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Fertility in Cebú × brown Swiss cows treated with prostaglandins, progesterone and eCG

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ABSTRACT

Objective: To evaluate the application of one or two doses of prostaglandin F2α (PGF2α), the application of a progestogen on different days of the estrous cycle and the usage of eCG in the estrus synchronization and fertility of Zebu × Brown Swiss cows.

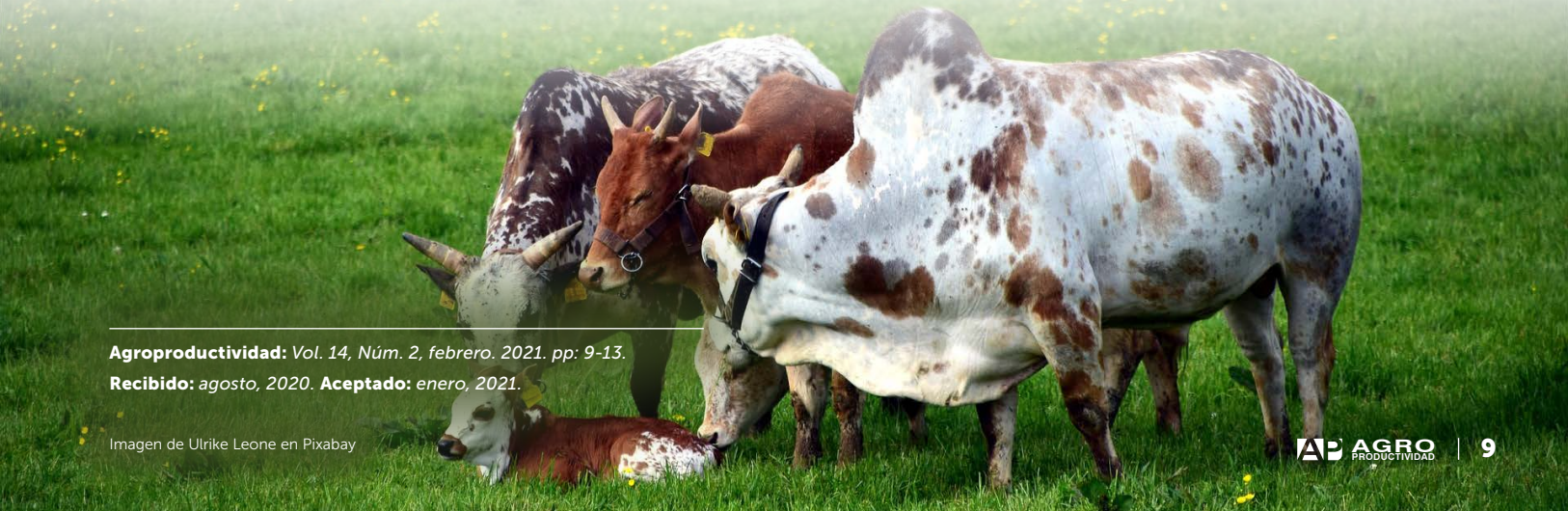
Design/methodology/approach: The study was based on three protocols. The first consisted of two treatments: PGI) 26 cows were injected with a single 25 mg dose of PGF2α and 10 cows with two 25 mg doses of PGF2α at a 14-day interval. Protocol 2 consisted of two treatments: NG14) 11 cows were implanted with 3 mg of Norgestomet on day 7 of their estrous cycle and NG7) 11 animals received the same dose on day 14. In protocol 3 all cows were implanted with 3 mg of Norgestomet for 9 days, 48 h before removing the implant, 25 mg of PGF2α was applied. Once the implants were removed, they were distributed into two treatments. Norgestomet (n=11) without eCG and Norgestomet + eCG (500 IU) (n=11).

Results: The application of PGF2α at two times had no influence ($p>0.05$) in the estrous percentages and conception. The NG7 achieved estrous synchronization in 81.8 % of the cows, in between 24 and 36 h, compared to 45.4 % of the NG14; however, the conception rate was lower ($p\leq0.05$). The eCG application synchronized 90.9 % of estrous between 24 and 36 h, compared with 36.4 % of the group with no eCG application.

Study limitations/implications: Transrectal ultrasounds are required to assess the ovarian structures present at the time of the estrus onset in a synchronization protocol.

Findings/conclusions: Cows that present *corpus luteum* do not require more than one injection of PGF2α, the pregnancy percentage increases when Norgestomet is implanted on day 14 of the estrous cycle, in addition the application of eCG increases the synchronization percentage of the heat between 24 to 36 h after the progestogen withdrawal.

Key Words: Estrus synchronization, Fertility, eCG, PGF2α, Norgestomet, Zebu × Swiss brown.



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INTRODUCTION

The use of estrus synchronization protocols (ESC) and artificial insemination (AI) in cows are common practices to improve fertility, reproductive parameters and accelerate genetic improvement of a herd. The objective of the estrus synchronization protocols is to control the life of the *corpus luteum*, as well as the cycle and emergence of follicular waves (Ayres *et al*., 2013). To achieve this, hormones as PGF2α, GnRH (Wiltbank *et al*., 2015), progestogens (Filho *et al*., 2009) and equine chorionic gonadotropin (eCG) (Lucy *et al*., 2004) are used. In tropical regions of Mexico, given their aptitudes for meat and milk production, Brown Swiss cattle and their crosses with zebu breeds, resistant to parasites and heat stress, are preferred by cattle ranchers; however, the reproductive efficiency of the herds in the tropics is usually low. For Mexico, a 14-month interval between births is reported (Hernández *et al*., 2001), which may be due to longer gestation periods, low postpartum body conditions and the calf's presence, which causes reduced frequency LH pulses in the cows (Filho *et al*., 2009). Consequently, the use of estrus synchronization protocols is particularly useful, as it guarantees that the cows are inseminated at detected estrus or under a fixed-time insemination scheme (IATF). Yet, the information regard to the use of synchronization and fertility protocols for Zebu × Brown Swiss cattle is limited. The objective here was to evaluate the application of 1 or 2 prostaglandins doses, the use of progestogen on a different day of the estrous cycle and the eCG application in the estrus synchronization and fertility of Zebu × Brown Swiss cows.

MATERIALS AND METHODS

Location: The study took place at the Experimental Unit "El Gargaleote", of the Universidad Autónoma Chapingo. The experimental unit is in Tamuín, San Luis Potosí, Mexico; between 21° 10' and 24° 29' N and 98° 20' and 102° 18' W, at 20 masl and with a 25.8 °C average temperature (García, 2004).

Animals and feeding: Zebu × Brown Swiss cows (n=83) from 3 to 6 years of age, with a live weight of 517±43 kg, healthy and with *corpus luteum* (CL) greater than 12 mm were used. The size of the CL was determined by transrectal ultrasonography (SONOVET 600®, Medison equipped with a 7.5 - Mhz transducer, Aloka 210, Corometrics Medical Systems, Wallingford, CT). The cows diet was based on forage, in a pasture of Star of Africa (*Cynodon nlemfuensis*) and Bermuda (*Cynodon dactylon*) grasses.

Protocols and experimental design

Three estrus synchronization protocols were evaluated.

Protocol 1: 36 cows were used, assigned to one of two groups, PGI and PGII. The PGI cows (n=26) received an I.M. shoot of 25 mg of dinoprost (Lutalyse, Pfizer), on a random day of their estrus cycle. The PGII cows (n=10) received two I.M. at 14-day intervals. The first injection was given at the same time as the PGI group cows.

Protocol 2: Twenty-two cows received a 25 mg dinoprost I.M. injection. The cows were monitored for estrus detection during the morning (06:00 to 10:00 h) and afternoon (16:00 to 19:00 h) for three days after the dinoprost application. All 22 cows showed estrus, considering estrus as day zero of their estrous cycle and were assigned to one of two groups: NG7 and NG14. The NG7 group cows (n=11) were subcutaneously implanted in the middle part of the ear with 3 mg of norgestomet (Crestar®, Intervet México, S.A.) on day seven after the estrus. The implant was removed nine days after placement and each received an injection of 25 mg of dinoprost. The cows of the NG14 group received the same treatment as those of the NG7, except that the application of the implant was carried out on day 14 of the estrous cycle.

Protocol 3: Cows (n=25) received a 25 mg dinoprost I.M. injection. The cows were monitored for estrus detection in the morning (06:00 to 10:00 h) and the afternoon (16:00 to 19:00 h) for three days, after the application of dinoprost. Estrus was detected in 23 of the 25 cows injected with dinoprost. The day of estrus was designated as day zero. Cows that presented estrus were assigned to one of two groups: Norgestomet (n=11) and Norgestomet + eCG (n=12). The cows of both groups received an implant with 3 mg of norgestomet (Crestar®, Intervet México, S.A.), on day 14 of the estrous cycle, by s.c. in the middle of the ear. The implant was removed nine days after its placement. The cows received a 25 mg injection of dinoprost seven days after implant application. On the implant removal day, the cows in the NG + eCG group received an I.M. of 500 IU of eCG hormone (500 IU; Folligon, Intervet México, S. A.), while those in the NG group received an injection of saline solution as a placebo.

In the three evaluated synchronization protocols, heat detection took place during the three consecutive days after the dinoprost application (protocol - 1), or after removing the implant (protocol 2 and 3), during the morning (06:00 at 10:00 a.m.) and afternoon (4:00 p.m. to 7:00 p.m.). Cows were then inseminated, following the a.m. - p.m. system, by the same inseminator, using conventional semen from a certified company. The pregnancy diagnosis was made via ultrasound 45 days after the artificial insemination.

The estrus percentage, distribution and conception percentage were evaluated. The estrus percentage was defined as the number of cows that presented estrus among the number of synchronized cows per hundred. The estrus distribution was defined as the number of cows that showed oestrus between 24 and 36 h and 37 to 48 h after the prostaglandins application (experiment 1) and the progestin withdrawal (experiments 2 and 3); while the conception percentage was defined as the number of pregnant cows among the number of inseminated cows.

Statistical analysis

The data were analysed with a model adjusted for a completely randomized design, where the incidence of estrus and percentage of conception variables were analysed using the Chi-Square test in the SAS statistical software for Windows version 9.3 (SAS, 2011).

RESULTS AND DISCUSSION

Protocol 1

Table 1 shows the estrus (84.6 *vs*. 100%) and conception rates (54.5 *vs*. 50%) under protocol 1. There was no statistical difference ($p \geq 0.05$) between treatments when applying one or two PGF2α doses, respectively.

Protocol 2

Prior to the treatment's application, all the cows had an average 14 mm *corpus luteum*. The PGF2α application achieved that all the animals presented estrus. After this, it was noted that the Norgestomet application on day 7 or 14 of the estrous cycle did had no influence on the number of cows that presented estrus ($p > 0.05$); although in 81.8% of the cows to which the progestogen was implanted on day 7 of the estrous cycle reported estrus between 24 and 36 h after the progestogen withdrawal, compared to 45.4% of the cows to which Norgestomet was implanted on day 14 of the estrous cycle ($p = 0.07$; Table 2). The remaining 18.2% of the cows implanted on day 7 of the estrus cycle presented estrus 37 to 48 h after the implant removal, compared with 54.6% of the cows implanted on day 14 of the cycle ($p = 0.07$). A higher conception percentage was observed in cows implanted with Norgestomet on day 14 of the cycle ($p < 0.05$; 81.8%) compared to those that were implanted on day 7 of the estrous cycle (36.4%).

Protocol 3

No effect was found of the application of 500 IU of eCG to the withdrawal of the progestin in the number of cows that reported estrus ($p > 0.05$); however, the eCG grouped 90.9% of cows in estrus between 24 and 36 h compared with 36.4% of cows without the eCG application ($p \leq 0.05$; Table 3). The 9.1 % of the remaining cows with the application of eCG showed estrus between 37 and 48 h after the removal of the implant compared to 63.6% of the cows without the application

Table 1. Estrus incidence and conception percentage in dual-purpose cows treated with one or two prostaglandins injections.

	PGI	PGII
Estrus percentage	22/26 (84.6%)[a]	10/10 (100%)[a]
Conception rate	12/22 (54.5%)[a]	5/10 (50%)[a]

[a,b] Means in the same row with the same literal are statistically different ($p < 0.05$). PGI: single injection of 25 mg prostaglandin (dinoprost) PGII: double injection of 25 mg of prostaglandin (dinoprost) with an interval of 14 days.

Table 2. Estrus distribution and pregnancy percentage in dual-purpose cows treated with Norgestomet on day 7 and 14 of their estrus cycle.

Estrus distribution	NG7	NG14
24-36 h	9/11 (81.8%) [a]	5/11 (45.4%) [a]
37-48 h	2/11 (18.2%) [a]	6/11 (54.6%) [a]
Total	11/11 (100%) [a]	11/11 (100%) [a]
Pregnancy percentage	4/11 (36.4%)[b]	9/11 (81.8%)[a]

[a,b] Means in the same row with different literals are statistically different ($p < 0.05$). NG7: Norgestment implant on day 7. NG14: Norgestment implant on day 14.

Table 3. Estrus distribution and percentage of pregnancy in dual-purpose cows treated with eCG at progestin withdrawal.

Estrus distribution	Norgestomet	Norgestomet + eCG
24-36 h	10/11 (90.9%)[a]	4/11 (36.4%)[b]
37-48 h	1/11 (9.1%)[b]	7/11 (63.6%)[a]
Total	11/12 (91.6%)[a]	11/11 (100%)[a]
Conception rate	9/11 (81.81%)[a]	6/11 (54.54%)[a]

[a,b] Means in the same row with different literals are statistically different ($p < 0.05$). T1: Norgestoment. T2: Norgestoment more 500 IU of eCG.

of eCG ($p \leq 0.05$). Although no statistical differences ($p > 0.05$) were observed in the percentage of conception, a numerical trend of 81.8 *vs*. 54.5% of cows that became pregnant with the application of eCG compared to the cows that did not receive an application of eCG, each.

A 100% of the cows that received double PGF2α application showed estrous compared to 84.6% in the cows that received a single dose. Previously, 100% of estrus in cows has been reported when applying two injections of prostaglandins at 11 days intervals (Sahatpure and Patil, 2008), although the fertility rate with the usage of prostaglandins is lower (Sales *et al*., 2011); likewise, the percentage of estrus were higher than the results reported by Ramana *et al*. (2013) who obtained 82% of estrus, when two injections of PGF2α were applied. PGF2α is effective when supplied between days 8 and 17 of the estrous cycle and when there is a functional *corpus luteum* in one of the two ovaries (Dejanette and Marshall, 2003). Using 35 mg of PGF2α after the application of GnRH (Pursley *et al*., 1997) can cause luteolysis, whereas using PGF2α in cyclic cows one day before and on the day of progestin insertion, reported no difference ($p > 0.05$) in the conception rate (Xu and Burton, 2000). At the same time, two PGF2α applications in Zebu cattle modify the conception rate by 67% (Ramana *et al*., 2013). The results in the present study show that in cows with the presence of a *corpus luteum* greater than 12 mm, two prostaglandins application doses are not necessary to increase the conception percentage; however, when the day of the estrous cycle is unknown, two PGF2α injections can be applied at 14 days intervals to increase the number of individuals in estrus.

The placing of the Norgestomet implant on day 7 or 14 of the estrous cycle had no influence the number of cows that showed estrus, however, it did modify the time of estrus onset, were 81.8% of the cows that were implanted on day 7 of their cycle showed estrus 24 to 36 h after the progestin withdrawal. In another study in Zebu cows implanted with Norgestomet, the estrus percentage was of 78 to 100%, with heats happening at 84.3% of the individuals 72 h after the removal of the implant (Singh *et al*., 1998). The application of PGF2α 48 h before withdrawing the progestogen also reports an increase in the pregnancy and delivery rate (Mialot *et al*., 1998). Due the fact that the conception percentage increased when inserting the progestogen on day 14 of the estrous cycle. The application of the eCG hormone was tested upon withdrawal of the progestin, reporting that the estrus presence did not change ($p > 0.05$). However, the eCG application when the progestin was withdrawn succeeded in synchronizing 90.9% of the cows between 24 and 36 h after withdrawal. Likewise, the percentage of conception increased from 54.5 to 81.8% with the application of eCG upon withdrawal of the progestogens. eCG hormone should be used when removing the implant (Baruselli *et al*., 2004) because it improves the ovulatory and conception rates (Duffy *et al*., 2004). In another study, on Zebu × Holstein cows treated with a Norgestomet implant for 9 days plus 500 IU of eCG, the presence of estrus during the first 26 h after the end of hormonal treatment was observed, and the conception rate was 61.5% (Soto *et al*., 2002), which is lower than the 81.8% found in the present study. In *Bos indicus* cows, the administration of 300 IU of eCG at the time of progestin withdrawal increased the conception rate by 76.6% compared to other hormonal protocols (Campos *et al*., 2013). Likewise, the results of the present study were superior to those reported in another research where the conception rate of zebu cows was 55.7% when 400 IU of eCG was supplied at the progestogen withdrawn (Villa *et al*., 2007); however, the higher conception percentage found in the present experiment could be because cycling cows were used. The administration of eCG at the end of the treatment with progestogens allows to advance and synchronize the estrus, so its usage is recommended to improve the pregnancy rate, because it increases the diameter of the dominant follicle and the concentration of progesterone (Dorneles *et al*., 2013).

CONCLUSIONS

Ovaries with active *corpus luteum* do not require two injections of PGF2α, while the use of Norgestomet in Zebu × Brown Swiss cycling cows is recommended to be applied on day 14 of the estrous cycle, but when greater estrus synchronization is required, the use of eCG is desirable at withdraw the progestogen.

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