Tropical and Subtropical Agroecosystems, 15 (2012): 685 - 692

COMPARISON BETWEEN ESTRADIOL CYPIONATE AND GONADOTROPIN RELEASING HORMONE AS OVULATION SYNCHRONIZATION TREATMENTS FOR FIXED-TIME ARTIFICIAL INSEMINATION PROGRAMS IN BRAHMAN-CROSS HEIFERS IN A SUBTROPICAL REGION OF NORTHEASTERN MEXICO

[COMPARACIÓN ENTRE CIPIONATO DE ESTRADIOL Y HORMONA LIBERADORA DE GONADOTROPINAS COMO PARTE DE LOS TRATAMIENTOS PARA SINCRONIZAR LA OVULACIÓN PARA INSEMINACIÓN ARTIFICIAL A TIEMPO FIJO EN VAQUILLAS CRUZADAS DE BRAHMAN EN UNA REGIÓN SUBTROPICAL DEL NORESTE DE MEXICO]

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SUMMARY

Synchronization protocols with intravaginal progesterone releasing devices (CIDR) and PGF2α were evaluated, with GnRH or estradiol cypionate (ECP) added for fixed-time artificial insemination (FTAI) in five counties in Tamaulipas, Mexico. Brahman-cross heifers (≥15 months old) were selected based on body condition (≥3 on a 5-point scale) and confirmed ovarian activity. The six treatments (n = 320 each) were: T1 (9-d CIDR, ECP on insertion, PGF2α on day 9, ECP on day 10, FTAI 54 h after removal); T2 (7-d CIDR, ECP on insertion, PGF2α on day 7, ECP on day 8, FTAI 54 h after removal); T3 (7-d CIDR, GnRH on insertion, PGF2α on day 7, FTAI and GnRH 48 h after removal); T4 (7-d CIDR, GnRH on insertion, PGF2α on day 6, FTAI and GnRH 48 h after removal); T5 (7-d CIDR, GnRH on insertion, PGF2α on day 7, FTAI and GnRH 60 h after removal); and T6 (7-d CIDR, GnRH on insertion, PGF2α on day 7, FTAI alone 48 h after removal). Pregnancy was diagnosed ultrasonically 45 days after FTAI. Analyses included pregnancy rates and treatment costs (hormones and handling). Pregnancy rates ranged from 31.6 ± 3.9 to 48.0 ± 10.6%; neither treatment nor county affected these rates ($p > 0.05$). In conclusion, the inclusion of treatment costs showed two more economical treatments (T2 using estradiol or T3 using GnRH) under the nutritional, weather and handling conditions present in the tropical region of southeastern Tamaulipas.

Key words: ovulation synchronization; Brahman cattle; fixed-time AI; GnRH; estradiol

RESUMEN

Se evaluaron protocolos de sincronización con un dispositivo intravaginal (CIDR) y PGF2α, comparando la inclusión de GnRH o cipionato de estradiol (ECP) para realizar inseminación artificial a tiempo fijo (IATF) en cinco municipios del sureste de Tamaulipas, México. Se seleccionaron vaquillas de cruza Brahman (≥15 meses de edad) por condición corporal (≥3 en la escala de 5 puntos) y actividad ovárica. Los seis tratamientos (n = 320 cada uno) fueron: T1 (CIDR por 9 d, ECP al insertar, PGF2α el día 9, ECP el día 10, IATF 54 h post-retiro); T2 (CIDR por 7 d, ECP al insertar, PGF2α el día 7, ECP el día 8, IATF 54 h post-retiro); T3 (CIDR por 7 d, GnRH al insertar, PGF2α el día 7, IATF y GnRH 48 h post-retiro); T4 (CIDR por 7 d, GnRH al insertar, PGF2α el día 6, IATF y GnRH 48 h post-retiro); T5 (CIDR por 7 d, GnRH al insertar, PGF2α el día 7, IATF y GnRH 60 h post-retiro); y T6 (CIDR por 7 d, GnRH al insertar, PGF2α el día 7, IATF sola 48 h post-retiro). Se diagnosticó la gestación mediante ultrasonido 45 días después de la IATF. Se calculó la tasa de gestación a la IATF y el costo del tratamiento (hormonas y manejo). La tasa de gestación varió entre

31.6 ± 3.9 y 48.0 ± 10.6%; ni el tratamiento ni el municipio afectaron esta tasa (P>0.05). En conclusión, la inclusión de costos al análisis mostró dos tratamientos (T2 con estradiol o T3 con GnRH) más económicos y efectivos bajo las condiciones nutricionales, climáticas y de manejo del sureste de Tamaulipas.

Palabras clave: sincronización de la ovulación; Brahman; inseminación artificial a tiempo fijo; GnRH; estradiol.

INTRODUCTION

Mexico is an important beef producer, ranking eighth in the world, and its northeastern state of Tamaulipas is a major contributor to this production. Most beef cattle farms use extensive management, with animals on pasture under relatively low monitoring which tends to reduce reproductive efficiency. Recent advances in reproductive technology allow for improvements in this efficiency; for instance, artificial insemination (AI) accelerates genetic improvement by introducing semen from genetically superior animals. However, the introduction of AI alone requires detecting estrus which is impractical in range cattle. Treatments using progestogens and/or prostaglandin F2α (PGF2α) lead to estrus synchronization, which may still vary within a few days, and the incorporation of gonadotropin releasing hormone (GnRH) or estradiol cypionate regulates follicle wave dynamics and brings ovulation within a short period, allowing the elimination of estrus detection, so the treated animals can be inseminated at a fixed time (FTAI), regardless of estrus behavior. Several studies using these methods in dairy or beef cattle of European breeds have been published (Martínez *et al.,* 2002; Lamb *et al.,* 2006; Kim *et al.,* 2007; Schafer *et al.,* 2007; Busch *et al.,* 2007, 2008; Bucher *et al.,* 2009; Colazo *et al.,* 2009; Larson *et al.,* 2009; Lamb *et al.,* 2010; Martínez *et al.,* 2012), proving their efficacy with pregnancy rates that ranged from 41 to 61% in heifers and from 26 to 69% in cows. There are also extensive studies on ovulation synchronization in Brahman cattle in the U.S.A. (Saldarriaga *et al.,* 2007), Australia (Butler *et al.,* 2011) and South America (Meneghetti *et al.,* 2009; Peres *et al.,* 2009; Sá Filho *et al.,* 2009, 2010a, 2010b) with comparable pregnancy rates (36 to 52% in heifers and 32 to 54% in cows), but to the best of our knowledge, not in northeastern Mexico, particularly in the state of Tamaulipas; we were interested in confirming whether treatments used elsewhere would be effective under the nutritional, weather and handling conditions present in regional ranches. Thus, the objective of the present study was to compare different treatments using GnRH or estradiol cypionate in progesterone- and PGF2α-based ovulation synchronization for FTAI in Brahman heifers in southeastern Tamaulipas, a subtropical region in northeastern Mexico; treatment costs were incorporated to find the most economically efficient protocol for regional Brahman cattle. Treatments were chosen based on those used for *Bos taurus* or *Bos indicus* cattle, with variations such as timing of GnRH or PGF2α, or the possible exclusion of the final GnRH. Our hypothesis was that either GnRH or estradiol could be used successfully in progesterone-PGF2α based synchronization protocols, so the selection of a treatment might also depend on protocol details such as the timing of the different hormonal treatments, the number of times animals were to be handled for the treatments, and the relative costs of hormones and handling. Preliminary results from this study were presented at the "10º Congreso Internacional de Médicos Veterinarios Especialistas en Bovinos de la Comarca Lagunera", Torreón, Coah., Mexico, in November 2010.

MATERIALS AND METHODS

Animals

A total of 1,920 heifers of Brahman, Swiss-Brahman and Brangus breeds were used. All the ranches included a mixture of breeds with varying proportions of *Bos taurus* and *Bos indicus* blood, so results were pooled by ranch, and the evaluation of breed effects was not possible. The animals belonged to 23 ranches in southeastern Tamaulipas. Most of the ranches were located in the counties of Aldama, Altamira and Soto la Marina (n = 8, 7 and 4 ranches, respectively); there were two more in the county of Güémez and one in the county of Casas. Every participating ranch carried out extensive management with rotation lots on Bermuda grass (*Cynodon dactylon*) pastures. This region of Tamaulipas is located between 22°30' and 24°5' north, and between 97°45' and 99° west; it has subtropical climate, with a mean annual temperature of 27.6 ºC and mean annual precipitation of 750.3 mm (INEGI, 2008). All the treatments were performed between July 2009 and January 2010.

The heifers were ≥15 months of age; only animals exhibiting a body condition score of ≥3 on a simple 5-point scale used for dairy cattle, where 1 = thin and 5 = fat (Edmonson *et al.,* 1989), and evidence of ovarian activity during an ultrasonographic evaluation

(≥10 mm follicles or presence of a corpus luteum) were included in the study. Within each ranch, heifers were randomly allocated to the experimental treatments. To ease animal handling during treatments, several replicates were planned (7 to 13 per treatment), with 11 to 65 animals per replicate (mean = 28.8 ± 10.1 heifers per replicate).

Synchronization treatments

The study included two experiments. In both cases, the treatment for ovulation synchronization began (d 0) with the insertion of an intravaginal controlled internal drug release device containing 1.9 g of progesterone (CIDR; Pfizer, Mexico City, Mexico), accompanied by an intramuscular injection of 25 mg PGF2α (Lutalyse; Pfizer, Mexico City, Mexico) at the time of CIDR removal or 24 h earlier. Variations between treatments included the duration of CIDR treatment (7 or 9 d); the timing of PGF2α treatment relative to CIDR removal (at removal or 24 h before removal); the interval from CIDR removal to AI (48, 54 or 60 h); and treatments with either estradiol cypionate (ECP; Pfizer, Mexico City, Mexico), using 1 mg im on day 0 and 0.5 mg im 24 h after CIDR removal, or GnRH (Cystorelin; Merial, El Marqués, Qro., Mexico) using 100 µg on day 0, and 48 or 60 h after CIDR removal. The details of each treatment group (T) are shown in Figure 1. Although estradiol benzoate is frequently used for ovulation synchronization programs (e.g., Martinez *et al.,* 2002), estradiol cypionate was chosen because it has been used in Brahman cattle (Meneghetti *et al.,* 2009; Peres *et al.,* 2009; Phillips *et al.,* 2010; Sales *et al.,* 2012) and it has been shown to be as effective as estradiol benzoate in ovulation synchronization (Meneghetti *et al.,* 2009; Sales *et al.,* 2012). On the day of CIDR removal, any animal that had lost the device was not inseminated and was excluded from the calculation of pregnancy rates; however, they were included in the calculation of treatment costs (see below). Routine AI was performed at the described time without detecting estrus; to prevent technician effects, all the inseminations were performed by two trained technicians working together. Semen from several bulls was used for AI; however, they were all from proven fertile bulls, and samples were evaluated to confirm viability at the time of AI. Pregnancy was diagnosed using ultrasound (ALOKA SSD-500) 45 days after FTAI.

Experiment 1 compared the use of ECP vs. GnRH as part of the synchronization treatment, and included Treatments T1 to T4. T1 incorporated a longer exposure to CIDR (Sá Filho *et al*., 2009) which may affect fertility by modifying the time from follicle-wave synchronization at first ECP treatment to CIDR removal, and T4 included an earlier treatment with PGF2α (Lucy *et al*., 2001), which would give more time for endogenous progesterone to decrease before CIDR removal, but giving an additional handling session for the heifers.

Experiment 2 evaluated the use of GnRH at the end of the synchronization treatment, comparing T5 and T6, to determine whether the final GnRH treatment is required for FTAI. The final dose of GnRH is used to synchronize ovulation, and if not given during synchronization, it may be necessary to detect estrus in the treated animals (Lane *et al*., 2008).

Cost analysis

In addition to the analysis of gestation rates, the cost of each treatment was calculated, including the price of each hormone used per animal (CIDR, PGF2α, estradiol cypionate or GnRH), costs for materials used (such as rectal sleeves, insemination material, and a portion of the investment cost for a semen tank and similar equipment) and the number of times the animals were handled (3 or 4 times for treatments and insemination, plus one time for pregnancy diagnosis), using the following formula: *Treatment cost per treated animal = [cost of hormones and materials + (4.62 X number of manipulations)]*. All costs are expressed in US dollars, using the equivalent price at the beginning of the study, in July 2009. The costs for hormones and materials were taken from the purchase price, and the cost of each handling session was set at a constant US$4.62 per handling session per animal, which was equivalent to 60 Mexican pesos (MN$; MN$13=US$1).

The cost per pregnancy (TCP= was calculated from the cost per treatment, using the following formula:

$$TCP = \frac{(\text{cost per treatment})(\text{n treated animals})}{\text{n pregnant animals.}}$$

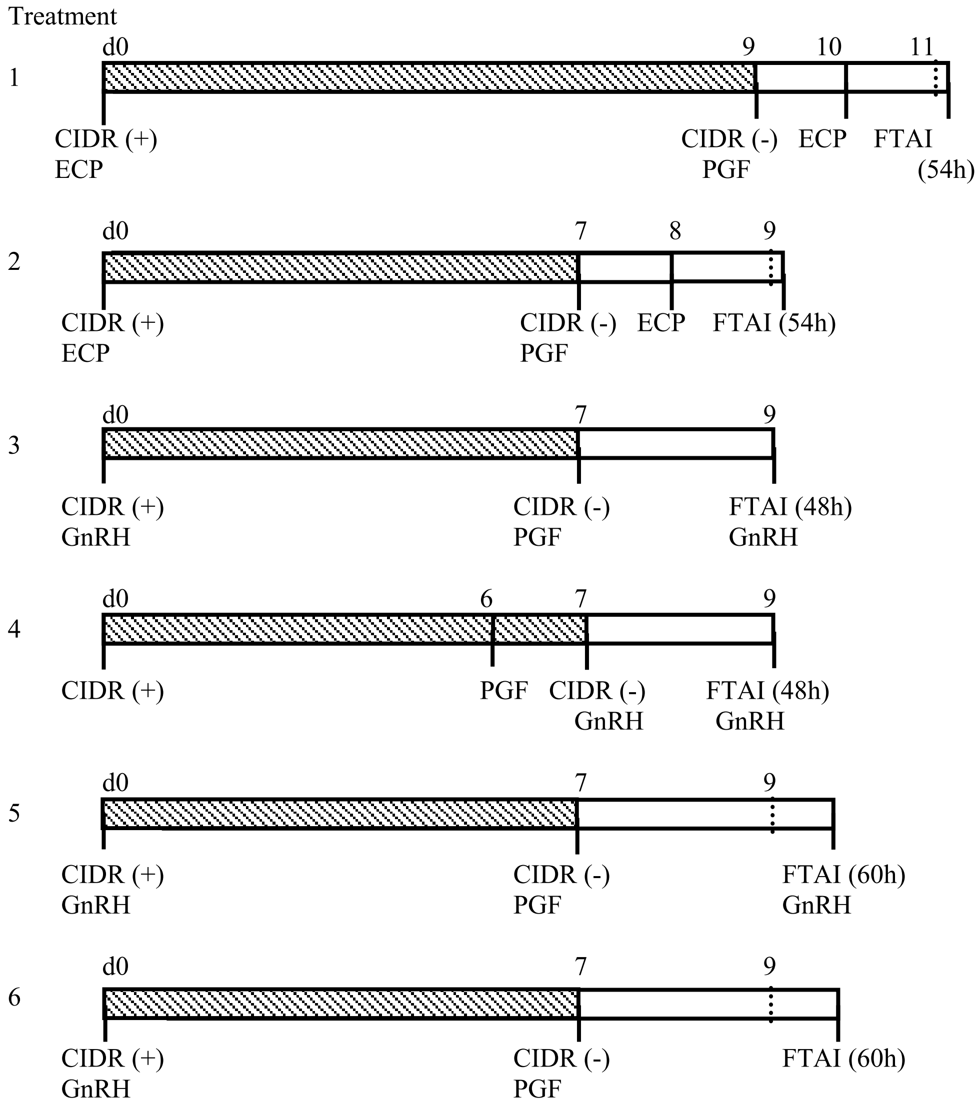

Figure 1. Diagrams for all synchronization treatments. The shaded bar represents the duration of CIDR treatment. CIDR (+) and CIDR (-) = CIDR insertion and removal, respectively.

Statistical analysis

Since there were several replicates in each treatment, the mean pregnancy rate and standard deviation were calculated for each treatment. To evaluate differences in mean pregnancy rates between synchronization treatments, and possible regional effects on treatment performance, a nested analysis of variance (ANOVA) was carried out using treatment as the main factor and county as a nested factor within treatments; the effect of county was examined to exclude regional variations in treatment response that would preclude comparing treatments from different regions. Analyses were performed using Statistica, v6.1 (Statsoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Table 1 summarizes the results. The replicates in Aldama, Altamira and Soto la Marina counties were nested to analyze pregnancy rates in heifers. There was no significant effect of county on pregnancy rate, and no significant difference between the six treatments ($p > 0.05$). Pregnancy rates in heifers in treatments 2 to 6 were similar, ranging between 42.6 ± 8.1 and 48.0 ± 10.6%. Treatment 1 showed a lower pregnancy rate, at 31.6 ± 3.9%. The retention rate for the CIDR was 99.01% (only 19 out of 1920 animals lost the device). The pregnancy rates observed in the treatments were comparable to those described by other authors. Among the reviewed literature, there is a large variety of results in pregnancy rates after synchronization treatments for FTAI. For instance, studies using estrogens obtained pregnancy rates ranging from 28.3% (Phillips *et al.,* 2010) to 61.8% (Sales *et al.,* 2012), while those using GnRH ranged between 26% (Colazo *et al.,* 2009) and 69% (Busch *et al.,* 2008); other authors have described results within these ranges (Martinez *et al.,* 2002; Lamb *et al.,* 2006; Busch *et al.,* 2007; Kim *et al.,* 2007; Saldarriaga *et al.,* 2007; Bucher *et al.,* 2009; Larson *et al.,* 2009; Meneghetti *et al.,* 2009; Peres *et al.,* 2009; Sá Filho *et al.,* 2009; Martinez *et al.,* 2012). The results of the present studies were also within these ranges, with small variations between treatments, except for T1 (Table 1).

The evaluation of the first four treatments to compare the effects of ECP vs. GnRH (Experiment 1) and the evaluation of GnRH at the end of synchronization (Experiment 2) showed no significant differences between treatments ($p = 0.22$). Similarly, the effect of the different counties on pregnancy rates was not significant ($p = 0.09$), validating the comparison of treatments from different counties.

Since the six treatments produced statistically similar results, any of them may be used. However, if the cost of each treatment is considered, in combination with pregnancy rates (Table 1), T2 (using estradiol) or T3 (using GnRH) would be recommended, since they balance desirable pregnancy rates (47.1 - 48%) with costs lower than those of other treatments.

Although no significant differences were found between treatments ($p = 0.22$), the numerically lower pregnancy rate observed in T1 may be due to the longer duration of the CIDR treatment (9 d), compared with all other treatments (7 d). The longer interval from follicle wave synchronization (stimulated by the first injection of estradiol cypionate) to the induction of ovulation (by the second injection of estradiol cypionate) could lead to an older follicle at ovulation, possibly past its optimal dominance period, and thus, to lower potential fertility (Peres *et al.,* 2009). However, Peres *et al.* (2009) reported 36.4 and 41.3% pregnancy rates in two groups of Nelore heifers after a treatment similar to that used for T1 (except for the use of estradiol benzoate instead of ECP, and the fact that it was given on day 0 and 9 instead of 0 and 10). If they applied PGF2α on day 7 instead of day 9, pregnancy rates improved to 52% (Peres *et al.,* 2009), suggesting that an earlier induction of luteolysis would allow a better follicle maturation, a change that was not observed in the present study.

In the protocol used for T4, PGF2α was given one day before CIDR removal, to allow more time for luteolysis (Lucy *et al*., 2001). However, fertility was not improved by this change in treatment timing, and it did result in a higher cost due to the additional handling session.

Table 1. Mean pregnancy rates and cost of each treatment. Costs are shown in US dollars.

Treatment	1	2	3	4	5	6
Hormone evaluated	ECP	ECP	GnRH	GnRH	GnRH	GnRH
CIDR duration (d)	9	7	7	7	7	7
PGF2α given (d)	9	7	7	6	7	7
CIDR-FTAI Interval (h)	54	54	48	48	60	60
GnRH given at FTAI	N/A[3]	N/A	+	+	+	-
Handling sessions (n)	5	5	4	5	4	4
Replicates (n)	12	11	12	13	11	7
Treated animals (n)	320	320	320	320	320	320
Inseminated animals (n)	318	320	315	318	315	317
CIDR loss (%)	0.6	0	1.6	0.6	1.6	0.9
Mean pregnancy rate (%)[1]	31.6	47.1	48.0	44.0	44.3	42.6
± Std. Deviation	3.9	18.5	10.6	15.1	13.4	8.1
Costs per treated animal:						
Materials ($)[2]	0.80	0.80	0.80	0.80	0.80	0.80
Hormones ($)	12.56	12.56	17.91	17.91	17.91	14.83
Handling ($)	23.08	23.08	18.46	23.08	18.46	18.46
Total cost ($)	36.43	36.43	37.16	41.78	37.16	34.09
Pregnant animals (n)	100	147	151	144	139	135
Cost per pregnancy ($)	116.59	79.31	78.76	92.84	85.56	80.80

[1] ($p > 0.05$).
[2] Includes costs for insemination material and equipment.
[3] Does not apply to these treatments.

The use of ECP or GnRH after CIDR removal is intended to reduce the variation in interval from CIDR removal to ovulation due to individual follicle status, thus synchronizing ovulation (Crowe *et al.,* 1993). However, T6 (which did not receive GnRH after CIDR removal) showed a pregnancy rate comparable to that of the other groups (Table 1). This could suggest that the final GnRH treatment can be avoided without losing fertility. However, other studies showed that the full treatment leads to better results (Lane *et al.,* 2008).

CONCLUSION

Under the climatic, nutritional and management conditions found in southeastern Tamaulipas, six synchronization treatments for Brahman heifers showed statistically similar results in pregnancy rates, and therefore, can be used in regional cattle. Considering the costs of the hormones and animal handling, Treatments 2 and 3 would be recommended to synchronize ovulation for fixed-time insemination in heifers; these combine adequate pregnancy rates at a reduced cost in heifers selected for body condition and proven ovarian activity.

Acknowledgment

This study was financed by the Consejo Nacional de Ciencia y Tecnología - CONACYT (FOMIX – Gobierno del Estado de Tamaulipas, Project 107405). The authors would like to thank Roberto Pérez-Castañeda and Jorge Loredo-Osti for their advice on statistical analysis, as well as the ranch owners for allowing the use of their facilities, the collaboration of their personnel and the use of their animals for the present study. The names of the ranches are, in alphabetical order: in Aldama County: El 18, El Chicloso, El Ombligo, La Esperanza, Los Carriles, San Antonio, San Gerardo and San Juan; in Altamira County: Cazaderos, El Apuro, El Toruno, La Gloria, La Rosita, San Andrés and San Juan; in Casas County: Santa Teresa; in Güémez County: La Providencia and Palomas Blancas; and in Soto la Marina County: El Huasteco, La Jauja, Las Brisas and San Juan.

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Submitted October 31, 2011– Accepted October 10, 2012
Revised received October 19, 2012