

**UNIVERSIDAD AUTÓNOMA AGRARIA  
ANTONIO NARRO  
UNIDAD LAGUNA**

**DIRECCIÓN DE POSGRADO**

**FACTORES QUE INFLUYEN EN LA PREÑEZ DE VACAS REPETIDORAS  
TRATADAS CON rbST E INDUCIDAS A OVULAR CON UN PROTOCOLO  
BASADO EN CIDR**

**TESIS**

**PRESENTADA COMO REQUISITO PARCIAL  
PARA OBTENER EL GRADO DE:**

**MAESTRA EN CIENCIAS EN PRODUCCIÓN AGROPECUARIA**

**POR:**

**MVZ ARACELY ZÚÑIGA SERRANO**

**DIRECTOR DE TESIS: DR. MIGUEL MELLADO BOSQUE**

**TORREÓN, COAHUILA, MÉXICO  
MAYO 2012**

Universidad Autónoma Agraria Antonio Narro  
Unidad Laguna  
Subdirección de posgrado

**FACTORES QUE INFLUYEN EN LA PREÑEZ DE VACAS REPETIDORAS  
TRATADAS CON rbST E INDUCIDAS A OVULAR CON UN PROTOCOLO  
BASADO EN CIDR  
POR**


**ARACELY ZÚÑIGA SERRANO**

Elaborado bajo la supervisión del Comité Particular de Asesoría y aprobada como  
requisito parcial, para optar al grado de:

**MAESTRA EN CIENCIAS EN PRODUCCIÓN AGROPECUARIA**

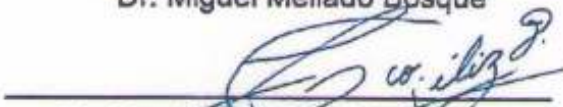
Comité Particular de Asesoría

Asesor principal:



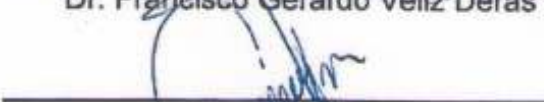
Dr. Miguel Mellado Bosque

Asesor:



Dr. Francisco Gerardo Vélz Deras

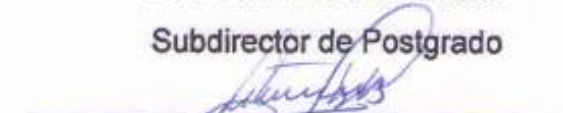
Asesor:



Dra. Ma. de los Angeles de Santiago Miramontes

Dr. Fernando Ruíz Zarate

Subdirector de Postgrado



Dr. Pedro Antonio Robles Trillo

Jefe del Departamento de Postgrado U.L.

Torreón, Coahuila, México

Mayo de 2012

## DEDICATORIA

*Este trabajo está dedicado a quienes forman la base de mi vida y por quienes soy la persona que soy ahora:*

*A mis padres:*

*Ramón Zúñiga Romero y Ma del Carmen Serrano Padilla, a mi padre por inculcarme el amor al estudio, la disciplina, a fijarme metas , conseguir las a base de esfuerzo y a ser perseverante; a mi madre por su incansable lucha porque mis hermanos y yo tuviéramos una carrera profesional y anteponer siempre nuestras necesidades a las suyas , por enseñarme a luchar sin darme por vencida a pesar de las circunstancias en contra y pese a todos los pronósticos...estoy sumamente orgullosa de ella y me siento feliz de tenerla.*

*A mis hermanos:*

*José Ramón, Luz del Carmen, Dolores Josefina, Adriana y Miguel Ángel, por todas las tristezas y alegrías compartidas, porque todo lo que pasamos valió la pena, por ser buenos hijos y buenos hermanos, y por estar siempre unidos...los quiero mucho. Especialmente a Luz del Carmen y José Ramón, gracias por todo su apoyo, ustedes son una parte muy importante en este logro.*

*A mi tío:*

*Profesor Jesús Zúñiga Romero, por su apoyo incondicional y por estar a nuestro lado siempre.*

*A mi Abuelo Sr Francisco Zúñiga Granados †, por ser como un padre y estar con mis hermanos y conmigo en todo momento.*

*A mi Abuelita Soledad Padilla García †, por su ejemplo de trabajo, por su apoyo incondicional y todo el amor que nos dio a mis hermanos y a mí. Siempre estará presente en mi corazón y nunca dejaré de extrañarla.*

*A dos personitas que llegaron a mi vida para que yo conociera el amor más grande e incondicional que se puede conocer y que me hicieron comprender mucho a mi madre: a mis hijos Jair y Paulina, son el motor de mi vida y todos mis logros son pensando en ellos, son mi adoración.*

*A mi esposo: Jair Méndez Lara...Por su invaluable apoyo para que este proyecto fuera una realidad, por ser un excelente compañero y amigo.*

**GRACIAS A TODOS ELLOS**

*Porque Jehová da la sabiduría.  
Y de su boca viene el conocimiento y la inteligencia.  
El provee de sana sabiduría a los rectos;  
Es escudo a los que caminan rectamente.  
Cuando la sabiduría entrare en tu corazón,  
Y la ciencia fuere grata a tu alma,  
La discreción te guardará;  
Te preservará la inteligencia,  
Para librarte del mal camino,  
De los hombres que hablan perversidades.  
Proverbios 2, 6:12*

## *AGRADECIMIENTOS*

*A Dios, por concederme la vida y darme la oportunidad de llegar hasta aquí, por manifestarse en mí cuando más he necesitado de Él.*

*A mis padres y hermanos por estar presentes en mi vida en todo momento.*

*A mi esposo e hijos, por su comprensión y apoyo.*

*A mi “Alma Mater”, por abrirme sus puertas después de 15 años y darme la oportunidad de ser alguien.*

*Quiero hacer especial este agradecimiento a estas personas que Dios puso en mi camino, en el tiempo y el lugar correctos:*

*Doctor Miguel Mellado Bosque, Doctor Francisco Gerardo Véliz Deras y Doctora María de los Ángeles de Santiago Miramontes, mis asesores en este proyecto. Especialmente al Doctor Miguel Mellado Bosque, mi asesor principal y pieza clave en este proyecto. Nunca podré terminar de agradecer todo el apoyo, me siento muy orgullosa de haberlos conocido y pertenecer a este gran grupo de trabajo.*

*A mis queridos amigos, Manuel Guillén Muñoz, Oscar Ángel García, Edgar Sepúlveda González, Sonia Ibarra y Sandra Marcelino León, me siento muy feliz de haberlos conocido y*

*compartir con ustedes momentos amargos y de mucha felicidad, y de formar este equipo tan bonito, a mis queridas amigas además de compañeras Luz María Tejada Ugarte y Leticia Gaytán Alemán; Lucy te agradezco todo lo que me has dado aparte de tu gran amistad, tus ocurrencias, tu apoyo incondicional, tu inteligencia que muchas veces era la solución a mis problemas, te admiro y te quiero mucho; Lety, te empecé a conocer para mi gusto muy tarde, porque me perdí de muchas cosas bonitas que pudimos haber vivido, pero no lo lamento, porque he aprendido a quererte, a admirarte por tu nobleza e inteligencia y por lo divertida que eres.*

*Al Doctor Esteban Favela Chávez...gracias por todo Doctor, porque sin conocerme me tendió la mano, tiene mi agradecimiento por siempre.*

*Al M.V.Z. Rodrigo Isidro Simón Alonso nunca terminaré de agradecerle todo lo que ha hecho por mí, le reitero mi admiración, mi cariño y apoyo incondicional. GRACIAS!!!*

*Al Doctor Fernando Ulises Adame de León, por todo el apoyo brindado y la confianza que depositó en mí persona.*

*A la Doctora María de los Ángeles de Santiago Miramontes...le agradezco todo su apoyo y la confianza, porque me ha permitido conocer a la persona tan linda que es.*

*A la MC María Hortensia Cepeda Eizalde, por ser un ejemplo en mi carrera y en mi vida, gracias por su apoyo, por sus consejos y por todo el tiempo que me ha dedicado...la quiero mucho maestra.*

*Al Señor Gildardo López nunca podré terminar de agradecer la confianza que puso en mí y todo lo que usted hizo para que ahora esté donde me encuentro, Dios lo bendiga.*

## ÍNDICE DE CONTENIDO.

DEDICATORIA .....	III
AGRADECIMIENTOS.....	V
ÍNDICE DE CONTENIDO.....	VII
ÍNDICE DE CUADROS .....	IX
COMPENDIO .....	X
ABSTRACT .....	XII
INTRODUCCIÓN. ....	XIV
OBJETIVO .....	XVI
HIPÓTESIS .....	XVI
REVISIÓN DE LITERATURA .....	1
LITERATURA CITADA.....	9
MANUSCRITO EN REVISIÓN EN ANIMAL REPRODUCTION SCIENCE .....	13
ABSTRACT .....	13
1. INTRODUCTION.....	14

<b>2. MATERIALS AND METHODS.....</b>	<b>15</b>
2.2. STUDY HERD, HOUSING AND FEEDING.....	16
2.3. REPRODUCTIVE MANAGEMENT.....	17
2.3. DATA COLLECTION.....	18
2.4. Statistical analyses.....	19
<b>3. RESULTS .....</b>	<b>20</b>
<b>4. DISCUSSION .....</b>	<b>24</b>
<b>CONCLUSIONS.....</b>	<b>27</b>
<b>ACKNOWLEDGMENTS .....</b>	<b>28</b>
<b>REFERENCES.....</b>	<b>28</b>



## ÍNDICE DE CUADROS

**1.- ODDS RATIO (OR) PARA LA PROBABILIDAD DE PREÑEZ EN VACAS REPETIDORAS EN FUNCIÓN DE LOS COMPONENTES DE LA LECHE, LA DURACIÓN DE LAS LACTANCIAS, LA DURACIÓN DEL PERÍODO SECO EN LA LACTANCIA ANTERIOR Y THI EL DÍA DE LA IATF.....VIII**

**2.- EL EFECTO DE LA TEMPORADA EN LA TASA DE PREÑEZ EN VACAS HOLSTEIN REPETIDORAS SOMETIDAS A UN PROGRAMA DE SINCRONIZACIÓN DE CELO Y CIDR-AITF.....**

## **COMPENDIO**

**Factores que influyen en la preñez de vacas repetidoras tratadas con rbst  
e inducidas a ovular con un protocolo basado en cidr**

**Por**

**Aracely Zúñiga Serrano**

**MAESTRÍA EN CIENCIAS EN PRODUCCIÓN AGROPECUARIA**

**UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO**

**UNIDAD LAGUNA**

**Torreón, Coahuila, MAYO 2012**

El objetivo de este estudio fue determinar, utilizando modelos logísticos múltiples, los factores que afectan la preñez por IA (P/IA) a tiempo fijo (IATF) en vacas repetidoras (VR) con intervalos de parto prolongados y tratadas con rbST durante la lactancia. 498 vacas de diferentes partos, que no habían quedado preñadas alrededor de los 200 días en leche (DEL), recibieron un dispositivo de CIDR y 100 mg de GnRH el día 0. La remoción del CIDR y el tratamiento de PGF2 $\alpha$  (25 mg) se realizaron simultáneamente el día 7. El día 8 se inyectó

benzoato de estradiol (EB 1 mg) y GnRH el día 9, las vacas fueron inseminadas a las 16-20 horas después. Las vacas con un promedio de grasa en leche <3% tuvieron 43% más de probabilidades ( $P \leq 0.05$ ), de quedar preñadas con la IATF que las vacas con un contenido de grasa en la leche >3%. Las vacas con <6 servicios aumentaron significativamente las probabilidades de quedar preñadas que las vacas con >6 servicios a la IATF (P/IA 36 vs. 27%,  $p < 0.05$ ). Vacas con menos de 3 lactancias tratadas con CIDR tuvieron 1.7 veces más probabilidades (P/IA 35 comparado con 21%,  $p < 0.05$ ) para quedar preñadas que las vacas de más edad. Las vacas cuyo período seco fue >62 días tuvieron más probabilidades de quedar preñadas ((P/IA 35 vs. 30%;  $p < 0.05$ ) que las vacas sometidas a la IATF, con períodos secos <62 días. Las vacas cuyo pico de producción de leche fue inferior a 55 kg fueron 1.5 veces más propensas a quedar preñadas que las vacas cuyo pico de leche fue superior a 55 kg (P/IA 37 vs. 28%,  $p < 0.05$ ). Vacas sometidas a la IATF con un índice de temperatura-humedad (THI) <76 tuvieron 45% más de probabilidades ( $p < 0.05$ ) para quedar preñadas que las vacas inseminadas con una THA >76. Las vacas tratadas con CIDR mostraron una depresión ( $p < 0.05$ ) en la fertilidad durante el verano en comparación con otras estaciones del año. Se concluyó que una proporción aceptable (32%) de las vacas repetidoras (VR) quedaron preñadas con el protocolo utilizado en el presente estudio. Así mismo, la IA alrededor de los 200 DIM es económicamente justificable en establos lecheros con 3 ordeñas por día el uso de rbST a lo largo de la lactancia, porque la duración de las lactancias se puede extender más de 15 meses. Este estudio también demostró que la subfertilidad en vacas tratadas con CIDR se asocia con altos rendimientos al pico de la lactancia, el alto contenido de proteína de leche, un mayor número de servicios, edad avanzada, alta temperatura ambiente el día de la IA y a cortos períodos secos.

**Palabras clave:** desempeño reproductivo, sincronización de celo, pico de lactancia, proteína en leche, período seco.

## **Abstract**

### **Factors influencing pregnancy per AI in repeat breeder cows treated with rbST and induced to ovulate with a CIDR-based protocol**

**By**

**Aracely Zúñiga Serrano**

**MASTER OF SCIENCE IN AGRICULTURAL PRODUCTION**

**UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO**

**UNIDAD LAGUNA**

**Torreón, Coahuila, MAYO 2012**

The aim of this study was to determine, using multiple logistic models, factors affecting pregnancy per AI (P/AI) following fixed-time artificial insemination (FTAI) in repeat breeders cows (RBC) with extended postpartum intervals and treated with rbST throughout lactation. 498 Holstein cows of all parities, unable to become pregnant around 200 days in milk (DIM) received a CIDR device and 100 µg of GnRH on Day 0. CIDR removal and PGF<sub>2α</sub> (25 mg) treatment were done concurrently on day 7. Estradiol benzoate (EB, 1 mg) was injected on day 8 and GnRH on day 9; cows were inseminated 16-20 h later. Cows with an average milk fat <3% were 43% more likely ( $P<0.05$ ) to become pregnant at FTAI than cows with milk fat >3%. Cows with <6 services had significantly increased chances of becoming pregnant than cows with >6 services at FTAI

(P/AI 36 vs. 27%;  $P<0.05$ ). CIDR-treated cows with less than three lactations older cows. Cows with dry period length  $>62$  days were more likely to become pregnant (P/AI 35 vs. 30%;  $P<0.05$ ) than cows subjected to FTAI with dry periods  $<62$  days. Cows with peak milk yields lower than 55 kg were 1.5 times more likely to get pregnant than cows with peak milk yields greater than 55 kg (P/AI 37 vs. 28%;  $P<0.05$ ). Cows subjected to FTAI with a temperature-humidity index (THI)  $<76$  were 45% more likely ( $P<0.05$ ) to become pregnant than cows inseminated with a THA $>76$ . CIDR-treated were 1.7 times more likely (P/AI 35 vs. 21%;  $P<0.05$ ) to become pregnant than cows showed a depression ( $P<0.05$ ) in fertility during summer compared to other seasons of the year. It was concluded that an acceptable proportion (32%) of RBC can become pregnant with the protocol used in the present study. Also, AI around 200 DIM is economically justifiable in dairy operations with 3x milking and the use of rbST throughout lactations, because lactation length can be extended beyond 15 months. This study also demonstrated that subfertility in CIDR-treated cows is associated with high peak lactational yields, high milk protein content, increased service, increased age, high ambient temperature at AI and short dry periods.

**Keywords:** reproductive performance; estrus synchronization; peak lactation, milk protein; dry period.

## **Introducción.**

En la industria lechera los retornos económicos se maximizan cuando las vacas conciben con menos de tres inseminaciones en la lactancia temprana. Este escenario está lejos de alcanzarse en los establos lecheros de la región lagunera, debido a estrés calórico crónico de esta zona y la intensificación extrema de la producción de leche en las explotaciones lecheras de esta región, consistente en tres ordeñas diarias, mas de dos comidas por día, y el uso rutinario de la hormona del crecimiento a través de lactancias que usualmente rebasan los 400 días de lactancia. Aunado a lo anterior, la producción de leche por lactancia en años recientes ha rebasado los 9,000 kg de leche en lactancias de 300 días, lo cual repercute negativamente en la eficiencia reproductiva de las vacas (Royal et al., 2000; Berry et al., 2008).

Algunos factores que impiden que las vacas lecheras queden gestantes antes de los 100 días de lactancia son errores en la detección de estros (Heuwieser et al., 1997; Pursley et al., 1998), inflamaciones o impedimentos anatómicas del canal reproductivo de las vacas (Senosy et al., 2009; Rizos et al., 2010), imbalances hormonales (Waldmann et al., 2001; Bage et al., 2002), defectos en la ovulación (Bage et al., 2002), anormalidades en los gametos (Maurer and Echterkamp, 1985), deficiencias en el funcionamiento del cuerpo lúteo (Mann and Lamming, 2001), desórdenes nutricionales (Thatcher et al., 2010) y la mortalidad embrionaria antes de los 40 días de gestación (Zavy et al., 1994; Peters, 1996) y estrés calórico (Al-Katanani et al., 1999; Sartori et al., 2002).

En vacas con producciones de leche sobresaliente y que no quedan gestantes después de repetidos servicios, puede resultar rentable tratar de

fecundarlas alrededor de los 300 días de lactancia. Lo anterior se debe a que los altos niveles de producción de leche de estas vacas permiten que estas tengan lactancias prolongadas, y de esta forma estas vacas no experimentan periodos secos prolongados.

Existen muchos protocolos para inducir la ovulación en vacas lecheras sin detección de celo (Lane et al., 2008). El uso de estos protocolos es una alternativa para fecundar vacas con un historial de fallas en su fecundación en su lactancia temprana. El objetivo de este estudio es determinar los factores involucrados en el éxito de la fecundación de vacas “repetidoras” con un alto nivel de producción de leche, inducidas a la ovulación con una combinación de progestágenos, PGF2 $\alpha$ , GnRH y benzoato de estradiol e inseminadas sin detección de celo. Un objetivo adicional es comparar la producción de leche y duración de la lactancia en aquellas vacas que queden preñadas alrededor de los 300 días de lactancia y las no preñadas, para precisar si económicamente es conveniente fecundar las vacas de un alto potencial lechero en un estado avanzado de la lactancia.

## **Objetivo**

El objetivo de este estudio fue determinar los factores involucrados en el éxito de la fecundación de vacas “repetidoras” con un alto nivel de producción de leche, inducidas a la ovulación con una combinación de progestágenos, PGF2 $\alpha$ , GnRH y benzoato de estradiol e inseminadas a tiempo fijo sin detección de celo. Un objetivo adicional fue comparar la producción de leche y duración de la lactancia en aquellas vacas que quedaron preñadas alrededor de los 300 días de lactancia y las no preñadas, para precisar si económicamente es conveniente fecundar las vacas de un alto potencial lechero en un estado avanzado de la lactancia.

## **Hipótesis**

En vacas cuya producción de leche es sobresaliente y que no quedan gestantes después de repetidos servicios, puede resultar rentable tratar de fecundarlas alrededor de los 300 días de lactancia. Lo anterior se debe a que los altos niveles de producción de leche de estas vacas permiten que estas tengan lactancias prolongadas, y de esta forma éstas no experimentan periodos secos prolongados.





## Revisión de literatura

En un estudio de Kim et al. (2007) se compararon dos protocolos basados en el uso de CIDR (dispositivo vaginal de progesterona) e inseminación a tiempo fijo en vacas Holstein “repetidoras”, sin anormalidades aparentes en su aparato reproductivo. En un primer experimento, un grupo de 55 vacas se asignaron a dos tratamientos: (1) Aplicación intravaginal de CIDR; a las vacas se les administró una inyección de 1 mg de benzoato de estradiol (BE) más 50 mg de progesterona (P4; Día 0). El día 7 recibieron una inyección de PGF2 $\alpha$  y el CIDR fue removido. Las vacas recibieron una inyección de 1 mg EB el día 8 y fueron inseminadas 30 h después. El segundo grupo de vacas se les aplicó el CIDR y fueron inyectadas con 250 ug gonadorelina (GnRH; Día 0). El día 7 recibieron una inyección de PGF2 $\alpha$  y el CIDR fue removido. Las vacas fueron inyectadas con 250 ug gonadorelina el día 9 y fueron inseminadas 17 horas después.

En un segundo experimento, vacas repetidoras (no preñadas) del primer experimento fueron asignadas a los mismos tratamientos del primer experimento. Los ovarios de las vacas fueron examinados por ultrasonografía y se colectaron muestras de sangre para análisis de progesterona.

Las tasas de preñez en el primer (18.5 vs. 32.1%) y segundo (40.0 vs. 38.1%) experimento y los datos combinados (27.7 vs. 34.7%) no difirieron entre grupos. La proporción de vacas con emergencia de olas foliculares no difirieron entre vacas tratadas con benzoato de estradiol (12/15) y GnRH (13/15). Se concluyó que los tratamientos con benzoato de estradiol o GnRH en vacas tratadas con CIDR son igualmente efectivos para sincronizar olas foliculares, sincronizar la ovulación y tener aceptables tasas de preñez en vacas repetidoras.

En un estudio de Souza et al. (2008) se evaluó la aplicación del protocolo Ovsynch antes del Ovsynch con inseminación a tiempo fijo (Doble-Ovsynch) el cual se compare con el protocolo Presynch-Ovsynch. Para este estudio se usaron 337 vacas Holstein las cuales se dividieron en dos grupos: Presynch (n = 180), dos inyecciones de PGF2 $\alpha$  a intervalos de 14 d, seguido por el Ovsynch con inseminación 12 d más tarde; (2) Doble-Ovsynch (n = 157), con aplicación de GnRH, PGF2 $\alpha$  7 d más tarde, y GnRH 3 d después, seguido de Ovsynch con inseminación a tiempo fijo, 7 d después. Los tratamientos se aplicaron alrededor de 70 días postparto. Doble-Ovsynch incrementó las gestaciones pos IA (G/IA) comparado con Presynch-Ovsynch (49.7% vs. 41.7%). Doble-Ovsynch incrementó G/IA solo en primíparas (65.2% vs. 45.2%) y no vacas multíparas (37.5% vs. 39.3%). En un subgrupo de 87 vacas se llevaron a cabo estudios de ultrasonografía y mediciones de los niveles de P4 al aplicar GnRH y 7 días después. Doble-Ovsynch disminuyó el porcentaje de vacas con baja P4 (<1 ng/mL) al aplicar GnRH (9.4% vs 33.3%) e incrementó el porcentaje de vacas con altos niveles de P4 ( $\geq 3$  ng/mL) al aplicar PGF2 $\alpha$  (78.1% vs 52.3%). Entonces, la presincronización de las vacas con Doble-Ovsynch incrementa la fertilidad en vacas primíparas comparado con el procedimiento Presynch, posiblemente debido a la inducción de la ovulación en vacas no ciclando.

Suh et al. (2007) llevaron a cabo un estudio con vacas repetidoras, donde probaron el efecto de benzoato de estradiol (BE) o GnRH sobre las olas foliculares, desarrollo folicular y tasas de preñez de vacas tratadas con CIDR (dispositivo vaginal de progesterona) e inseminación a tiempo fijo. Las vacas recibieron CIDR con una inyección de 1 mg BE mas 50  $\mu$ g de progesterona o 250  $\mu$ g de Gonadorelin (GnRH) al inicio del experimento (día 0) En el día 7 el CIDR fue removido y todas las vacas recibieron PGF2 $\alpha$ . Las vacas en el grupo BE + P4 recibieron una inyección de EB el día 8 y AI 30 horas después. Las vacas del grupo GnRH recibieron una inyección de 250  $\mu$ g GnRH el día 9 y fueron inseminadas 19 horas más tarde. En un subgrupo de vacas los ovarios fueron estudiados con ultrasonografía cada 24 h. La emergencia de las olas

foliculares ocurrió dentro de 7 días en 12/15 de las vacas tratadas con EB + P4 y 13/15 en las vacas tratadas con GnRH. La media del diámetro de los folículos preovulatorios fue más pequeña en el grupo EB + P4 ( $12.1 \pm 0.1$  mm) que las vacas tratadas con GnRH ( $13.8 \pm 0.1$  mm). Las vacas con ovulación sincronizada no difirió entre grupos de vacas: EB + P4 (11/15 y 19.4%, respectivamente) y para GnRH (13/15 y 34.3%, respectivamente). Se concluyó que con tratamientos con EB o GnRH en vacas tratadas con CIDR se logra una misma sincronización folicular, desarrollo folicular, ovulación y tasas de preñez.

En un estudio de Dewey et al. (2010) se evaluaron tres protocolos de sincronización para vacas lecheras. A los  $32 \pm 3$  d después de enrolarse en el programa de inseminación artificial (AI; d -7), una semana antes del diagnóstico de preñez, las vacas de dos explotaciones lecheras se distribuyeron en tres protocolos de sincronización de la ovulación. Las vacas fueron revisadas para detectar preñez a los  $39 \pm 3$  d después de enrolarlas en los programas de IA. Las vacas Testigo que no fueron detectadas preñadas fueron resincronizadas (d 0-GnRH, d 7-PGF<sub>2α</sub>, y d 10-GnRH y AI) el mismo día. Las vacas en el tratamiento GGPG (GnRH-GnRH-PGF<sub>2α</sub>-GnRH) recibieron una inyección de GnRH al momento de enrolarse (d -7) y si se diagnosticaban no preñadas eran sometidas al tratamiento de resincronización de las vacas testigo en el día 0. Las vacas en el tratamiento CIDR que eran diagnosticadas no preñadas recibían el protocolo de resincronización del testigo más CIDR del día 0 al 7. Después de la IA resincronizada, la preñez de las vacas fue detectada a los  $39 \pm 3$  y  $67 \pm 3$  d (hatos de California) o  $120 \pm 3$  d (hatos de Arizona). Las vacas del grupo GGPG presentó más cuerpos lúteos que las del grupo CIDR y testigo el d 0 ( $1.30 \pm 0.11$ ,  $1.05 \pm 0.11$ , y  $1.05 \pm 0.11$ , respectivamente) y d 7 ( $1.41 \pm 0.14$ ,  $0.97 \pm 0.13$ , y  $1.03 \pm 0.14$ , respectivamente). A los  $39 \pm 3$  d después de la resincronización de la IA, la preñez por IA (P/IA) se incrementó en las vacas en los grupos GGPG (33.6%) y CIDR (31.3%) comparado con el testigo (24.6%).

Kim et al. (2006) evaluaron si el uso de CIDR (dispositivo vaginal de progesterona) e IA a tiempo fijo podría usarse como una herramienta para el tratamiento de quistes foliculares en vacas lecheras. En un primer experimento vacas lecheras lactantes diagnosticadas con quistes foliculares fueron asignadas a dos tratamientos: una inyección de GnRH al momento del diagnóstico (día 0) e IA al estro dentro de 21 d. A otro grupo se le insertó CIDR y una inyección de GnRH el día 0, PGF<sub>2α</sub> al momento de la inserción del CIDR el cual se removió el día 7, GnRH se inyectó el día 9, y TAI 16 h después de la inyección de GnRH. La tasa de concepciones después de aplicar el tratamiento de CIDR e IA a tiempo fijo fue mayor (52.3%) que cuando sólo se aplicó GnRH (26.9%). En un segundo experimento vacas lecheras diagnosticadas con quistes foliculares y vacas con ciclos estruales normales recibieron los mismos tratamientos: CIDR y una inyección de GnRH el día 0, PGF<sub>2α</sub> al remover CIDR el día 7, y GnRH el día 9. La proporción de vacas con emergencia de olas foliculares y el intervalo entre el tratamiento y la ola folicular no difirió entre grupos. La media del diámetro del folículo dominante los días 4 t 7 así como el folículo preovulatorio el día 9, y la sincronización de la ovulación después de la segunda inyección de GnRH no difirió entre grupos. Estos autores concluyeron que el tratamiento con CIDR resulta en una aceptable tasa de concepciones en vacas lecheras con quistes foliculares.

El objetivo de un estudio de Bartolomé et al. (2005b) fue comparar la tasa de preñez a la resincronización e IA a tiempo fijo en vacas lecheras que recibieron GnRH a los 23 d y fueron diagnosticadas no preñadas a los 30 d después de enrolarse en el programa de IA. Las vacas no preñadas al día 30 (día 0 del estudio) se clasificaron como diestro (74.8%), metestro (5.6%) y sin cuerpo lúteo (CL) (19.5%). Las vacas en diestro se distribuyeron al grupo GnRH (PGF<sub>2a</sub> el día 0, GnRH el día 2 e IA 16 h más tarde, o al grupo de cipionato de estradiol (CE) (PGF<sub>2a</sub> el día 0, ECP el día 1, e IA 36 h después.

Las vacas en metestro se asignaron al grupo de Heatsynch modificado (GnRH el día 0, PGF2a el día 7, CE el día 8 e IA el día 9) Las vacas sin CL se clasificaron como proestro (10.6%), con quistes ováricos (7.5%) o en anestro (1.4%), y se asignaron a recibir los tratamientos GnRH versus CIDR ya sea en grupo GnRH (GnRH el día 0, PGF2a el día 7, GnRH el día 9 IA 16 h más tardar), el grupo CIDR (CIDR días 0 a 7, PGF2a el día 7, GnRH el día 9 e IA 16 h después), el grupo GnRH + CIDR (GnRH el día 0, CIDR del día 0 al 7, PGF2a el día 7, GnRH el día 9 e IA 16 h después), y el grupo testigo (PGF2a el día 7, GnRH el día 9 e IA 16 h después).

Para las vacas en diestro no existió diferencia en tasa de preñez los días 30, 55 and 90 para las vacas en el grupo GnRH (27.5, 26.5 y 24.2%) o CE (29.1, 25.5 y 24.1%). Para las vacas sin CL GnRH el día 0 incrementó la proporción de vacas con CL los días 7 y 17 y la concentración de P4 en plasma el día 17 en vacas con quistes ováricos pero no en vacas en proestro. Este estudio mostró que el CIDR incrementó la tasa de preñez en vacas con quistes ováricos pero disminuyó la tasa de preñez en vacas en proestro.

El objetivo de otro estudio de Bartolomé et al. (2005a) fue comparar la tasa de preñez y pérdida de preñez en vacas diagnosticadas no preñadas y reinseminadas después de aplicar los protocolos Ovsynch o Heatsynch. También se evaluaron los efectos los estados del ciclo estrual, quistes ováricos y anestro sobre la tasa de preñez para ambos protocolos. Vacas no preñadas se asignaron al grupo Ovsynch recibieron GnRH el día 0, PGF<sub>2α</sub> el día 7, GnRH el día 9, e IA 16 h después (día 10). Las vacas en el grupo Heatsynch recibieron GnRH el día 0, PGF<sub>2α</sub> el día 7, cipionato de estradiol (CE) el día 8, e IA 48 h después (día 10). Las tasas de preñez a los 27, 45 y 90 días después de la resincronización de la IA no difirieron para el grupo Ovsynch (25.2, 17.5, and 13.9%) y Heatsynch (25.8, 19.9, and 16.1%). No hubo diferencia para pérdidas de preñez los días 27 a 45 y días 45 a 90 para vacas en el grupo Ovsynch (25.0 y 17.9%) y Heatsynch (14.7 y 10.3%). Sin embargo, las tasas de preñez se

incrementaron en las vacas en metaestro con el protocolo de Heatsynch y las vacas con quistes ováricos sometidas al protocolo de Ovsynch.

Fricke et al. (2003) llevó a cabo un estudio en un establo comercial de Wisconsin donde las vacas recibieron un tratamiento hormonal para su primera IA. Este tratamiento consistió en dos inyecciones de 25 mg  $\text{PGF}_{2\alpha}$  a los  $32 \pm 3$  d y  $46 \pm 3$  d (Presynch); 100  $\mu\text{g}$  GnRH a los  $60 \pm 3$  d; 25 mg  $\text{PGF}_{2\alpha}$  a los  $67 \pm 3$  d; y 100  $\mu\text{g}$  GnRH + AI a los  $69 \pm 3$  d (Ovsynch). Las vacas inseminadas se distribuyeron para recibir el protocolo de resincronización (Resynch; 100  $\mu\text{g}$  GnRH, d 0, 25 mg  $\text{PGF}_{2\alpha}$ , d 7, 100  $\mu\text{g}$  GnRH + AI, d 9) a los 19 (D19), 26 (D26), o 33 d (D33) después de la primera AI para proceder con una segunda AI que no concibieron con el Ovsynch. La tasa de preñez (TP) pos IA (TP/IA) para Ovsynch a los 68 d después de la IA fue 31% sin diferencias entre grupos. Para Resynch TP/IA se determinó 26 d después de AI para las vacas D19 y D26 y 33 d después de AI para vacas D33. La TP/IA para Resynch fue 32%. Sin embargo, TP/IA para las vacas tratadas con Resynch para D26 (34%) y D33 (38%) fue mayor que para vacas D19 (23%) con un cuerpo lúteo al momento de la inyección de  $\text{PGF}_{2\alpha}$  o a la primera inyección de GnRH (vacas D26 + D33). Estos autores indican que aunque la administración de GnRH a vacas preñadas 19 días después de primer servicio no parece inducir pérdida embrionaria, la iniciación de Resynch 19 d después de la inseminación resultó en una reducción de TP/IA con la iniciación de Resynch 26 o 33 d después de la primera inseminación.

Brusveen et al. (2008) utilizaron vacas Holstein, las cuales recibieron GnRH seguido de 7 d después de  $\text{PGF}_{2\alpha}$ ; y después recibieron alguno de los siguientes tratamientos: 1) GnRH + IA a tiempo fijo 48 h después de  $\text{PGF}_{2\alpha}$ ; (Cosynch-48); 2) GnRH 56 h después de  $\text{PGF}_{2\alpha}$ ; + IA a tiempo fijo 72 h después de  $\text{PGF}_{2\alpha}$ ; (Ovsynch-56); o 3) GnRH + IA a tiempo fijo 72 h después de  $\text{PGF}_{2\alpha}$ ; (Cosynch-72). La preñez se diagnosticó por ultrasonido a los 31-33 y nuevamente a los 52-54 días posinseminación. La tasa de preñez para los

grupos Cosynch-48 (29.2%) y Cosynch-72 (25.4%). El grupo Ovsynch-56 presentó una mayor tasa de preñez (38.6%) que Cosynch-48 o Cosynch-72. Las vacas presincronizadas en su primer servicio presentaron mayores tasas de preñez que las vacas con varios servicios en Cosynch-48 (36.2 vs. 23.0%) y Ovsynch-56 (44.8 vs. 32.7%) pero no en Cosynch-72 (24.6 vs. 26.2%). Igualmente, las vacas primerizas presentaron tasas de preñez más altas que las vacas multíparas en Cosynch-48 (34.1 vs. 22.9%) y Ovsynch-56 (41.3 vs. 32.6%), pero no en Cosynch-72 (29.8 vs. 25.3%). Estos investigadores no encontraron ventajas del Cosynch a las 72 h vs. 48 h. Por otra parte, se encontró una clara ventaja al utilizar GnRH a las 56 h, 16 h antes a 72 h de la inseminación artificial, probablemente debido a un mejor momento de la inseminación con relación a la ovulación.

McDougall et al. (2010) llevó a cabo un estudio para inducir la ovulación en vacas lecheras en anestro. La presencia o ausencia de cuerpo lúteo se determinó con ultrasonido. Las vacas se distribuyeron en 4 tratamientos: 1) 100 µg of gonadorelin, seguido de 500 µg de cloprostenol 7 d más tarde, seguido de 54 a 56 h después de 100 µg of gonadorelin, seguido de Inseminación artificial a tiempo fijo a las 13 - 18 h después de la última inyección de GnRH (Ovsynch). (2) Similar a (1) pero con la aplicación de P4 intravaginal entre GnRH inicial y PGF<sub>2α</sub> (Ovsynch-56+P4); (3) similar a (2) pero con la inyección de GnRH retrasada hasta 71 h después de PGF<sub>2α</sub> y retiro de P4 con Inseminación artificial a tiempo fijo 0 a 5 h después del tratamiento con GnRH y con inseminación de todas aquellas vacas detectadas en estro antes de la segunda inyección de GnRH (Cosynch-72+P4); y (4) testigo. Las vacas en todos los tratamientos presentaron intervalos más cortos entre el primer día de inseminaciones a la concepción comparadas con el testigo. El tratamiento Ovsynch-56+P4 presentó intervalos entre el primer día de inseminaciones a la concepción 3, 6, y 16 d más cortos que Cosynch-72+P4, Ovsynch, o testigo, respectivamente, y el efecto positivo de Ovsynch-56+P4 se presentó tanto en las vacas con o sin cuerpo lúteo. Las vacas tratadas con Ovsynch-56+P4



presentaron menos intervalos cortos que Ovsynch (ejemplo, <18 d; 16 vs. 31%) y más vacas con elevados niveles de P4 en leche (>1 ng/mL) en el d 7 (88 vs. 74%) y d 14 (80 vs. 60%). Estos investigadores concluyeron que el tratamiento de vacas en anestro resultó en preñeces más tempranas, pero no se mejoró la tasa de preñez.

## Literatura citada

- Al-Katanani, Y., Webb M.D.W. Hansen, P.J. 1999. Factors affecting seasonal variation in 90 day non-return rate to first service in lactating Holstein cows in a hot climate. *J. Dairy Sci.* 82, 2611–2615.
- Bage, R., Masironi, B., Sahlin L., Rodriguez-Martinez, H. 2002. Deviant peri-oestral hormone patterns affect the epithelium of the uterine tube in repeat-breeder heifers. *Reprod. Fertil. Dev.* 14, 461-469.
- Bartolome, J.A., Silvestre, F.T., Kamimura, S., Arteche, A.C.M., Melendez, P., Kelbert, D., McHale, J., Swift, K., Archbald, L.F., Thatcher, W.W. 2005. Resynchronization of ovulation and timed insemination in lactating dairy cows: I: use of the Ovsynch and Heatsynch protocols after non-pregnancy diagnosis by ultrasonography. *Theriogenology* 1617-1627.
- Bartolome, J.A., Sozzi, A., McHale, J., Swift, K., Kelbert, D., Archbald, L.F., Thatcher, W.W. 2005. Resynchronization of ovulation and timed insemination in lactating dairy cows III. Administration of GnRH 23 days post AI and ultrasonography for nonpregnancy diagnosis on day 30. *Theriogenology* 63, 1643–1658.
- Berry, D.P., Roche, J.R. Coffey, M.P. 2008. Body condition score and fertility – more than just a feeling. In *Fertility in dairy cows: bridging the gaps* (ed. MD Royal, NC Friggens and RF Smith), pp. 107–118. British Society of Animal Science, Cambridge University Press, Cambridge, UK.
- Brusveen, D., Cunha, A., Silva, C., Cunha, P., Sterry, R., Silva, E., Guenther, J., Wiltbank, M. 2008. Altering the time of the second Gonadotropin-Releasing

- Hormone injection and artificial insemination (AI) during Ovsynch affects pregnancies per AI in lactating dairy cows. *J. Dairy Sci.* 91, 1044-1052.
- Comisión Nacional del Agua (CONAGUA). 2008. Servicio Meteorológico Nacional. Datos climáticos del observador de Torreón, Coahuila.
- Dewey, S.T., Mendonça, L.G.D., Lopes Jr, G., Rivera, F.A., Guagnini, F.R.C., Chebel, Bilby, T.R. 2010. Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: I. Pregnancy rates and ovarian responses. *J. Dairy Sci.* 93, 4086-4095.
- Fricke, P.M., Caraviell, D.Z., Weigel, K.A., Welle, M.L. 2003. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. *J. Dairy Sci.* 86, 3941-3950.
- Heuwieser, W., Oltenacu P.A., Lednor A.J., Foote R.H. 1997. Evaluation of different protocols for prostaglandin synchronization to improve reproductive performance in dairy herds with low estrus detection efficiency. *J. Dairy Sci.* 80, 2766–2774.
- Kim U.H., Suh, G.H., Hur, T.Y., Kang, S.J., Kang, H.G., Park, S.B., Kim, H.S., Kim, I.H. 2007. Comparison of two types of CIDR-based timed artificial insemination protocols for repeat breeder dairy cows. *J. Reprod. Dev.* 53, 639-645.
- Kim, I.H., Suh, G.H., Kim, U.H., Kang, H.G. 2006. A CIDR-based timed AI protocol can be effectively used for dairy cows with follicular cysts. *Anim. Reprod. Sci.* 95, 4206-4213
- Lane, E.A., Austin, E.J., Crowe, M.A. 2008. Oestrous synchronisation in cattle- Current options following the EU regulations restricting use of oestrogenic

- compounds in food-producing animals: A review. *Anim. Reprod. Sci.* 109, 1-16.
- Mann, G.E., Lamming, G.E. 2001. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 121, 175–180.
- Maurer, R.R., Echterkamp, S.E. 1985. Repeat breeder females in beef cattle: influences and causes. *J. Anim. Sci.* 61, 624–636.
- McDougall, S. 2010. Effects of treatment of anestrus dairy cows with gonadotropin-releasing hormone, prostaglandin, and progesterone. *J. Dairy Sci.* 93, 1944-1959.
- Pursley, J.R., Silcox, R.W., Wiltbank, M.C. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *J. Dairy Sci.* 81, 2139–2144.
- Rizos, D., Carter, F., Besenfelder, U., Havlicek, V., Lonergan, P. 2010. Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows. *J. Dairy Sci.* 93, 1022-1029.
- Royal, M.D., Pryce, J.E., Woolliams, J.A., Flint, A.P.F. 2002. The Genetic Relationship between commencement of luteal activity and calving interval, body condition score, production, and linear type traits in Holstein-Friesian dairy cattle. *J. Dairy Sci.* 85, 3071–3080.
- Sartori, R., Sartor-Bergfelt, R., Mertens, S.A., Guenther, J.N., Parrish, J.J., Wiltbank, M.C. 2002 Fertilization and early embryonic development in

- heifers and lactating cows in summer and lactating and dry cows in winter. *J. Dairy Sci.* 85, 2803–2812.
- Senosy, W.S., Uchiza, M., Tameoka, N., Izaike, Y., Osawa T. 2009. Association between evaluation of the reproductive tract by various diagnostic tests and restoration of ovarian cyclicity in high-producing dairy cows. *Theriogenology* 72, 1153-1162.
- Souza, A.H., Ayres, H., Ferreira, R.M., Wiltbank, M.C. 2008. A new presynchronization system (Doble-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology* 70, 208–215.
- Suh, G.H., Kim, U.H., Hur, T.Y., Kang, S.J., Baek, K.S., Park, S.B., Kim, H.S., Kang, H.G., Kim, I.H. 2007. Effects of estradiol or GnRH on follicular wave emergence and pregnancy rates for a CIDR-based timed AI protocol in repeat breeder dairy cows. *Reprod. Fert. Dev.* 19, 128–128.
- Thatcher, W., Santos, J., Silvestre, F., Kim, I. Staples, C. 2010. Perspective on physiological/endocrine and nutritional factors influencing fertility in postpartum dairy cows. *Reprod. Dom. Anim.* 45, 2–14.
- Zavy, M.T. 1994. Embryonic mortality in cattle. In: Zavy M.T., Geisert R.D., editors. *Embryonic Mortality in Domestic Species*. CRC Press. p. 99–140.
- Waldmann, A., Reksen, O., Landsverk, K., Kommisrud, E., Dahl, E., Refsdal, A.O., Ropstad, E. 2001. Progesterone concentrations in milk fat at first insemination—effects on non-return and repeat-breeding. *Anim. Reprod. Sci.* 65, 33–41.
- Peters, A.R. 1996. Embryonic mortality in the cow. *Anim. Breed.* 64, 587–598.

**Factors influencing pregnancy per AI in repeat breeder cows treated with rbST and induced to ovulate with a CIDR-based protocol**

**ABSTRACT**

The aim of this study was to determine, using multiple logistic models, factors affecting pregnancy per AI (P/AI) following fixed-time artificial insemination (FTAI) in repeat breeders cows (RBC) with extended postpartum intervals and treated with rbST throughout lactation. 498 Holstein cows of all parities, unable to become pregnant around 200 days in milk (DIM) received a CIDR device and 100 µg of GnRH on Day 0. CIDR removal and PGF<sub>2α</sub> (25 mg) treatment were done concurrently on day 7. Estradiol benzoate (EB, 1 mg) was injected on day 8 and GnRH on day 9; cows were inseminated 16-20 h later. Cows with an average milk fat <3% were 43% more likely ( $P<0.05$ ) to become pregnant at FTAI than cows with milk fat >3%. Cows with <6 services had significantly increased chances of becoming pregnant than cows with >6 services at FTAI (P/AI 36 vs. 27%;  $P<0.05$ ). CIDR-treated cows with less than three lactations were 1.7 times more likely (P/AI 35 vs. 21%;  $P<0.05$ ) to become pregnant than older cows. Cows with dry period length >62 days were more likely to become pregnant (P/AI 35 vs. 30%;  $P<0.05$ ) than cows subjected to FTAI with dry periods <62 days. Cows with peak milk yields lower than 55 kg were 1.5 times more likely to get pregnant than cows with peak milk yields greater than 55 kg (P/AI 37 vs. 28%;  $P<0.05$ ). Cows subjected to FTAI with a temperature-humidity index (THI) <76 were 45% more likely ( $P<0.05$ ) to become pregnant than cows inseminated with a THA>76. CIDR-treated cows showed a depression ( $P<0.05$ )

in fertility during summer compared to other seasons of the year. It was concluded that an acceptable proportion (32%) of RBC can become pregnant with the protocol used in the present study. Also, AI around 200 DIM is economically justifiable in dairy operations with 3x milking and the use of rbST throughout lactations, because lactation length can be extended beyond 15 months. This study also demonstrated that subfertility in CIDR-treated cows is associated with high peak lactational yields, high milk protein content, increased service, increased age, high ambient temperature at AI and short dry periods.

Keywords: reproductive performance; estrus synchronization; peak lactation, milk protein; dry period.

## **1. Introduction**

In intensively managed dairy cows repeat breeding can be a major factor involved in infertility. This reproductive disorder leads to large economic losses for the dairy producer due to increased calving intervals, more inseminations (wasted semen and insemination costs), increased culling and replacement costs, and loss of genetic gain through the increased generation intervals (Bartlet et al., 1986; Lafi et al. 1992; Canu et al., 2010; Yusuf et al., 2010). In dairy herds of adequate fertility, where conception rates are commonly at 50-55%, about 14-24% of the cows present the repeat breeding syndrome (Bartlet et al., 1986; Moss et al., 2002; Yusuf et al., 2010). These figures are much higher in dairy operations in zones of intense heat load due to the adverse effect of high ambient temperature on all aspect of the reproductive process (Mellado et al., 2012). In the particular case of dairy operation of northern Mexico, the chronic high ambient temperature coupled with the use of rbST throughout lactation results in a high number of cows unable to become pregnant around 200 DIM (Mellado et al., 2012).

Cows under these circumstances become candidates to be culled, but high culling rates are very costly to dairy producers. There is a tremendous

opportunity to improve profitability by decreasing the number of subfertile cows culled when lactations can be extended beyond 15 months. With prolonged lactations it could be economically advantageous to get cows pregnant even after >200 DIM.

One possible alternative to avoid culling of subfertile cows unable to become pregnant after multiple services is to get them pregnant with fixed-time artificial insemination (TFAI) using a CIDR-based protocol. This technique allows for 100% of the cows to be submitted to AI, without the need for estrous detection (Pursley et al., 1995; Kim et al., 2005). Moreover, an advantage in pregnancy rate of TFAI over insemination at detected estrus has been observed in some cases (Pursley et al., 1997a,b; Cartmill et al., 2001; Amiridis et al., 2009), but not in others (Lima et al., 2009).

This reproductive tool applied to cows with advanced lactation can only be commercially viable if lactations can be substantially extended by increasing persistency and rebreeding cows to calve every 17 rather than 12 months. This can be achieved with simple management procedures such as chronic application of rbST (in countries where this hormone is legally permitted) throughout lactation and three milkings per day. Thus, the aim of this study was to establish the factors influencing P/AI of subfertile cows unable to become pregnant around 200 DIM, and treated with a CIDR-based protocol for fixed-time artificial insemination (FTAI) in a hot arid environment. An additional objective was to assess the suitability of extended lactations (around 450 days) for farms where cows are chronically treated with rbST, so that inseminations of low-fertility cows around 200 d of lactation can be justified.

## **2. Materials and methods**



## 2.2. Study herd, housing and feeding

The experimental procedures and animal care conditions were approved by the Ethics Committee of the Research Department of the Agrarian Autonomous University Antonio Narro. This study was conducted on a commercial dairy farm located in northern Mexico (latitude 26° 23' N, longitude 104° 47' W; mean annual temperature 27° C) during 2010 and 2011.

The herd consisted of approximately 3,000 lactating Holstein cows housed in open-dirt pens with fans and sprinklers for forced evaporative cooling during the warm weather. A total of 498 Holstein cows of all parities that calved between January and December 2010, and that were unable to get pregnant with more than three services were included in the study

Cows were fed diets formulated to provide recommended total daily nutrients for 670-kg dairy cows producing >33 kg of milk/d (NRC, 2001). Cows were fed total mixed diets (49% forage and 51% concentrate; DM basis) that were formulated to provide at least 1.62 Mcal/kg NEI and contained 18% crude protein. Diets contained soybean meal and ground shelled corn as the base ingredients of the concentrate mix; the forage portion of the diet was 50% corn silage and 50% alfalfa hay (DM basis). Cows were fed ad libitum for a daily *feed* refusal of approximately 10% of that offered, 4 times daily at 0600, 1000, 1200, and 1600 h. Only during summer, 80% of diet is offered in the evening and 20% in the morning.

Lactating cows were allocated to 3 lactation stage groups ( $70 \pm 13$ ,  $145 \pm 12$  and  $\geq 210 \pm 9$  d in milk; mean  $\pm$  SD). Cows included in the study were in their first to eight lactation, with body condition score ranging from 2.75 to 3.5 (scale 1 to 5).

Cows were milked thrice daily (0400, 1200, and 2000 hours), and the annual rolling herd average was 13600 kg throughout the duration of the study. All cows were subjected to recombinant bovine somatotropin (rbST; Lactotropin, 500 mg of zinc bovine somatotropin, Elanco Animal Health, Mexico) administration

(subcutaneous) every 14 d beginning at 60 DIM and continuing until 2 weeks before drying off.

### 2.3. Reproductive management

All cows were routinely vaccinated against diseases that impair reproduction functions, such as bovine viral diarrhea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, para-influenza and leptospirosis (5-varieties). Herd personnel examined fresh cows weekly to identify and treat cows with postpartum reproductive disorders such as retained placenta, metritis, and pyometra. Cows became eligible for insemination after exceeding the voluntary waiting period of 50 DIM.

Cows had an average of 239 days in milk (DIM; SD=42) when subjected to the FTAI treatment, and the range of unsuccessful inseminations was 3 to 12; mean=5.6). The average ( $\pm$  SD) duration of the dry period was  $79 \pm 33$ , 12–223) days. Detection of estrus was initiated at the end of the voluntary waiting period, with the aid of pedometers, and AI was conducted based on visual observation of estrous behavior, following the standard a.m./p.m. rule. Commercial frozen-thawed semen from multiple sires was used across all months of the year.

Cows not pregnant around 200 DIM and with more than 3 services without subsequent calving, with clear estrous signs and no clinically detectable reproductive disorders were considered repeat breeders and submitted for FTAI (CIDR-based protocol). Cows were induced to ovulate by inserting, at a random stage of the estrous cycle, an intravaginal device containing 1.9 g of progesterone (CIDR, Pfizer, Cambridge, USA) and 100 mg GnRH (Fertagyl, Intervet, Mexico, im, Day 0). Seven days later, the CIDR was removed and cows were treated with 25 mg *PGF2 $\alpha$*  (5 ml of Lutalyse, Upjhon, Mexico, im., day 7). At 24 hours after *PGF2 $\alpha$* , cows received 1 mg of estradiol benzoate (day 8); one day later (day 9) a second dose of 100 mg GnRH was administered and FTAI was performed 16-20 hours later. The designated farm inseminator performed all AI, using commercial frozen-thawed bull semen of known acceptable fertility.

Pregnancy was detected by palpation of the uterus per rectum about 50 days post AI. All pregnancy examinations were performed by the same veterinarian throughout the study period.

### 2.3. Data collection

Data from reproductive examinations were collected by the herd veterinarian during daily herd health revision for cows included in this study. Milk yields were electronically recorded at every milking using the AfiFarm management program. Milk yield was expressed as total milk yield per lactation or adjusted to 305 days in milk.

Individual milk samples were also collected every 3 weeks from three consecutive milkings (then composited) during the milk recording day, and analyzed for somatic cell counts (SCC) using a Foss 303 Milk-O-Scan® analyzer (Foss Foods Inc., Eden Prairie, MN). Fat and protein concentrations were also recorded electronically at each milking. Persistency was calculated as the month's milk divided by last month's expressed as percentage.

For each cow the following variables were recorded: occurrence of dystocia, ovarian cysts, retention of fetal membranes, metritis, hypocalcemia, ketosis, static ovaries and abortion. It is worth mentioning that cows experiencing these calving-related disorders were appropriately treated so that they were healthy before the start of the service period. Additional variables were season of AI, number of services prior to FTAI, milk yield at FTAI, peak milk yield, days to peak, parity, lactation length, persistency, dry period length previous to the current lactation and temperature and humidity the day of FTAI.

Climatic data were obtained from a meteorological station located two km away from the barn for the duration of the study. Information consisted of daily maximum temperatures and relative humidity. Air temperature was recorded with a mercury thermometer under full shade and at 1.5 m above the ground.

This information was used to calculate the temperature-humidity index (THI) for each day, using the following equation (highest daily temperature in Celsius degrees; RH refers to maximum relative humidity):

$$\text{THI} = (0.8 \times \text{temperature}) + [(\% \text{ RH}/100) \times (\text{temperature} - 14.4)] + 46.4.$$

#### 2.4. Statistical analyses

To analyze factors contributing to the probability of pregnancy (binary outcome), a multivariable logistic regression model of SAS was used, applying a backward stepwise logistic model to eliminate all nonsignificant explanatory variables. Variables were continuously removed from the model by the Wald statistic criterion if the significance was greater than 0.20. To construct the final statistical model, a preliminary full model was conducted for the following potentially explanatory variables of interest: season of FTAI, THI at FTAI, number of services prior to FTAI, DIM at FTAI, lactation number, milk fat and protein content (full lactation composition results), milk persistency, somatic cell counts (logtransformed:  $\ln(\text{SCC} + 1)$ ), occurrence of mastitis, retained placenta, hypocalcemia, metritis, abortion, static ovaries and ovarian cysts, dry period length before the current lactation, days at peak lactation, milk production at peak yield, 300-d milk yield, lactation length, total milk production during the current lactation and bulls. The Wald  $\chi^2$  statistic was used to determine the significance of each variable that remained in the reduced model. The full statistical model included main effects and all interactions, except for the days open x times inseminated because of the close correlation between these terms. No significant interactions were found and the final model included only main effects.

The GENMOD procedure of SAS was also used to assess the statistical significance of month of breeding on pregnancy rate. THI at FTAI was included in the model as a covariate. Treatment means were separated using the probability of a statistical difference (PDIFF option of SAS). Statistical differences were considered significant at  $P < 0.05$ .

The SCC was classified as being  $\leq 180000$  or  $> 180000$  prior to FTAI. Total milk yield was categorized as lower or greater than 18000 kg. 305-d milk yield was defined as less or higher than 13000 kg. Milk yield at FTAI was classed as lesser or greater than 38 kg. Lactation number was organized into less than three lactations or third or greater lactation. DIM at FTAI was divided into two classes: lesser or greater than 220 days. Categories for number of times inseminated prior to the FTAI were less or more than 6. Classes for THI at FTAI were defined as less or greater than 76 units. The records for milk fat and protein were grouped into two classes: lesser or greater than 3%. Dry period was divided into two classes corresponding to  $< 62$  and  $> 62$  days. Persistency was grouped into two categories:  $< 77$  and  $> 77\%$ . Monthly values were grouped into seasons, winter months being December–February; spring, March–May; summer, June–August; and fall, September–November.

### **3. Results**

The combined administration of CIDR, GnRH, EB, and PGF2 $\alpha$  was effective in treating RBC, reaching a 32% P/AI. Overall P/AI (cows pregnant after FTAI and a subsequent AI) was 52%. Average ( $\pm$  SD) total milk production per lactation was  $17882 \pm 4975$  kg with a lactation length of  $478 \pm 117$  days.

The majority of variables included in the model were discarded because of their limited influence on P/AI. Listed in Table 1 are factors which significantly affected the likelihood of cows becoming pregnant with the FTAI protocol used in the present study. Cows experiencing a peak milk yield  $< 55$  kg were 1.5 times more likely to become pregnant than cows producing more than 55 kg at peak lactation.

Compared to cows with  $> 6$  services, cows with  $< 6$  services were 1.5 times more likely to become pregnant (Table 1). Young cows were more likely ( $P < 0.05$ ) to become pregnant at FTAI as were multiparous cows.

All variables previously mention, except number of lactation, dry period length and milk protein content were also significantly influenced the likelihood of cows getting pregnant when considering the outcome of the FTAI plus an additional service immediately after the estrus brought about by the hormonal treatment (Table 1).

Table 1. Odds ratios (OR) for the likelihood of pregnancy in repeat breeder cows as a function of milk-related traits, number of services previous to FTAI, number of lactations, length of dry period in previous lactation and THI the day of FTAI.

Variable	Outcome at FTAI			Outcome with FTAI + an additional AI next natural estrus		
	Pregnancy rate (%)	Odds ratio <sup>a</sup>	95% CI	Pregnancy rate (%)	Odds ratio <sup>a</sup>	95% CI
Yield to peak						
>55 kg	28			46		
<55 kg	37	1.50*	1.02 - 2.20	58	1.57*	1.10 – 2.24
Services						
>6	27			46		
<6	36	1.51*	1.03 - 2.21	57	1.57*	1.10 – 2.24
Lactations						
>3	21			46		
<3	35	2.04*	1.16 - 3.59	53	1.33	0.83 – 2.15

ITH at FTAI						
>76	25			45		
<76	35	1.63*	1.09 - 2.46	58	1.66*	1.15 – 2.40
Milk protein						
>3 %	26			50		
<3 %	36	1.37*	0.95 - 2.01	53	1.13	0.80 – 1.62
Dry period						
>62 days	35			50		
<62 days	30	0.79*	0.54 – 1.16	53	1.12	0.78 – 1.60
Bull (n=47)	Range 0-55					

---

<sup>a</sup>Odds ratios measure how much more or less likely the outcome is among variables with a given risk factor, compared with those without it, or reference category (odds ratio of 1.0).

\* $P < 0.05$ .

Another important factor affecting pregnancy rate with FTAI and FTAI plus an additional service was season of breeding (Table 2). Cows that were inseminated in summer were less likely ( $P<0.05$ ) to become pregnant than were cows that were inseminated in all other seasons. Service sire influenced the proportion of CIDR-treated cows diagnosed as pregnant at 50 days post FTAI ( $P = 0.01$ ).

Table 2. The effect of season on pregnancy rate in repeat breeder Holstein cows subjected to a CIDR-estrus synchronization program and FTAI.

Season	FTAI	FTAI + AI next natural estrus
Spring	34 (40/116) <sup>a</sup>	52 (60/116) <sup>a</sup>
Summer	19 (11/58) <sup>b</sup>	29 (17/58) <sup>b</sup>
Fall	32 (47/145) <sup>a</sup>	45 (65/144) <sup>a</sup>
Winter	34 (61/179) <sup>a</sup>	64 (115/180) <sup>c</sup>

Values with different superscript differ ( $P<0.05$ ).



#### **4. Discussion**

The protocol used in the present study for ovulation induction was effective in treating the RBC, reaching a 32% P/AI. This figure was very close to that observed by Ui-Hyung et al. (2007) and Alnimer and Husein (2007) in RBC subjected to a similar estrus synchronization protocol. P/AI in herds in this hot-arid zone is 33% (Mellado et al., 2012); thus, comparable P/AI were achieved than those observed with the conventional reproductive programs for dairy cows. The long lactation length observed in cows in the present study ( $478 \pm 117$  days) support the practice of getting RBC pregnant around 200 days in milk, because these 3x milked cows treated with rbST throughout lactation experience extended lactation cycles, which makes this reproductive management economically feasible.

Cows attaining a peak milk yield  $>55$  kg d were less likely to become pregnant as cows achieving peak milk production less than this figure. This response is similar to that found by Lean et al. (1989) where cows with peak milk yields greater than the median of the herd were less likely to conceive than cows with lower peak milk yields.

This response seems to be associated, with greater negative energy balances (NEB) during early lactation in those cows with high peak milk yield. Although most inseminations in cows in the present study were carried out after the altered metabolic state associated with poor fertility early in lactation (Fenwick et al., 2008; Wathes et al., 2011), NEB have carry-over effects on the success of subsequent inseminations (Wathes et al., 2007). NEB during early postpartum can affect fertility later in lactation by reducing the number of ovarian cycles, because ovulatory cycles preceding AI is essential for an adequate priming of the uterus (Butler, 2003). Further carry-over effects of NEB can result in

deficiencies in oocyte and embryo developmental competence as well as inadequate corpus luteum function (Leroy et al., 2008a). Additionally, cows in an extreme state of NEB during early lactation show higher incidences of diseases such as mastitis (Santos et al., 2004; Heringstad et al., 2006), locomotive problems (Collard et al., 2000) and metabolic disorders (Collard et al., 2000; Ingvarsen et al., 2003), and these diseases decrease reproductive performance of cows.

Cows with low milk protein content had higher odds of becoming pregnant than cows with milk protein levels >3%. Milk protein content is linked to the energy status of the dairy cow, with a drop of this milk component with an energy deficit (Fulkerson et al., 2001; Kessel et al., 2008; Reksen et al., 2002).

In fact, milk protein content (Tena-Martinez et al., 2009) or fat-protein ratio (Buttchereit et al., 2011) are better indicators of negative energy balance than milk fat content, DMI, BCS or other milk components. Most inseminations in these cows occurred when animals had regained a positive energy balance status (about 20 weeks postpartum; Taylor et al., 2003), but there are subsequent effects on fertility that lead to poor conception rates (Wathes et al., 2009). Other researchers have found that milk protein level has no negative influence on reproductive performance of Holstein cows (Demeter et al., 2010).

A significant effect of age group on P/AI was detected. Young cows had higher P/AI than cows with >3 lactation. These findings are in agreement with related studies reported in the literature in countries with temperate climate (Balendran et al., 2008; Souza et al., 2008; Friedman et al., 2011) showing reduced pregnancy rates in mature cows compared with heifers.

Correlations between fertility and production traits are generally negative (Neuenschwander et al., 2005; Tsuruta et al., 2009) and these values become more negative with increasing number of lactation, as a consequence of the increased energy requirements with increased milk productions. Also, profiles of IGF-I, urea and body condition score usually are higher in first lactation cows than mature animals, and these variables are positively related to fertility (Wathes et al., 2007). An additional explanation for decreased fertility with

increasing age is that, compared to first-parity cows, cows in second, third, and fourth parities had significantly higher odds of being a repeat breeder (Bonneville-Hébert et al., 2011).

A marked decline of pregnancy rate was observed with increasing service number (Table 1). A reduction in fertility with increasing number of services has been amply documented (Rajala-Schultz et al., 2001; Brusveen et al., 2008; Chebel et al., 2007). Lower fertility with advancing service number may be ascribed to increasing percentage of repeat breeders that accumulate as cows are presented for successive services.

Cows with long dry periods had higher odds of getting pregnant with the FTAI protocol used in the present study than cows with <62 days dry. This is contrary to the notion that reducing the length of the dry period (<60 days) improves early lactation energy balance and consequently fertility indices (Opsomer et al., 2000; Gümen et al., 2005; Watters et al., 2009). However, the improved reproductive efficiency after shorter dry periods has been observed early during the breeding period and only in older cows. In the present study all cows had repeatedly ovulated before the hormonal treatment, and the beneficial effect of short dry period on fertility diminishes as lactation lengthens (Watters et al., 2009). Other researchers have not found a consistent improvement in reproductive variables (Pezeshki et al., 2007) or have observed lower fertility (Pinedo et al., 2011) with increased dry period length. One possible explanation for the beneficial effect of long dry period in the present study could be that cows with longer dry periods tend to have lower milk yields (Kuhn et al., 2007), and higher milk yield alone is associated with reduced health and fertility (Leroy et al., 2008a,b).

In the present study P/AI was sensitive to THI >76 at FTAI. Fertility in Holstein cows has been found to be responsive to heat stress exerted prior (AL-Katanani et al., 1999), the day of breeding (Cavestany et al., 1985), and shortly after breeding (Mellado et al., 2012). The detrimental effect of elevated heat load at AI is the consequence of a series of events, such as the delay in ovulation and follicular persistency, which leads to the ovulation of poor quality oocyte, which

in turn causes low fertilization rate and increased embryonic mortality (Sartori et al., 2002; Roth, 2008). High heat load also lowers diameter of dominant follicle and alters of the biochemical composition in the follicular fluid of this follicle (Shehab-El-Deen et al., 2010), which result in inferior oocyte and granulosa cell quality (Payton et al., 2011). Hyperthermia of oocytes also hampers embryonic development, which compromises fertility (Edwards et al., 2009).

P/AI was greater in winter, spring and fall in CIDR-treated cows than in summer (by about 14 percentage points). Several environmental components affect female fertility; among these heat stress is particularly significant. Summer heat stress is a main factor related to low conception rate in high producing dairy herds in warm areas worldwide (García-Ispuerto et al., 2007; Ben Salem and Bouraoui, 2009; Ferreira et al., 2011). In the present study P/AI was higher in CIDR-treated cows in the fall than in summer, which suggests that the effects of heat stress had no a carry-over effect into subsequent less warm months, as it has been observed in this zone (Mellado et al., 2012). Also, spring months in this zone are hot enough to cause a measurable stress response in cows (mean THI= 70, 76 and 84, for March, April and May, respectively), but this heat load during spring did not impair reproductive performance of CIDR-treated cows. The combination of hormones used in the ovulation induction protocol in the present study apparently somehow overrode the negative effect of hyperthermia on fertility of cows. This supposition is based on studies demonstrating that injection of GnRH at detection or shortly after estrus enhances secretion of luteal progesterone and embryo survival under intense summer heat in subtropical areas (Ullah et al., 1996; Cruz-Velázquez et al., 2009). Also under subtropical conditions, FTAI programs based on GnRH increased pregnancy rate during summer heat stress (De la Sota et al., 1998; Alnimer and Usein, 2007).

## **Conclusions**

These results indicate that the FTAI program in RBC used in the present study was efficacious in attaining an acceptable P/AI. This data also indicate that breeding cows around 200 DIM is a feasible practice under the conditions of the present study, because with 3x milking and rbST treatment throughout lactation, extended lactation (15-month cycles) can be achieve, which makes this practice economically viable in intensive dairy operations. Additionally, these results show that milk protein, peak milk yield, parity, dry period length and high ambient temperatures at breeding are associated with the fertility response of CIDR-treated subfertile Holstein cows in a hot–arid environment.

### **Acknowledgments**

We acknowledge the financial support from SAGARPA-CONACYT (Mexico), project 144591. The authors thank Martha Alicia de la Rosa for help with ordering the data.

### **References**

- AL-Katanani, Y.M., Webb, D.W., Hansen, P.J., 1999. Factors affecting seasonal variation in 90-day nonreturn rate to first service in lactating Holstein cows in a hot climate. *J. Dairy Sci.* 82, 2611–2616.
- Alnimer, M.A., Husein, M.Q., 2007. The effect of progesterone and oestradiol benzoate on fertility of artificially inseminated repeat-breeder dairy cows during summer. *Reprod. Dom. Anim.* 42, 363–369.
- Amiridis, G.S., Tsiligianni, TH., Dovolou, E., Rekkas, C., Vouzaras, D., Menegatos, I., 2009. Combined administration of gonadotropin-releasing hormone, progesterone, and meloxicam is an effective treatment for the repeat-breeder cow. *Theriogenology* 72, 542–548.

- Balendran, A., Gordon, M., Pretheeban, T., Singh, R., Perera, R., Rajamahedran, R., 2008. Decreased fertility with increasing parity in lactating dairy cows. *Can. J. Anim. Sci.* 88, 425-428.
- Bartlett, P.C., Kirk, H.J., Mather, E.C., 1986. Repeated insemination in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. *Theriogenology* 26, 309-322.
- Ben Salem, M., Bouraoui, R., 2009. Heat stress in Tunisia: Effects on dairy cows and potential means of alleviating it. *S. African J. Anim. Sci.* 39, 256-259.
- Ben Salem, M., Bouraoui, R., 2009. Heat stress in Tunisia: Effects on dairy cows and potential means of alleviating it. *S. African J. Anim. Sci.* 39, 256-259.
- Brusveen, D.J., Cunha, A.P., Silva, C.D., Cunha, P.M., Sterry, R.A., Silva, E.P.B., Guenther, J.N., Wiltbank, M.C., 2008. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during ovsynch affects pregnancies per AI in lactating dairy cows. *J. Dairy Sci.* 91, 1044-1052.
- Buttchereit, N., Stamer, E., Junge, W., Thaller, G., 2011. Genetic relationships among daily energy balance, feed intake, body condition score, and fat to protein ratio of milk in dairy cows. *J. Dairy Sci.* 94, 1586-1591.
- Butler, W.R., 2003. Energy balance relationships with follicular development, ovulation and fertility in pp dairy cows. *Livest. Prod. Sci.* 83, 211-218.
- Canu, S., Boland, M., Lloyd, G.M., Newman, M., Christie, M.F., May, P.J., Christley, R.M., Smith, R.F., Dobson, H., 2010. Predisposition to repeat breeding in UK cattle and success of artificial insemination alone or in combination with embryo transfer. *Vet. Rec.* 167, 44-51.
- Cartmill, J.A., El-Zarkouny, S.Z., Hensley, B.A., Rozell, T.G., Smith, J.F., Stevenson, J.S., 2001. An alternative AI breeding protocol for dairy cows exposed to elevated ambient temperatures before or after calving or both. *J. Dairy Sci.* 84, 799–806.
- Cavestany D., El-Wishy A.B., Foote R.H., 1985. Effect of season and high environmental temperature on fertility of Holstein cattle *J. Dairy Sci.* 68, 1471-1478.

- Chebel, R.C., Braga, F.A., Dalton, J.C., 2007. Factors affecting reproductive performance of Holstein heifers. *Anim. Repr. Sci.* 101, 208-224.
- Collard, B.L., Boettcher, P.J., Dekkers, J.C.M., Petitclerc, D., Schaeffer, L.R., 2000. Relationships between energy balance and health traits of dairy cattle in early lactation. *J. Dairy Sci.* 83, 2683-2690.
- Cruz-Velázquez, J.E., Elizondo-Vázquez, C.A., Ulloa-Arvizu, R., Fernández-García, I.G., 2009. The effect of GnRH after insemination on progesterone concentrations and conception rates in repeat-breeding Holstein cows under heat stress conditions. *Téc. Pec. Méx.* 47, 107-115.
- De la Sota, R.L., Burke, J.M., Risco, C.A., Moreira, F., DeLorenzo, M.A., Thatcher, W.W., 1998. Evaluation of timed insemination during summer heat stress in lactating dairy cattle. *Theriogenology* 49, 761-770.
- Demeter, R.M., Markiewicz, K., van Arendonk, J.A.M., Bovenhuis, H., 2010. Relationships between milk protein composition, milk protein variants, and cow fertility traits in Dutch Holstein-Friesian cattle. *J. Dairy Sci.* 93, 5495-5502.
- Edwards, J.L., Bogart, A.N., Rispoli, L.A., Saxton, A.M., Schrick, F.N., 2009. Developmental competence of bovine embryos from heat stressed ova. *J. Dairy Sci.* 92, 563-570.
- Fenwick, M.A., Llewellyn, S., Fitzpatrick, R., Kenny, D.A., Murphy, J.J., Patton, J., Wathes, D.C. 2008. Negative energy balance in dairy cows is associated
- Ferreira, R.M., Ayres, H., Chiaratti, M.R., Ferraz, M.L., Araújo, A.B., Rodrigues, C.A., Watanabe, Y.F., Vireque, A.A., Joaquim, D.C., Smith, L.C., Meirelles, F.V., Baruselli, P.S., 2011. The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *J. Dairy Sci.* 94, 2383-2392.
- Friedman, E., Voet, H., Reznikov, D., Dagoni, I., Roth, Z., 2011. Induction of successive follicular waves by gonadotropin-releasing hormone and prostaglandin F<sub>2α</sub> to improve fertility of high-producing cows during the summer and autumn. *J. Dairy Sci.* 94, 52393-2402.

- Fulkerson, W.J., Wilkins, J., Dobos, R.C., Hough, G.M., Goddard, M.E., Davison, T., 2001. Reproductive performance in Holstein-Friesian cows in relation to genetic merit and level of feeding when grazing pasture. *Anim. Sci.* 73, 397-406.
- García-Ispuerto, F.G., Lopez-Gatius, P., Bech-Sabat, G., Santolaria, P., Yaniz, J.L., Nogareda, C., De Rensis, F., Lopez-Bejar, M., 2007. Climate factors affecting pregnancy rate of high-producing dairy cows in northeastern Spain. *Theriogenology* 67, 1379-1385.
- Gümen, A., Rastani, R.R., Grummer, R.R., Wiltbank, M.C., 2005. Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. *J. Dairy Sci.* 88, 2401-2411.
- Heringstad, B., Andersen-Ranberg, I.M., Chang, Y.M., Gianola, D., 2006. Genetic analysis of nonreturn rate and mastitis in first-lactation Norwegian Red cows. *J. Dairy Sci.* 89, 4420-4423.
- Ingvarsen, K.L., Dewhurst, R.J., Friggens, N.C., 2003. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. *Livest. Prod. Sci.* 83, 277-308.
- Kessel, S., Stroehl, M., Meyer, H.H., Hiss, S., Sauerwein, H., Schwarz, F.J., Bruckmaier, R.M., 2008. Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. *J. Anim. Sci.* 86, 2903-2912.
- Kim, U.H., Suh, G.H., Nam, H.W., Kang, H.G., Kim, I.H., 2005. Follicular wave emergence, luteal function and synchrony of ovulation following GnRH or estradiol benzoate in a CIDR-treated, lactating Holstein cows. *Theriogenology* 63, 260-268.
- Kuhn, M.T., Hutchison, J.L., Norman, H.D., 2007. Dry period length in US Jerseys: Characterization and effects on performance. *J. Dairy Sci.* 90, 2069-2081.



- Lafi, S.Q., Kaneene, J.B., Black, J.R., Lloyd, J.W., 1992. Epidemiological and economic study of the repeat breeder syndrome in Michigan dairy cattle. II Economic modeling. *Prev. Vet. Med.* 14, 99-114.
- Lean, I.J., Galland, J.C., Scott, J.L., 1989. Relationships between fertility, peak milk yields and lactational persistency in dairy cows. *Theriogenology* 31, 1093-1103.
- Leroy, J.L.M.R., Opsomer, G., Van Soom, A., Goovaerts, I.G.F., Bols, P.E.J., 2008a. Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part I. The importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high-yielding dairy cows. *Repr. Dom. Anim.* 43, 612-622.
- Leroy, J.L., Van Soom, A., Opsomer, G., Goovaerts, I.G., Bols, P.E., 2008b. Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part II. Mechanisms linking nutrition and reduced oocyte and embryo quality in high-yielding dairy cows. *Repr. Dom. Anim.* 43, 623-632.
- Lima, F.S., Risco, C.A., Thatcher, M.J., Benzaquen, M.E., Archbald, L.F., Santos, J.E.P., Thatcher, W.W., 2009. Comparison of reproductive performance in lactating dairy cows bred by natural service or timed artificial insemination. *J. Dairy Sci.* 92, 5456-5466.
- Mellado, M., Sepulveda, E., Meza-Herrera, C., Veliz, F.G., Arevalo, J.R., Mellado, J., de Santiago A., 2012. Effects of heat-stress and sex-sorted semen on reproductive efficiency in Holstein cows treated with rbST throughout lactation. *Repr Dom Anim* (in press).
- Moss, N., Lean, I.J., Reid, S.W.J., Hodgson, D.R., 2002. The epidemiology of subfertility in non-seasonal calving dairy herds in the Camden region of New South Wales: description of population and incidence. *Aust. Vet. J.* 80, 425-431.
- National Research Council., 2001. Nutrient requirements of dairy cattle. 7<sup>th</sup> rev. ed. National Academy Press, Washington, DC.
- Neuenschwander, T., Kadarmideen, H.N., Wegmann, S., de Haas, Y., 2005. Genetics of parity-dependant production increase and its relationship with

- health, fertility, longevity, and conformation in Swiss Holsteins. *J. Dairy Sci.* 88, 1540-1551.
- Opsomer, G., Gröhn, Y.T., Hertl, J., Coryn, M., Deluyker, H., de Kruif, A., 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: A field study. *Theriogenology* 53, 841-857.
- Payton, R.R., Rispoli, L.A., Saxton, A.M., Edwards, J.L., 2011. Impact of heat stress exposure during meiotic maturation on oocyte, surrounding cumulus cell, and embryo RNA populations. *J. Repr. Dev.* 57, 481-491.
- Pezeshki, A., Mehrzad, J., Ghorbani, G.R., Rahmani, H.R., Collier, R.J., Burvenich, C., 2007. Effects of short dry periods on performance and metabolic status in Holstein dairy cows. *J. Dairy Sci.* 90, 5531–5541.
- Pinedo, P., Risco, C., Melendez, P., 2011. A retrospective study on the association between different lengths of the dry period and subclinical mastitis, milk yield, reproductive performance, and culling in Chilean dairy cows. *J. Dairy Sci.* 94, 106-115.
- Pursley, J.R., Kosorok, M.R., Wiltbank, M.C., 1997a. Reproductive management of lactating dairy cows using synchronization of ovulation. *J. Dairy Sci.* 80, 301–306.
- Pursley, J.R., Mee, M.O., Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF<sub>2</sub> $\alpha$  and GnRH. *Theriogenology* 44, 915–923.
- Pursley, J.R., Wiltbank, M.C., Stevenson, J.S., Ottobre, J.S., Garverick, H.A., Anderson, L., 1997b. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.* 80, 295–300.
- Rajala-Schultz, P.J., Saville, W.J.A., Frazer, G.S., Wittum, T.E., 2001. Association between milk urea nitrogen and fertility in Ohio dairy cows. *J. Dairy Sci.* 84, 482-489.
- Reksen, O., Havrevoll, Ø., Gröhn, Y.T., Bolstad, T., Waldmann, A., Ropstad, E., 2002. Relationships among body condition score, milk constituents, and postpartum luteal function in Norwegian dairy cows. *J. Dairy Sci.* 85, 1406-1415.

- Roth, Z., 2008. Heat stress, the follicle, and its enclosed oocyte: Mechanisms and potential strategies to improve fertility in dairy cows. *Reprod. Dom. Anim.* 43, 238–244.
- Ullah, G., Fuquay, J.W., Keawkhong, T., Clark, B.L., Pogue, D.E., Murphey, E.J., 1996. Effect of gonadotropin-releasing hormone at estrus on subsequent luteal function and fertility in lactating Holsteins during heat stress. *J. Dairy Sci.* 79, 1950-1953.
- Wathes, D.C., Cheng, Z., Fenwick, M.A., Fitzpatrick, R., Patton, J., 2011. Influence of energy balance on the somatotrophic axis and matrix metalloproteinase expression in the endometrium of the postpartum dairy cow. *Reproduction* 141, 269-281.
- Wathes, D.C., Cheng, Z., Chowdhury, W., Fenwick, M.A., Fitzpatrick, R., Morris, D.G., Patton, J., Murphy, J.J., 2009. Negative energy balance alters global gene expression and immune responses in the uterus of postpartum dairy cows. *Physiol. Genomics* 39, 1-13.
- Wathes, D.C., Fenwick, M., Cheng, Z., Bourne, N., Llewellyn, S., Morris, D.G., Kenny, D., Murphy, J., Fitzpatrick, R., 2007. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. *Theriogenology* 68 Suppl 1, 232-241.
- Watters, R.D., Wiltbank, M.C., Guenther, J.N., Brickner, A.E., Rastani, R.R., Fricke, P.M., Grummer, R.R., 2009. Effect of dry period length on reproduction during the subsequent lactation. *J. Dairy Sci.* 92, 3081-3090.
- Yusuf, M., Nakao, T., Ranasinghe, R.M.S. BK, Gautam, G, Long, ST, Yoshida, C, Koike, K, Hayashi, A., 2010. Reproductive performance of repeat breeders in dairy herds. *Theriogenology* 73, 1220-1229.