EMBRYO TRANSFER IN ANGUS AND BRAHMAN RECIPIENT COWS: EFFECT OF TWO METHODS OF ESTRUS SYNCHRONIZATION ON INDUCED ESTRUS AND PREGNANCY

Transferencia de Embriones en Vacas Receptoras Angus y Brahman: Efecto de Dos Métodos de Sincronización de Celos sobre el Celo Inducido y Preñez

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ABSTRACT

Over a 3-year period, 88 Angus and 87 Brahman cows were used as recipients to determine the effects of breedtype and method of estrous synchronization on induced estrus and subsequent pregnancy following embryo transfer. Estrus was synchronized in recipients using either prostaglandin-$F_{2\alpha}$ (PGF; Lutalyse) or progestogen (PRO; Syncro-Mate-B). Recipients were treated (im) with PGF on day -11 (25 mg), 0 (12.5 mg), and 1 (12.5 mg). Recipients were treated with PRO on day -9 (6 mg norgestomet ear implant and 2 mL [im] of 3 mg of norgestomet and 5 mg of estradiol valerate) and the ear implant was removed on day 0. Embryos were randomly transferred to recipients synchronous +24 to -24 h with a 7-d embryo. Both synchronization methods (PGF vs. PRO) were similarly effective in inducing estrus (89.8 vs. 86.7%). Percentage of induced estrus was similar between Brahman (91.0%) and Angus (85.6%). Recipients treated with PGF had a longer (P = 0.001) interval to estrus than PRO treated recipients (77.4 vs. 60.1 h). Estrus response in Angus was earlier (P = 0.043) than in Brahman (65.4 vs. 72.2 h). Neither breed (P = 0.97; Angus 49% 35/72 and Brahman 54% 38/70) nor estrus synchronization treatment (P = 0.23; PRO 49% 35/72 and PGF 54% 38/70) affected pregnancy rate. Pregnancy rates in recipients closely synchronized (0 h) was 60.0%, within +12 and -12 h was 56.0%, and within +24 and -24 h was 51.5%. Four-year-old recipients had lower pregnancy rates (34.8%) than did 5-year-old (60.9%) or greater than 6-year-old (70.0%) recipients. These results indicate that PGF, when administered as a split-second dose, is as effective in synchronizing estrus in Angus and Brahman as PRO. Asynchrony of embryo age with recipient stage of cycle up to +24 or -24 h did not significantly affect pregnancy rates.

Key words: Embryo transfer, induced estrus, pregnancy, progestogen, prostaglandin.

RESUMEN

Durante tres años, vacas receptoras Angus (88) y Brahman (87) se utilizaron para determinar los efectos de raza y método de sincronización sobre las características del celo inducido y la preñez subsecuente a la transferencia embrionaria. El celo fue sincronizado en las receptoras utilizando $PGF_{2\alpha}$ (Lutalyse®) o progesterona (PRO; Syncro-Mate-B). Un grupo de receptoras fue inyectado (im) con PGF los días 11 (25 mg), 0 (12,5 mg), y 1 (12,5 mg). Otro grupo fue tratado con PRO el dia -9 (implante auricular con 6 mg norgestomet e inyectadas (im) con 2 mL con 3 mg de norgestomet y 5 mg de valerato de estradiol), el implante fue removido el día 0. Los embriones fueron transferidos al azar a las receptoras en sincronía +24 a -24 h con embriones de 7-d de edad. Ambos métodos de sincronización (PGF vs. PRO) fueron similares en la inducción del celo (89,8 vs. 86,7%). El porcentaje de celos inducidos fue similar en Brahman (91,0%) y Angus (85,6%). Las receptoras tratadas con PGF presentaron un intervalo al celo más largo (P = 0,001) que aquellas tratadas con PRO (77,4 vs. 60,1 h).

El intervalo al estro post-tratamiento fue más corto en la vacas Angus expresaron celo más temprano (P = 0,043) que las Brahman (65,4 vs. 72,2 h). La tasa de preñez no fue afectada por la raza (Angus 49% 35/72 y Brahman 54% 38/70; P = 0,97) ni por el tratamiento de sincronización (PRO 49% 35/72 y PGF 54% 38/70; P = 0,23). Las tasas de preñez en receptoras de sincronización cercana (0 h) fue del 60,0%, entre +12 y -12 h fue del 56,0%, y entre +24 y -24 h fue del 51,5%. Las receptoras de cuatro años de edad presentaron tasas de preñez menores (34,8%) que las de cinco (60,9%) o las de 6 o más años de edad (70,0%). Los resultados indican que cuando la segunda aplicación de PGF se administra dividida en dos dosis, es tan efectiva para sincronizar el celo en Angus y Brahman como lo es el PRO. La asincronía de la edad del embrión con el ciclo de la receptora entre +24 y -24 h no afectó significativamente la tasa de preñez.

Palabras clave: Transferencia de embriones, celo inducido, preñez, progesterona, prostaglandina.

INTRODUCTION

Embryo transfer technology accelerates the genetic improvement of cattle and can also support genetic conservation programs through the enhanced movement of cattle germplasm world-wide [26, 27]. A requisite for the use of embryo transfer in the tropics and subtropics is success using tropically-adapted cattle, primarily *Bos indicus*. Estrus synchronization programs have mainly been tested in *Bos taurus* females and may not be as efficacious in *B. indicus*. Two commonly used methods of estrus synchronization include prostaglandin $F_{2\alpha}$ and progestogen-based programs. The progestogen used in this study, Syncro-Mate-B, was widely used and studied but currently is not available in the U.S.A. A similar product, Crestor, is available in Europe. Previous estrus synchronization studies using prostaglandin $F_{2\alpha}$ or Syncro-Mate-B in *B. indicus* or crossbred *B. indicus* females were not as successful as would be expected from *B. taurus* females [5, 9, 16, 19]. However, using a modified three injection prostaglandin $F_{2\alpha}$ regimen (i.e., split 2nd and 3rd injection 24 h apart), acceptable pregnancy rates were reported for Brahman-influenced females bred by AI that were greater than those obtained with the traditional double injection prostaglandin $F_{2\alpha}$ regimen [21]. Furthermore, there are few published studies from the subtropics or tropics that have directly compared estrous synchronization methods between purebred *B. indicus* and purebred *B. taurus* recipient cows used in an embryo transfer program.

The primary objectives in this study were to determine the effect of two estrus synchronization protocols (prostaglandin and norgestomet) in Angus and Brahman recipients on estrus synchronization and pregnancy when using embryo transfer.

MATERIALS AND METHODS

Location and Animal Management

This study was conducted over three years (1990 to 1992) at the Subtropical Agricultural Research Station (STARS), Brooksville, Florida, U.S.A. Each year the study was initiated in May and continued through July. Geographical coordinates of STARS (Main Station) are 28 37 00 North Latitude and 82 21 30 West Longitude. Average annual rainfall is 1,372 mm, and over one-half of that falls in June to September. Average temperature during the study was approximately 22C. Throughout the study cows grazed bahiagrass (*Paspalum notatum* Flügge) or mixed bahiagrass-legume (*Arachis glabrata*) pastures. A custom mineral mixture (25 to 32% salt, 15 to 18% Ca, 5 to 8% P, 0.94% Fe, 0.15% Fl, 0.10% Cu, 0.01% Co, and 0.0010 to 0.0015% Se) was offered. Each year, recipients were divided into two work groups, such that activities were carried out on two consecutive days. Husbandry followed recommended guidelines [4].

Recipient Cows and Estrus Synchronization Treatments

Purebred Angus (n = 88) and Brahman (n = 87) cows from 4 to 12 years of age and weighing 300 to 690 kg were used as recipients (TABLE I). According to previous year lactation status, recipient cows were open (n = 65) or lactating (n = 110), with a mean of 106 d postpartum (range 66 to 172 d). Recipient dams at random stages of the estrous cycle were synchronized using either prostaglandin-$F_{2\alpha}$ (PGF; Lutalyse, Pharmacia-Upjohn Co., Kalamazoo, MI, U.S.A.) or progestogen (PRO; Syncro-Mate-B, Merial Limited, Iselin, NJ, U.S.A.) treatments. The prostaglandin treatment included administration (im) of PGF on day 11 (25 mg), day 0 (12.5 mg), and 24 h later or day 1 (12.5 mg). This sequence of PGF administration had previously been shown to result in a higher percentage of pregnancies in artificially inseminated cows when compared with the standard double application sequence of 25 mg of PGF 11 d apart [21]. The PRO regimen consisted of a 6 mg norgestomet ear implant and 2 mL (im) of 3 mg of norgestomet and 5 mg of estradiol valerate on day -9. The ear implant was removed on day 0. Calves were not separated from their dams. Procedures were timed so that the second injection of PGF coincided with removal of the PRO ear implant.

Recipients were observed for signs of estrus at least twice daily (morning and evening) for a minimum of 30 min on days 0 through 5. Two sterile (epididymectomized) marker bulls fitted with chin ball marker devices were present at a 2:30 ratio to aid in the detection of estrus. Homo- and heterosexual mounting activity were used to determine time of estrus. Estrus was recorded when a cow was observed standing to be mounted or when ink marks indicated that the cow had been mounted. The number of recipient cows assigned each year to the experimental groups according to breed (Angus and Brahman) and to method of estrus

NUMBER OF ANIMALS BY YEAR, BREED OF RECIPIENT, AND SYNCHRONIZATION TREATMENT / NUMERO DE ANIMALES POR AÑO, RAZA DE LA RECEPTORA Y TRATAMIENTO DE SINCRONIZACION

Year	n	Breed of recipient	n	Synchronization treatment	n
1	60	Angus	30	Prostaglandin-$F_{2\alpha}$	15
				Progestogen	15
		Brahman	30	Prostaglandin-$F_{2\alpha}$	15
				Progestogen	15
2	60	Angus	30	Prostaglandin-$F_{2\alpha}$	15
				Progestogen	15
		Brahman	30	Prostaglandin-$F_{2\alpha}$	15
				Progestogen	15
3	55	Angus	28	Prostaglandin-$F_{2\alpha}$	14
				Progestogen	14
		Brahman	27	Prostaglandin-$F_{2\alpha}$	14
				Progestogen	13

synchronization (PGF and PRO), are presented in TABLE I. Synchronized estrus (EST) was defined as an estrus event within 120 h after day 0 (i.e., after second injection for the PGF group and after implant removal for the PRO group). Interval to estrus (ITE) was defined as the period (h) between day 0 and the manifestation of standing estrus. To assess estrus distribution, ITE was blocked into 12 h periods: 24 = 13 to 24; 36 = 25 to 36; 48 = 37 to 48; 60 = 49 to 60; 72 = 61 to 72; 84 = 73 to 84; 96 = 85 to 96; 108 = 97 to 108; 120 = 109 to 120; 132 = 121 to 132; and 144 = 133 to 144. Distribution of estrus (DOE) over time during the period of synchronized estrus was analyzed to evaluate synchrony of estrus associated with each synchronization protocol. Estrus synchrony (ESY) or the relationship between the recipient estrous cycle (e.g., development stage of the maternal uterine environment) and a 7 d embryo, was categorized as follows: 1) Synchrony, recipient-embryo closely (0 h) synchronized (e.g., uterine environment was appropriate for a 7 d embryo); 2) Plus asynchrony, recipient ahead (+) of the 7 d embryo, recipient came into estrus before the donor (e.g., uterine environment is either +12 or +24 h in a more advanced stage of development relative to a 7 d embryo); and 3) Minus asynchrony, recipient behind (-) the 7 d embryo, recipient came into estrus after the donor (e.g., uterine environment is premature either -12 or -24 h relative to a 7 d embryo).

Progesterone

Four blood samples from each recipient treated with PGF were collected via jugular venipuncture to determine concentrations of progesterone. The first was collected on day -11 just before PGF administration (25 mg). A second sample was obtained on day 0, immediately before PGF administration (12.5 mg). A third sample was collected on day 1, just prior to PGF administration (12.5 mg). The fourth sample was collected at embryo transfer. Three blood samples were collected from each recipient treated with PRO. The first was collected on day -9 just before norgestomet implantation. A second sample was ob-

tained on day 0, at implant removal. The third sample was collected at embryo transfer. Blood samples were allowed to clot and were centrifuged (Sorvall, RT6000, DuPont, U.S.A.) within 2 h of collection at 1800 x g for 30 min at 4C. Serum was separated and stored at -20C (Bohn, Beacon II, Heatcraft Refrigeration Products Inc., U.S.A.) until concentrations of progesterone were quantified using a solid-phase radioimmunoassay procedure (RIA; Coat-A-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.). All samples were run in 100-ΦL duplicates. Intra- and interassay coefficients of variation (CV) for six assays were 4.12 and 7.21%, respectively. Approximate sensitivity of the assay was 0.01 ng/mL.

Embryo Management

Embryos from Romosinuano donors (a criollo breed native to Colombia were imported from Costa Rica [years 1 and 3]) and from Brahman donors (year 2; collected at STARS) were used. Embryos were collected and frozen by a different embryo transfer specialist in Costa Rica than at STARS. Embryos were recovered between approximately 6 and 8 d following the onset of estrus using nonsurgical techniques and were handled according to the IETS standard/conventional approach [26]. Embryos were frozen using glycerol [6]. Romosinuano embryos were exported from Costa Rica and imported to the USA according to regulations from each country. Costa Rica is geographically north of Panama and hence is free of Foot-and-Mouth Disease, thus embryo import regulations for countries with endemic Foot-and-Mouth Disease did not apply [1]. At the time of transfer, embryos were thawed (4-step glycerol) [6] and evaluated according to IETS [26] for stage of development and quality code.

Embryos were randomly transferred to recipients synchronous +24 to -24 h with a 7 d (6 to 8 d) embryo. Under commercial conditions recipients out of this time-range would not have received an embryo. However, except for year 3 in this

study, all recipients that exhibited estrus were eligible to receive an embryo (i.e., recipient had estrus late compared to a 7 d embryo; -36 and -48 h). The same embryo transfer specialist performed all the nonsurgical transfers [29]. Prior to transfer, the recipients were palpated for the presence of a corpus luteum (CL). Embryos were deposited approximately one-third of the way into the uterine horn ipsilateral to the CL of ovulation. At the time of embryo transfer, each recipient was weighed and body condition scored [20]. Approximately 45 to 60 d following the transfer of embryos, recipients were palpated per rectum by an experienced professional to determine pregnancy status. Pregnancy rate was defined as the proportion of all recipients receiving an embryo that became pregnant.

Statistical Analysis

Data were analyzed using the General Linear Model (GLM) procedure, Chi-square analysis and contingency tables using the Frequency Procedure (PROC FREQ) of SAS [22, 24]. The model to analyze the proportion of recipients with an induced estrus included effects for year, breed, age of cow, previous year lactation status (open or lactating), synchronization method and all two-way interactions with synchronization method, BCS was included as a covariable. Higher order interactions were included in the error term. When only lactating recipients were analyzed, days postpartum was included as a covariable. Interval from day 0 to onset of induced estrus was analyzed using a model that included year, breed, age of cow, previous lactation status, synchronization method and all two-way interactions with synchronization method, BCS was included as a covariable. The distribution of estrus, measured in blocks of 12 h intervals from days 0 to 5 and estrus synchrony were analyzed relative to year, breed, age of cow, synchronization method, BCS, and days postpartum.

Pregnancy rate was analyzed using a statistical model that included year, breed, age of cow, previous year lactation status, synchronization method, synchrony status, and embryonic factors before transfer (stage of development and quality code). Concentrations of progesterone, measured on days -11, -9, 0, 1, and at embryo transfer were analyzed using a model that included year, breed, age, previous lactation status, BCS, and all two-way interactions. Mean concentrations of progesterone were compared between synchronized and nonsynchronized recipients. Similarly, mean concentrations of progesterone measured at embryo transfer were compared between pregnant and nonpregnant recipients. Nonsignificant effects detected in preliminary models were dropped from the final model.

RESULTS AND DISCUSSION

The overall estrus response, i.e., the proportion of recipients that exhibited signs of induced or synchronized estrus following treatment was 87 ± 2.5% (152/175; TABLE II). Expression of estrus was affected (P = 0.020) by year. Overall estrus response did not differ (P = 0.294) between Brahman (91%) and Angus (86%) recipient cows and did not differ (P = 0.546) between PGF (90%) and PRO (87%) synchronization treatments. No difference was found due to age of cow (P = 0.712), previous lactation status (P = 0.270), or BCS (P = 0.123).

Some studies using *B. indicus* females suggested that estrus after the standard PGF treatment is expected to be under 50% [9, 19]. However, this study supports the results of other studies that reported an estrus response over 70% [8, 21]. In the present study, splitting the standard PGF dose (25.0 mg) into two doses 12.5 mg each 24 h apart resulted in similar estrus synchronization between Angus and Brahman. Applying the same PGF protocol, 96% of cows were detected in estrus

TABLE II
LEAST SQUARES MEANS ± SE FOR INTERVAL TO ESTRUS, OVERALL ESTRUS RESPONSE, AND NON-ADJUSTED CUMULATIVE ESTRUS RESPONSE FROM 36 TO 120 H (12 H INTERVALS) POST TREATMENT, BY BREED AND SYNCHRONIZATION TREATMENT / MEDIAS DE CUADRADOS MÍNIMOS ± EE DE INTERVALO AL CELO, RESPUESTA GENERAL AL CELO, Y RESPUESTA ACUMULATIVA AL CELO NO-AJUSTAD A DESDE LAS 36 A 120 H (INTERVALOS DE 12 H), POR RAZA Y TRATAMIENTO DE SINCRONIZACIÓN.

Main effect	Interval to estrus (h)	Overall estrus response (%)	Non-adjusted cumulative estrus response (%)							
			36	48	60	72	84	96	108	120
Breed										
Angus	65.3 ± 2.48[a]	85.6 ± 3.84	8.0	28.0	50.5	69.5	75.1	78.4	78.4	84.1
Brahman	72.2 ± 2.37[b]	91.0 ± 3.75	3.4	23.3	44.2	60.5	66.4	75.9	80.5	83.9
Treatment[c]										
PGF	77.4 ± 2.45[d]	89.8 ± 3.71	0.0	8.0	28.0	56.8	63.6	72.7	76.1	83.0
PRO	60.1 ± 2.54[e]	86.7 ± 3.79	11.5	43.7	69.1	73.6	78.2	81.6	83.9	90.8

[a, b] Values within interval to estrus and breed with different superscripts differ (P = 0.043).
[c] Synchronization treatment. PGF = prostaglandin-$F_{2\alpha}$ administered on days -11 (25 mg), 0 (12.5 mg), and 1 (12.5 mg). PRO = standard Syncro-Mate-B regime.
[d, e] Values within interval to estrus and synchronization treatment with different superscripts differ (P = 0.001).

by radiotelemetry (HeatWatch®, DDx Inc., Denver, CO, U.S.A.) and 82% were detected in estrus by visual observation [14]. In *B. taurus*, an application of two 25 mg injections 11 or 14 d apart of PGF is reported to synchronize estrus in 70 to 90% of the females [18, 25]. A variable estrus response ranging from 66 to 100% has been reported in cows treated with the PRO protocol [7, 10, 11, 13, 18]. In a review it was reported that in most studies the percentage of female cattle detected in estrus using the PRO protocol was usually greater than 90% [18].

The overall mean interval to estrus was 69 ± 1.6 h (from day 0, second injection of PGF and implant removal in PRO). Interval to estrus was affected by breed (P = 0.043), synchronization method (P = 0.001), and the interaction of year by treatment (P = 0.006). This interaction of year by treatment on interval to estrus was due to a difference in magnitude between treatments each year but not to a difference in ranking between treatments. In year 1, there was a nonsignificant difference (P = 0.59) in interval to estrus of 3.3 h between PGF (71.0 ± 4.35 h) and PRO (67.7 ± 4.62 h) treatments; in year 2, interval to estrus differed (P = 0.001) by 19 h with PGF (72.8 ± 4.00 h) having a longer interval to estrus than PRO (54.1 ± 4.00 h); and in year 3, interval to estrus differed (P = 0.0001) by 30.1 h with interval to estrus being longer for PGF (88.5 ± 4.36 h) than PRO (58.4 ± 4.38) treated recipients. The interactions of breed by treatment (P = 0.078) and of age by treatment (P = 0.054) also occurred. Angus responded earlier (P = 0.043) to the estrus synchronization treatment than did Brahman recipients (65.3 vs. 72.2 h, respectively; TABLE II). Mean interval to synchronized estrus was later (P = 0.001) for recipient cows treated with PGF (77.4 h) than for those treated with PRO (60.1 h).

Longer ITE in Brahman than in Angus cows has been reported previously [3, 13, 14]. In the current study, recipients synchronized with PGF had a longer ITE than PRO treated recipients. This result agrees with previous findings [19] where Nelore (*B. indicus*) cows receiving two injections of PGF 11 d apart, exhibited estrus 70.5 ± 4.8 h after the second PGF injection and those receiving the PRO treatment showed estrus 57.7 ± 3.2 h after implant removal. Similarly, Brahman cows [3] exhibited estrus 72.0 h after the second PGF injection and 45.6 h after PRO implant removal. In purebred and crossbred *B. taurus* cows, an ITE of 71 ± 5 h after PGF (2 injections, 14 d apart) was reported [25]. Researchers [11] using females of various *B. taurus* breeds given PRO, reported a mean ITE of 37.2 ± 3.3 h.

The non-adjusted cumulative estrus response of recipients treated with PGF or PRO from 36 to 120 h post treatment is depicted in TABLE II. Few differences were observed between the percentages of Angus and Brahman recipients observed in estrus at any 12 h interval from 36 to 120 h post treatment. As might be expected from the observed interval to estrus, the non-adjusted cumulative estrus response for recipients synchronized with PRO was greater than for PGF at each of the 12 h intervals from 36 to 72 h post treatment. Recipients

treated with PRO began exhibiting estrus earlier than recipients treated with PGF. A clear peak in estrus of the PRO treated recipients occurred between 48 and 60 h, whereas a peak in estrus distribution of the PGF treated recipients was observed between 60 and 72 h. Thus, induced estrus was observed later and also was more dispersed over a greater time period in cows receiving the PGF regimen. The PRO protocol induced the earliest and tightest estrus response. Reports using the PRO protocol [12, 17] indicated a greater proportion (60%) of females observed in estrus between 24 and 48 h after implant removal.

Distribution of the number of induced estrus observed during the synchronized period is presented in FIG. 1. Within the +24 to -24 h synchronized period, 91% (138/152) of recipients showed estrus, and of those recipients that showed estrus, 49% (67/138) and 51% (71/138) were treated with PGF and PRO, respectively. Within the +12 to -12 h period, estrus was exhibited by 73% (111/152) of the recipients, of those 47% (52/111) and 53% (59/111) were treated with PGF and PRO (P = 0.04), respectively. Among recipients that were closely synchronized (33%; 50/152) relative to a 7 d embryo, 48% (24/50) had been treated with PGF and 52% (26/50) with PRO. Within recipients out of synchrony by -12 h (17%; 26/152), 27% (7/26) and 73% (19/26) had been treated with PGF and PRO, respectively (P = 0.02). Fourteen recipients (9%; 14/152) were out of phase by more than -36 h, of those 79% (n = 11) had been treated with the PGF and only 21% (n = 3) with the PRO protocol (P = 0.03).

A tighter synchrony of estrus was obtained in recipients treated with PRO than PGF. Although the ITE differed, there was not a difference between synchronization methods in the proportion of recipients that were closely synchronized (0 h). However, during the expanded period between +12 to -12 h, the PRO protocol was more effective than PGF in concentrating the estrus response. This difference was not evident inside the +24 to -24 h period, where estrus was exhibited by a similar proportion of recipients regardless of synchronization method.

Overall mean progesterone concentration at the beginning of the experiment was more than 3 ng/mL for recipients assigned to the PGF or PRO treatments (TABLE III). Serum progesterone concentration at the start of the experiment was affected only by the variable year (P = 0.001). At this time 73.9% (65/88) and 64.4% (56/87) of the Angus and Brahman recipients, respectively, had a progesterone concentration greater than 1,0 ng/mL. Also, of those recipients assigned to receive the PGF and PRO treatments, 65.9% (58/88) and 72.4% (63/87), respectively, had a progesterone concentration greater than 1.0 ng/mL.

Mean serum concentration of progesterone on day 0 was affected by year (P = 0.010) and synchronization method (P = 0.001). Immediately prior to the treatment procedures on day 0, concentration of progesterone was higher in PGF recipients (2.6
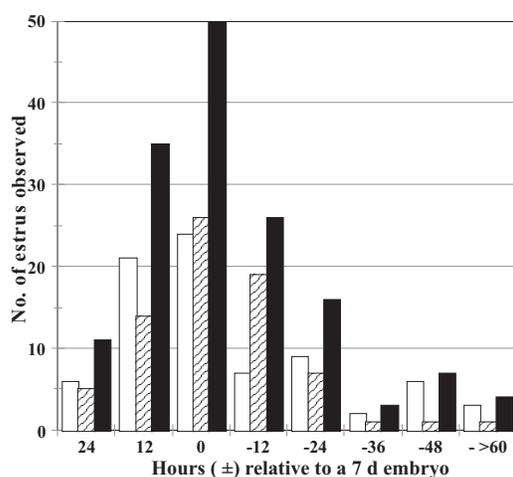
FIGURE 1. DISTRIBUTION OF ESTRUS OBSERVED WITHIN THE SYNCHRONIZATION PERIOD (+24 TO -60 H) AFTER SYNCHRONIZATION WITH PROSTAGLANDIN-F$_{2\alpha}$ [OPEN BARS], PROGESTOGEN [SYNCRO-MATE-B; CROSS-HATCHED BARS], AND TOTALS [SOLID BARS]/ DISTRIBUCIÓN DE CELOS OBSERVADO DURANTE EL PERIODO DE SINCRONÍA (+24 A -60 H) DESPUÉS DE LA SINCRONIZACIÓN CON PROSTAGLANDINA-F2$_{\alpha}$ [BARRAS ABIERTAS], PROGESTÁGENOS [SYNCRO-MATE-B; BARRAS CRUZADAS], Y TOTALES [BARRAS SÓLIDAS].

± 0.27 ng/mL) than in PRO recipients (0.5 ± 0.25 ng/mL; TABLE III). A progesterone concentration less than 1.0 ng/mL was detected in 76.7% (33/43) of Brahman and 88.6% (39/44) of Angus recipients under the PRO protocol. Regardless of treatment, recipients that later exhibited a synchronized estrus had a higher (P = 0.01) progesterone concentration (2.1 ± 0.16 ng/mL) at day 0 than recipients that did not exhibit a synchronized estrus (1.2 ± 0.34 ng/mL). Furthermore, just before the second injection of PGF (day 0), concentration of progesterone was higher (P = 0.001) in the synchronized recipients (3.6 ± 0.19 ng/mL) than in those non-synchronized (1.7 ± 0.48 ng/mL). Concentrations of progesterone at PRO implant removal (day 0) in synchronized and nonsynchronized recipients were 0.5 ± 0.20, and 0.4 ± 0.44 ng/mL (P = 0.890), respectively.

Low concentrations of progesterone at PRO implant removal were also found in previous work [2, 3]. Suckled Angus cows treated with PRO early in the estrous cycle had low progesterone concentrations (<1.0 ng/mL) at implant removal [2]. They attributed this response to a premature uterine release of PGF and early luteolysis. The PRO treatment consists of an ear implant that contains norgestomet (6 mg) and an intramuscular injection of estradiol valerate (5 mg) and norgestomet

TABLE III
LEAST SQUARES MEANS SE FOR SERUM PROGESTERONE VALUES ON SAMPLEDAY BY BREED, DEGREE OF SYNCHRONY, AND SYNCHRONIZATION METHOD/ MEDIAS DE CUADRADO MÍNIMOS EE DE LOS VALORES DE PROGESTERONA SÉRICA AL DÍA DE MUESTRA POR RAZA, GRADO DE SINCRONÍA Y MÉTODO DE SINCRONIZACIÓN

	Progesterone concentrations (ng/mL)[a]				
	Day -11[b]	Day -9[c]	Day 0	Day 1[b]	ET[d]
Breed					
Angus	3.4 ± 0.43	3.5 ± 0.43	1.5 ± 0.23	1.0 ± 0.16	2.2 ± 0.26
Brahman	3.2 ± 0.40	3.1 ± 0.42	1.6 ± 0.24	1.1 ± 0.17	2.2 ± 0.24
Synchrony[d]					
+24	-	-	1.5 ± 0.60	0.6 ± 0.26	3.3 ± 0.25
+12	-	-	2.2 ± 0.34	0.7 ± 0.14	2.6 ± 0.23
0	-	-	2.0 ± 0.28	0.8 ± 0.13	2.8 ± 0.20
-12	-	-	1.1 ± 0.39	1.0 ± 0.24	2.5 ± 0.26
-24	-	-	3.4 ± 0.50	1.2 ± 0.21	2.4 ± 0.28
Treatment[e]					
PGF	3.3 ± 0.32	-	2.6 ± 0.27[f]	1.0 ± 0.08	2.4 ± 0.25[h]
PRO	-	3.3 ± 0.33	0.5 ± 0.25[g]	-	2.0 ± 0.25[i]

[a] Day -11, first administration of prostaglandin-F$_{2\alpha}$ (PGF; 25 mg); Day -9, progestogen (PRO) implant insertion; Day 0, PRO implant removal, and second administration of PGF (12.5 mg); Day 1, third administration of PGF (12.5 mg); ET day, day of embryo transfer.
[b] Only recipients receiving PGF treatment were sampled this day.
[c] Only recipients receiving PRO treatment were sampled this day.
[d] 0 = synchronous; + = recipient in estrus before donor; = recipient in estrus after donor.
[e] Synchronization treatment. PGF = prostaglandin-F$_{2\text{á}}$ administered on Days -11 (25 mg), 0 (12.5 mg), and 1 (12.5 mg). PRO= Syncro-Mate-B regime.
[f, g] Values within synchronization treatment with different superscript differ (P = 0.001).
[h, i] Values within synchronization treatment with different superscript differ (P = 0.014).

(3 mg) mixed together. The norgestomet acts like an "artificial corpus luteum (CL)" and thus prevents luteinizing hormone surges and ovulation, CL formation and CL maintenance [28]. The lack of CL maintenance along with the estradiol valerate that is luteolytic induces CL regression. Cavalieri et al. [3] using Brahman cows with PRO implanted at random stages of the estrous cycle, reported that only 9.1% (1/11) had a progesterone concentration greater than 1.0 ng/mL at implant removal. They concluded that this low level of progesterone concentration at implant removal indicated functional luteolysis after PRO treatment.

Progesterone concentration immediately prior to the third injection of PGF (day 1) was not affected by year, breed, age, previous lactation status, or BCS (P>0.40). Twenty-four hours after the second injection of PGF (12.5 mg), the mean concentration of progesterone was 1.0 ± 0.11 ng/mL(day 1; TABLE III), and did not differ (P = 0.31) between synchronized (0.9 ± 0.11 ng/mL) and non-synchronized (1.2 ± 0.26 ng/mL) recipients. Thus, in PGF treated recipients, concentration of progesterone dropped precipitously from 2.6 ng/mL on day 0 after PGF administration to 1.0 ng/mL 24 h later (day 1).

Overall mean concentration of progesterone at embryo transfer was 2.6 ± 0.99 ng/mL. Progesterone concentration at embryo transfer was affected by year (P = 0.001), age (P = 0.019), and synchronization method (P = 0.014). Younger recipients (4- and 5- year-old) had higher progesterone levels (2.4 ± 0.28 ng/mL), than older (greater than 6- year-old) recipients (1.8 ± 0.27 ng/mL). Other researchers indicated that the variability often seen in progesterone concentrations at embryo transfer reflects a combination of different rates of CL development and the fluctuation of progesterone secretion during the early luteal phase of the estrous cycle [23]. Therefore, the results in the present study of younger cows having a higher concentration of progesterone at embryo transfer than older cows may be important but needs to be verified in larger data sets. There was no difference (P>0.13) in progesterone concentration at embryo transfer among recipients synchronized within the +24 to -24 h period (2.4 to 3.3 ng/mL; TABLE III). Levels of progesterone concentration in asynchronous recipients outside of the +24 to -24 h period were less than 2.0 ng/mL (e.g., 36 h [n = 3], 1.6 ± 0.62 ng/mL; 48 h [n = 7], 1.2 ± 0.76 ng/mL; 60 h [n = 3], 1.1 ± 1.05 ng/mL; and 72 h [n = 1], 0.6 ng/mL); however, the number of observations in these groups was small. Mean progesterone concentration at embryo transfer was higher (P = 0.014) in recipients under the PGF protocol (2.4 ± 0.25 ng/mL) than in recipients receiving the PRO protocol (2.0 ± 0.25 ng/mL). There was a tendency (P = 0.079) for recipients that became pregnant to have a higher mean progesterone concentration at embryo transfer (2.4 ± 0.25 ng/mL) than those found to be non-pregnant (2.0 ± 0.26 ng/mL).

On the day of embryo transfer, recipients treated with PRO had a progesterone concentration of 2.0 ng/mL and recipients 6- years-old and older had a mean progesterone con-

centration less than 2.0 ng/mL. As previously discussed progesterone concentrations at embryo transfer are variable and reflect a combination of different rates of CL development as well as fluctuation of progesterone secretion during the early luteal phase of the estrous cycle [23]. These results of progesterone concentrations at or less than 2.0 ng/mL support the conclusion of Spell et al. [23] that the minimum threshold at which progesterone concentration is essential for the establishment and maintenance of pregnancy at embryo transfer may be lower than that previously reported (2.0 to 5.0 ng/mL).

A total of 142 recipients were suitable for embryo transfer. Reasons for not receiving an embryo included the presence of a very small CL or no palpable CL, and an extreme out of estrus synchrony (greater than 48 h). Overall pregnancy rate was 51.4 ± 3.9% (73/142). Pregnancy rate was affected by age of recipient (P = 0.002) and embryo quality (P = 0.05). There was a tendency for year (P = 0.061) to affect pregnancy rate. Neither breed (P = 0.97; Angus 49% 35/72 and Brahman 54% 38/70) nor estrus synchronization treatment (P = 0.23; PRO 49% 35/72 and PGF 54% 38/70) affected pregnancy rate.

Young recipients (4-year-old) had a lower pregnancy rate (34,8%) than 5-year-old (60.9%) or greater than 6-year-old (70,0%) recipients. Just prior to transfer during the thawing procedure, embryos were classified according to quality code [26]; 90 embryos received a quality code 1 (excellent or good), 49 were classified as code 2 (Fair), and only one embryo received a quality code 3 (Poor). Pregnancy rates for quality groups 1, 2, and 3, were 58.9, 38.8, and 0.0%, respectively. Overall pregnancy rates were 65.2% in year 1, 42.1% in year 2, and 38.7% in year 3. Pregnancy rates for late morula (n = 86) and early blastocyst (n = 30) embryos were 56.5 and 50,0%, respectively (P = 0.54). Pregnancy rates relative to the other stages of embryo development (morula [33.3%; 2/3], blastocyst [37.5%; 3/8], expanded blastocyst [50.0%; 3/6], and hatched blastocyst [25.0%; 1/4]), were based on very small numbers of transfers.

There was a 52% (70/136) pregnancy rate for recipients synchronized within +24 to -24 h with a 7 d embryo (FIG. 2). The highest pregnancy rate (30/50; 60%) was obtained in recipients that were most closely synchronized (0 h) with a 7 d embryo. Pregnancy rates for recipients out of synchrony by +12, -12, and +24 h were 50% or greater; in contrast, at -24 h pregnancy rate declined to less than 20%.

Close recipient-donor synchronization in cattle is associated with higher pregnancy rates [10, 23]. In this study, pregnancy rate of recipients closely synchronized (0 h) was 60.0%. However, it has been reported that some degree of asynchrony between the recipient and the transferred embryo can still result in a pregnancy [10, 28]. In this study, recipients synchronized with a 7 d embryo within +12 and -12 h had a pregnancy rate of 56.0%, that declined to 51.5% when recipients were synchronized within +24 and -24 h. Pregnancy rate was compromised when the recipient came into estrus after the donor (-), that is, when a 7 d embryo was transferred into an uterine
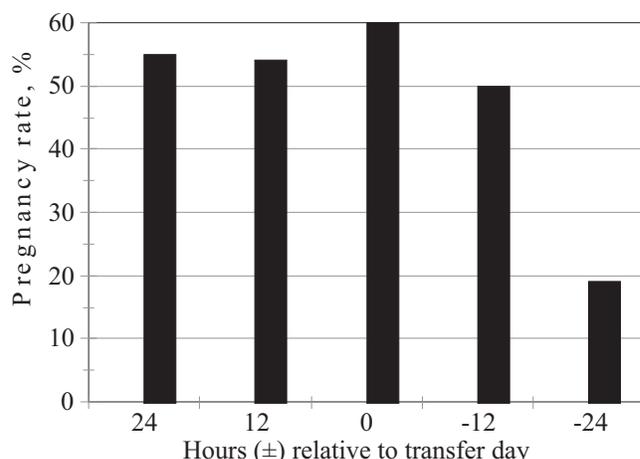
FIGURE 2. PREGNANCY RATES (%) OF RECIPIENTS RELATIVE TO TRANSFER OF A 7-D EMBRYO WITHIN +24 AND- 24 H. PLUS SYNCHRONY = RECIPIENTS IN ESTRUS BEFORE DONOR; MINUS (-) SYNCHRONY = RECIPIENTS IN ESTRUS AFTER THE DONOR / TASAS DE PREÑEZ (%) DE RECEPTORAS DE EMBRIONES DE 7-D DENTRO DE LAS +24 Y - 24 H. EN RELACIÓN A LA SINCRONÍA MAS (+) = RECEPTORAS EN CELO ANTES QUE LA DONADORA; SINCRONÍA MENOS (-) RECEPTORAS EN CELO DESPUÉS DE LA DONADORA.

environment that was early either by 12 or 24 h. Pregnancy rates declined to 50.0% in recipients out of synchrony by -12 h and to 18.8% when out of synchrony by -24 h.

There was a 20.1% difference in pregnancy rate among recipients receiving a quality code 1 or 2 embryo. Previous studies reported that a decrease in pregnancy rate is expected with each corresponding decrease in quality code [10, 23, 28]. Spell et al. [23] found no difference in pregnancy rate among cows receiving a code 1 or code 2 embryo. In the present study, there were not sufficient embryos in all different stages of development transferred to determine the effect of embryo development on pregnancy rate; however, a valid comparison was performed between pregnancy rate for late morula stage (56.5%) and early blastocyst (50.0%) embryos and the difference was not statistically significant. A previous report [23] noted that stage of embryo development did not significantly affect pregnancy rate after embryo transfer. However, higher pregnancy rates have been noted for early blastocyst compared to morula embryos [10, 15].

CONCLUSIONS AND IMPLICATIONS

A breed effect was evident on the interval to estrus where Angus responded earlier to the estrus synchronization treatment than did Brahman. Results also indicated that within the +24 to -24 h period, estrus responses were similar for recipients treated with PGF or PRO. However, a tighter synchrony of estrus was obtained with the PRO treatment. Thus, results support the use of a PRO protocol when a single, fixed-time activity (e.g., in-

semination, embryo transfer) is planned. Neither breed nor estrus synchronization method influenced pregnancy rates to embryo transfer in this study. Furthermore, asynchrony of the embryo and recipient of between +24 or -24 h does not appear to significantly affect pregnancy rates. The PGF protocol used in this research is as effective in synchronizing estrus in beef cattle as the PRO protocol. The PGF protocol requires an extra day of cattle handling but does not require the administration and removal of an implant. Thus, management preferences, product availability, and, of course, economics should each be considerations in the selection of PGF vs. PRO treatments for use in recipient cows for embryo transfer.

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Estrus synchronization minimizes problems associated with estrus detection, such as labor costs and estrus detection errors. While many synchronization programs involving one or a combination of several hormones have been developed and used on commercial farms, the basic principle of estrus synchronization involves manipulating the length of the luteal phase of the estrus cycle. The length of the luteal phase can be shortened by treatment with prostaglandin F2α or its analogues or it can be lengthened by treatment with exogenous progestogens. The indications for and methods of estrus synchronization in swine are varied, but all methods are based on controlling events leading to follicular maturation and ovulation or altering the luteal phase. The natural estrus method was conducted by estrus detection on recipients, and day-0 was calculated from the emergence of estrus expressions. The detection was performed 24 hours four times in 30 minutes of each observation (04.00, 10.00 AM and 04.00, 10.00 PM). On day-0, the observation was conducted using USG. Evaluating recipient and embryo factors that effect pregnancy rates of embryo transfer in beef cattle. Theriogenology. 2001; 56: 287-297. In agriculture, estrous synchronization is used (particularly in the dairy and beef industries) to facilitate breeding by artificial insemination. The term "estrus" refers to the phase of the estrous cycle in which a sexually mature, non-pregnant female is receptive to sexual advances from the male[3]. Ovulation occurs at approximately this time. Estrous synchronisation is the process of targeting female mammals to come to heat within a short time frame (36 to 96 hours). This is achieved through the use Estrus responses of recipient cows and heifers. The estrus response of females synchronized with estrous synchronization protocols are presented in Table 2. The proportion of Boran and Boran x HF crosses that responded to synchronization treatment was 48.72 and 44.59% respectively (P > 0.05). There was no significance difference among estrous synchronization protocols. Pregnancy rate per embryo transfer observed in present study is lower than the PR of 59.7% for Nellore and 68.3% for Gyr embryos that was reported by (Peixoto et al., 2007). Super-Ovulation, Estrous Synchronization and Embryo Transfer. [33]. 2003. Differences. between Brahman and Holstein cows in response to estrus synchronization, super-ovulation. Estrus synchronization is a key element of all the ART-protocols in livestock animals and has a major influence to increase the overall efficiencies of these programs (Baldassarre and Karatzas, 2004). Estrus synchronization plays a major role in fixed time breeding, AI, LOPU for oocyte or embryo collection and Embryo Transfer (ET). The value of estrus synchronization is vital in does as the duration of both estrous cycle and estrus is variable and estrus detection cannot be accomplished safely without a buck (Jainudeen et al., 2000). This technique has been developed in the early 1960s and sin... Principles of inducing superovulation in doe are the same as in cow and ewe.