional anestrus in beef cows: concentrations of glucose and nonesterified fatty acids in plasma and insulin in serum. J. Anim. Sci. 1989;67:2354–2362.

Richards JS, Sharma SC, Falender AE, Lo YH. Expression of FKHR, FKHRL1, and AFX genes in the rodent ovary: evidence for regulation by IGF-I, estrogen, and the gonadotropins. Mol. Endocrinol. 2002;16:580-599.

Robinson JJ, Ashworth CJ, Rooke JA, Mitchell LM, McEvoy TG. Nutrition and fertility in ruminant livestock. Anim. Feed. Sci. Tech. 2006;126:259-276.

Robker RL, Wu LL, Yang X. Inflammatory pathways linking obesity and ovarian dysfunction. J. Reprod. Immunol. 2011;88:142-8.

Rooke JA, Ewen M, Mackie K, Staine ME, McEvoy TG, Sinclair KD. Effect of ammonium chloride on the growth and metabolism of bovine ovarian granulosa cells and the development of ovine oocytes matured in the presence of bovine granulosa cells previously exposed to ammonium chloride. Anim. Reprod. Sci. 2004;1:53-71.

Roth Z, Meidan R, Braw-Tal R, Wolfenson D. Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. J. Reprod. Fertil. 2000;120:83–90.

Roth Z, Arav A, Bor A, Zeron Y, Braw-Tal R, Wolfenson D. Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. Reproduction 2001a;122:737–744.

Roth Z, Meidan R, Braw-Tal R, Shaham-Albalancy A, Wolfenson D. Delayed effect of heat stress on steroidogenesis in bovine medium-size and preovulatory follicles. Reproduction 2001b;121:745–751.

Roth Z, Bor A, Braw-Tal R, Wolfenson D. Carry-over effect of summer thermal stress on characteristics of the preovulatory follicle of lactating cows. J. Therm. Biol. 2004;29:681–685.

Roth Z, Hansen PJ. Involvement of apoptosis in disruption of developmental competence of bovine oocytes by heat shock during maturation. Biol. Reprod. 2004a;71:1898–1906.

Roth Z, Hansen PJ. Sphingosine-1-phosphate protects cultured bovine oocytes from heat shock during maturation. Biol. Reprod. 2004b;71:2072–2078.

Roth Z. Heat stress, the follicle, and its enclosed oocyte: mechanisms and potential strategies to improve fertility in dairy cows. Reprod. Domest. Anim. 2008;43: 238–244.

Roth Z, Aroyo A, Yavin S, Arav A. The antioxidant epigallocatechin gallate (EGCG) moderates the deleterious effects of maternal hyperthermia on follicle-enclosed oocytes in mice. Theriogenology 2008;70:887–897.

Roth Z. Physiology and endocrinology symposium: cellular and molecular mechanisms of heat stress related to bovine ovarian function. J. Anim. Sci. 2015;93:2034-2044.

Rubio I, Castillo E, Soto R, Alarcón F, Murcia C, Galina CS. Postpartum follicular development in Brahman cows under two stocking rates. Trop. Anim. Health. Prod. 2010;42:539–545.

Rusche WC, Cochran RC, Corah LR, Stevenson JS, Harmon DL, Brandt J. RT, Minton JE. Influence of source and amount of dietary protein on performance, blood metabolites, and reproductive function of primiparous beef cows. J. Anim. Sci. 1993;71:557–563.

Rutkowski DT, Kaufman RJ. A trip to the ER: coping with stress. Trends. Cell. Biol. 2004;14:20-28.

Ryan DP, Boland MP. Frequency of twin births among Holstein-Friesian cows in a warm dry climate. Theriogenology 1991;36:1–10.

Ryan NK, Woodhouse CM, Van der Hoek KH, Gilchrist RB, Armstrong DT, Norman RJ. Expression of leptin and its receptor in the murine ovary: possible role in the regulation of oocyte maturation. Biol. Reprod. 2002;66:1548-1554.

Sales JN, Carvalho JB, Crepaldi GA, Soares JG, Girotto RW, Maio JR, Souza JC, Baruselli PS. Effect of circulating progesterone concentration during synchronization for fixed-time artificial insemination on ovulation and fertility in *Bos indicus* (Nelore) beef cows. Theriogenology. 2015;83:1093-1100.

Samadi F, Phillips NJ, Blache D, Martin GB, D'Occhio MJ. Interrelationships of nutrition, metabolic hormones and resumption of ovulation in multiparous suckled beef cows on subtropical pastures. Anim. Reprod. Sci. 2013;137:137-144.

Sangsritavong S, Combs DK, Sartori R, Wiltbank MC. High feed intake increases blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J. Dairy Sci. 2002;85:2831-42.

Savio JD, Boland MP, Hynes N, Roche JF. Resumption of follicular activity in the early postpartum period of dairy cows. J. Reprod. Fertil. 1990;88:569-579.

Shrestha HK, Nakao T, Suzuki T, Akita M, Higaki T. Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high-producing dairy cows in a subtropical region in Japan. Theriogenology. 2005;64:855-866.

Scaramuzzi RJ, Campbell BK, Downing JA, Kendall NR, Khalid M, Munoz Gutierrez M, Somchit A. A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reprod. Nutr. Dev. 2006;46:339-54.

Shaham-Albalancy A, Nyska A, Kaim M, Rosenberg M, Folman Y, Wolfenson D. Delayed effect of progesterone on endometrial morphology in dairy cows. Anim. Reprod. Sci. 1997;48:159–174.

Shaham-Albalancy A, Folman Y, Kaim M, Rosenberg M, Wolfenson D. Delayed effect of low progesterone concentrations on bovine uterine PGF2α secretion in the subsequent oestrous cycle. Reproduction 2001;122:643–648.

Silva CF, Sartorelli ES, Castilho AC, Satrapa RA, Puelker RZ, Razza EM, Ticianelli JS, Eduardo HP, Loureiro B, Barros CM. Effects of heat stress on development, quality and survival of *Bos indicus* and *Bos taurus* embryos produced *in vitro*. Theriogenology. 2013;79:351-357.

Silveira PA, Spoon RA, Ryan DP, Williams GL. Evidence for maternal behavior as a requisite link in suckling-mediated anovulation in cows. Biol. Reprod. 1993;49:1338-1346.

Sirois J, Fortune JE. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: A model for studying ovarian follicular dominance. Endocrinology 1990;127:916–925.

Smith JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. Endocrinology. 2005a;146:3686–3692

Smith JT, Dungan HM, Stoll EA, Gottsch ML, Braun RE, Eacker SM, Clifton DK, Steiner RA. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. Endocrinology. 2005b;146:2976–2984.

Smith DL, Stinefelt BM, Blemings KP, Wilson ME. Diet-induced alterations in progesterone clearance appear to be mediated by insulin signaling in hepatocytes. J. Anim. Sci. 2006;84:1102-1109.

Soto CR, Rubio I, Galina CS, Castillo E, Rojas S. Effect of pre- and post-partum feed supplementation on productive performance of grazing primiparous Brahman cows. Trop. Anim. Health Prod. 2001;33:253–264.

Soto P, Smith LC. BH4 peptide derived from Bcl-xL and Bax-inhibitor peptide suppresses apoptotic mitochondrial changes in heat stressed bovine oocytes. Mol. Reprod. Dev. 2009;76:637–646.

Spencer TE, Sandra O, Wolf E. Genes involved in conceptus-endometrial interactions in ruminants: Insights from reductionism and thoughts on holistic approaches. Reproduction 2008;135:165–179.

Stagg K, Diskin MG, Sreenan JM, Roche JF. Follicular development in long-term anoestrous suckler beef cows fed two levels of energy postpartum. Anim. Reprod. Sci. 1995;38:49–61.

Stevenson JS, Knoppel EL, Minton JE, Salfen BE, Garverick HA. Estrus, ovulation, luteinizing hormone, and suckling-induced hormones in mastectomized cows with and without unrestricted presence of the calf. J. Anim. Sci. 1994;72:690-699.

Sturmey RG, Leese HJ. Energy metabolism in pig oocytes and early embryos. Reproduction. 2003;126:197-204.

Sullivan TM, Micke GC, Greer RM, Irving-Rodgers HF, Rodgers RJ, Perry VEA. Dietary manipulation of Bos indicus x heifers gestation affects the reproductive development of their heifer calves. Reprod. Fertil. Dev. 2009a;21:773-784.

Sullivan TM, Micke GC, Perry VE. Influences of diet during gestation on potential postpartum reproductive performance and milk production of beef heifers. Theriogenology. 2009b;72:1202-1214.

Swali A, Wathes DC. Influence of the dam and sire on size at birth and subsequent growth, milk production and fertility in dairy heifers. Theriogenology. 2006;66:1173-1184.

Takami M, Preston SL, Toyloy VA, Behrman HR. Antioxidants reversibly inhibit the spontaneous resumption of meiosis. Am. J. Physiol. 1999;276:684–688.

Tatman WR, Judkins MB, Dunn TG, Moss GE. Luteinizing hormone in nutrient restricted ovariectomized ewes. J. Anim. Sci. 1990;68:1097-1102.

Thatcher WW, Moreira F, Santos JEP, Mattos RC, Lopes FL, Pancarci SM, Risco CA. Effects of hormonal treatments on reproductive performance and embryo production. Theriogenology 2001;55:75–89.

Tancin V, Kraetzl W, Schams D, Bruckmaier RM. The effects of conditioning to suckling, milking and of calf presence on the release of oxytocin in dairy cows. Appl. Anim. Behav. Sci. 2001;72:235-246.

Trout JP, McDowell LR, Hansen PJ. Characteristics of the estrous cycle and antioxidant status of lactating Holstein cows exposed to heat stress. J. Dairy Sci. 1998;81:1244–1250.

Van Blerkom J, Antczak M, Schrader R. The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: Association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. Hum. Reprod. 1997;12:1047–1055.

Villa-Mancera A, Méndez-Mendoza M, Huerta-Crispín R, Vázquez-Flores F, Córdova-Izquierdo A. Effect of climate factors on conception rate of lactating dairy cows in Mexico. Trop. Anim. Health Prod. 2011;43:597-601.

Viñoles C, Paganoni B, Glover KMM, Milton JTB, Blache D, Blackberry MA, Martin GB. The use of a 'first wave' model to study the effect of nutrition on ovarian follicular dynamics and ovulation rate in the female sheep. Reproduction. 2010;140:865-874.

Von Keyserlingk MAG, Weary DM. Maternal behavior in cattle. Horm. Behav. 2007;52:106–113.

Wang G, Dayanithi G, Custer EE, Lemos JR. Adenosine inhibition via A (1) receptor of N-type Ca(2+) current and peptide release from isolated neurohypophysial terminals of the rat. J. Physiol. 2002;540:791–802.

Wettemann RP, Lents CA, Ciccioli NH, White FJ, Rubio I. Nutritional- and suckling-mediated anovulation in beef cows. J. Anim. Sci. 2003;81(E. Suppl. 2):E48-E59.

Williams GL. Suckling as a regulator of postpartum rebreeding in cattle: a review. J. Anim. Sci. 1990;68:831-852.

Wilson SJ, Kirby CJ, Koenigsfeld AT, Keisler DH, Lucy MC. Effects of controlled heat stress on ovarian function of dairy cattle.2. Heifers. J. Dairy. Sci. 1998a;81:2132–2138.

Wilson SJ, Marion RS, Spain JN, Spiers DE, Keisler DH, Lucy MC. Effects of controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows. J. Dairy Sci. 1998b;81:2124–2131.

Wise ME, Armstrong DV, Huber JT, Hunter R, Wiersma F. Hormonal alterations in the lactating dairy cow in response to thermal stress. J. Dairy Sci. 1988;71:2480–2485.

Wolfenson D, Flamenbaum I, Berman A. Hyperthermia and body energy store effects on estrous behavior, conception rate, and corpus luteum function in dairy cows. J. Dairy Sci. 1988;71:3497–3504.

Wolfenson D, Luft O, Berman A, Meidan R. Effects of season, incubation temperature and cell age on progesterone and prostaglandin F2a production in bovine luteal cells. Anim. Reprod. Sci. 1993;32:27–40.

Wolfenson D, Thatcher WW, Badinga L, Savio JD, Meidan R, Lew BJ, Braw-Tal R, Berman A. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. Biol. Reprod. 1995;52:1106–1113

Wolfenson D, Lew BJ, Thatcher WW, Graber Y, Meidan R. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. Anim. Reprod. Sci. 1997;47:9–19.

Wolfenson D, Roth Z, Meidan R. Impaired reproduction in heat-stressed cattle: Basic and applied aspects. Anim. Reprod. Sci. 2000;60–61:535–547.

Wolfenson D, Sonego H, Bloch A, Shaham-Albalancy A, Kaim M, Folman Y, Meidan M. Seasonal differences in progesterone production by luteinized bovine thecal and granulosa cells. Domest. Anim. Endocrinol. 2002;22:81–90.

Xu ZZ, McDonald MF, McCutcheon SN. The effects of nutritionally-induced live weight differences on follicle development, ovulation rate, oestrous activity and plasma follicle-stimulating hormone levels in the ewe. Anim. Reprod. Sci. 1989;19:67-78.

Yaakub H, O'Callaghan D, O'Doherty J, Hyttel P. Effect of dietary intake on follicle numbers and oocyte morphology in unsuperovulated and superovulated ewes. Theriogenology. 1997;47:182.

Yahav S, Straschnow A, Plavnik I, Hurwitz S. Blood system response of chickens to changes in environmental temperature. Poult. Sci. 1997;76:627–633.

Yahav S. Alleviating heat stress in domestic fowl – different strategies. World. Poult. Sci. J. 2009;65:719–732.

Yahav S, Shinder D, Ruzal M, Giloh M, Piestun Y. Controlling body temperature – the opportunities for highly productive domestic fowl. In Body temperature control (ed. AB Cisneros and BL Goins), pp. 65–98. NovaScience Publishers Inc., New York, USA. 2009.

Yamamoto S, Young BA, Purwanto BP, Nakamasu F, Matsumoto T. Effect of solar radiation on the heat load of dairy heifers. Aust. J. Agric. Res. 1994;45:1741–1749.

Yavas Y, Walton JS. Postpartum acyclicity in suckled beef cows: a review. Theriogenology 2000;54:25-55.

Younas M, Fuquay JW, Smith AE, Moore AB. Estrous and endocrine responses of lactating Holsteins to forced ventilation during summer. J. Dairy Sci. 1993;76:430–436

Zalesky DD, Forrest DW, Mc Atrhur NH, Wilson JM, Morris DL, Harms PG. Suckling inhibits release of luteinizing hormone releasing hormone from the bovine median eminence following ovarietomy. J. Anim. Sci. 1990;68:444-448.

Zeron Y, Ocheretny A, Kedar O, Borochov A, Sklan D, Arav A. Seasonal changes in bovine fertility: Relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. Reproduction 2001;121:447–454.

Zeron Y, Sklan D, Arav A. Effect of polyunsaturated fatty acid supplementation on biophysical parameters and chilling sensitivity of ewe oocytes. Mol. Reprod. Dev. 2002;61:271–278.

Zhu MJ, Ma Y, Long NM, Du M, Ford SP. Maternal obesity markedly increases placental fatty acid transporter expression and fetal blood triglycerides at midgestation in the ewe. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2010;299:R1224-1231.

“Cuando la ciencia ha hablado,
solo queda callar.”

Julio Verne
Viaje al centro de la tierra,
1864 AD.