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Pregnancy Rate in Anestrous *Bos taurus* x *Bos indicus* Crossbred Cows with Low Body Condition after Treatment with a Cidr Insert, Estradiol and Synthetic Progesterone in a Tropical Environment

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Abstract: The effect of treatment with natural and synthetic progesterone and estradiol on pregnancy rate in anestrous and lactating *Bos taurus* x *Bos indicus* crossbred cows with low body condition in a tropical environment was determined. On day 0, 107 anestrous cows were assigned randomly to five treatments; CIDR+ECP+P4 (n = 24), 1.9 g natural progesterone contained in a vaginal insert (CIDR), left 9 days *in situ* + i.m. 2 mg Estradiol Cypionate (ECP) and 100 mg synthetic Progesterone (P4); CIDR+ECP (n = 22): CIDR + i.m. 2 mg ECP; CIDR+EB+P4 (n = 20), CIDR + i.m. 10 mg Estradiol Benzoate (EB) and 100 mg P4; CIDR+EB (n = 21), CIDR + i.m. 10 mg EB and control (n = 20) i.m. 5 mL saline solution. The CIDR-treated cows received artificial insemination 48-56 h after CIDR removal and the control cows at 12 h after detected estrus. Pregnancy rate was 20.8% for CIDR+ECP+P4, 22.7% for CIDR+ECP, 10% for CIDR+EB+P4, 9.5% for CIDR+EB and 0% for the control group (p>0.05). Treatments used in this study resulted in similar pregnancy rates in anestrous *Bos taurus* x *Bos indicus* crossbred cows with low body condition.

Key words: Anestrous, *Bos taurus* x *Bos indicus*, CIDR, estradiol, progesterone, Mexico

INTRODUCTION

In *Bos taurus* x *Bos indicus* crossbred cows from tropical regions, the prolonged postpartum anestrus is the main limitation to improve reproductive efficiency (Ahuja and Montiel, 2005). These cattle are managed under extensive grazing and continuous suckling, making under-nutrition and suckling major factors contributing to delay the resumption of postpartum estrous cycles (Stevenson *et al*., 1994; Jolly *et al*., 1996). In addition, the short luteal phase that usually occurs following the 1st ovulation postpartum (Werth *et al*., 1996; Sa Filho *et al*., 2006) can further delay the interval from calving to conception and contribute to poor reproductive efficiency (Vasconcelos *et al*., 2009). Treatment with progesterone prior to 1st ovulation postpartum has reduced the occurrence of short oestrous cycles (Perry *et al*., 2004; Sa Filho *et al*., 2006).

Due to the 50% efficiency in estrus detection in *Bos taurus* x *Bos indicus* crossbred cows, the protocols that eliminate estrus detection by using fixed-Time Artificial Insemination (TAI) (Bader *et al*., 2005; Larson *et al*., 2006) are particularly attractive to be used in *Bos indicus* influenced cattle. The incorporation of an intravaginal insert impregnated with 1.9 g of progesterone (CIDR®, Controlled Internal Drug-releasing device) into TAI protocols has improved pregnancy rates in anestrous dairy (El-Zarkouny *et al*., 2004) and beef (Lamb *et al*., 2001) cows. In CIDR-treated cattle, synchronization rate is enhanced when oestrogen is administered during the progesterone period (Hanlon *et al*., 1996). The administration of oestrogen to progestogen-treated cattle suppresses the growth of the dominant follicle and induces the emergence (Bo *et al*., 2002) and synchronization (Taniguchi *et al*., 2007) of a new follicular wave. Different estradiol preparations have been used with this purpose (Stevenson *et al*., 2004; Kim *et al*., 2005; Martinez *et al*., 2005).

Cattle predominant in the Mexican tropics are *Bos taurus* x *Bos indicus* crosses which feed mainly on forages and commonly have a low body condition and long postpartum anestrus periods. Therefore, the objective of this study was to evaluate the pregnancy rate in anestrous and lactating *Bos taurus* x *Bos indicus*

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crossbred cows with low body condition induced to ovulation with a CIDR insert and estradiol with or without an injection of synthetic progesterone during the hot-dry season in a tropical environment.

MATERIALS AND METHODS

Area description: The study was conducted during the hot-dry season in five commercial herds of *Bos taurus* x *Bos indicus* crossbred cattle (with undefined percentage of *Bos taurus* and *Bos indicus*), located at Latitude 19°03'N, Longitude 96°09'W in Veracruz, Mexico at 10 m altitude with sub-humid tropical climate, mean annual temperature of 24.7°C and mean annual rainfall of 1510 mm.

Experimental animals and management: About 107 anestrous and lactating *Bos taurus* x *Bos indicus* crossbred cows at 60-120 days postpartum were used in the study. Cows averaged 59±10 months of age and 430±35 kg of weight and all were in their 3rd or 4th parity. Body Condition Score (BCS) was assessed using a 9 point scale (1 = emaciated to 9 = obese; Fox *et al*., 1992) and as a result of selection for anestrous females, BCS of the cows included in the study ranged from 2.5-4 because cows with BCS <2.5 were too thin and not suitable for an induction of ovulation program whereas cows with BCS >4 were cyclic. All cows were kept under similar management conditions being fed only by grazing in fields of *Cynodon plectostachyus*, *C. nlemfuensis*, *Digitaria decumbens*, *Brachiaria brizantha*, *B. decumbens*, *Paspalum* sp. and *Axonopus* sp. and being milked once a day in the morning. Calves were reared under a restricted suckling scheme in which the calf is allowed to suckle for 1-2 min immediately prior to morning milking to facilitate milk let-down and for 30 min of *ad libitum* suckling at midday; the rest of the day, the calf is kept separated from its mother.

Determination of anestrus status: Before the start of treatments, the anestrus status of the females included in the study was confirmed on days -9, -5 and 0 (day 0 = start of treatment) through transrectal Ultrasonography (US) to determine the ovarian structures present and through blood samples collected from the jugular vein to determine serum progesterone concentrations (solid phase radioimmunoassay; Srikandakumar *et al*., 1986). A cow was considered as anestrous when it had ovarian follicles <10 mm in diameter and no corpus luteum at each US and when serum progesterone concentrations from each of the three samples were <1 ng mL^{-1}. The US evaluations were performed at all locations by the same technician using a portable ultrasound scanner Aloka SSD 500 (Japan, Ltd.) with a 7.5 MHz transrectal transducer.

Induction of ovulation: On day 0 of the study, cows were assigned randomly to the following treatments; CIDR+ECP+P4 (n = 24), cows received 1.9 g of natural progesterone contained in a vaginal device (CIDR®, Pfizer Mexico) plus i.m. 2 mg of estradiol cypionate (ECP®, Pfizer Mexico) and 100 mg of synthetic progesterone (Progesterona®, Pfizer Mexico); CIDR+ECP (n = 22), a CIDR device plus i.m. 2 mg of estradiol cypionate; CIDR+EB+P4 (n = 20), a CIDR device plus i.m. 10 mg of estradiol benzoate (Estrol®, Loeffler Mexico) and 100 mg of synthetic progesterone; CIDR+EB (n = 21), a CIDR device plus i.m. 10 mg of estradiol benzoate and control group (n = 20) i.m. 5 mL of saline solution.

In all the treatments the CIDR device remained 9 days *in situ*. At each location, there were cows assigned to each of the five treatment groups and all of them cohabited within their herds during the study. At the time of the CIDR removal (9th day), calves of the treated and control cows were weaned for 48 h.

Estrus detection, artificial insemination and pregnancy diagnosis: In treated and control cows, estrus detection was performed twice daily (morning and afternoon) for 1 h with the aid of a teaser bull, starting 24 h after the CIDR removal until 3 days later. Estrus was defined as standing to be mounted by the bull or by another cow. All the cows that received a CIDR device were Artificially Inseminated (AI) within 48-56 h after its removal and the cows of the control group were AI 12 h after detected estrus. Pregnancy was diagnosed by US 30 days after AI.

Statistical analysis: The effect of treatment on pregnancy rate was analyzed by the Chi-square (χ^2) test using the SAS (Cody and Smith, 1991). The alpha value was set at 0.05.

RESULTS AND DISCUSSION

All the cows treated with a CIDR device received TAI whereas in the control group only three cows were detected in estrus and consequently AI 12 h later. There was no effect of treatment on pregnancy rate (p>0.05). Pregnancy rate for each of the treatment is shown in Table 1. In this study, the CIDR device was used to simulate a normal luteal phase so that follicular development and the manifestation of estrus and ovulation would occur at the CIDR removal (MacMillan and Peterson, 1993). The administration of 100 mg of synthetic progesterone at the CIDR insertion in some treatments was done to increase serum

Table 1: Pregnancy rate of anestrous and lactating *Bos taurus* x *Bos indicus* crossbred cows with poor body condition in a tropical environment induced to ovulation with natural progesterone (CIDR), Plus Estradiol Cypionate (PEC) or Estradiol Benzoate (EB) and synthetic Progesterone (P4)

Treatments	Cows artificially inseminated (n/N) (%)	Pregnant cows (n/N) (%)
CIDR+ECP+P4	24/24 (100)	5/24 (20.8)[a]
CIDR+ECP	22/22 (100)	5/22 (22.7)[a]
CIDR+EB+P4	20/20 (100)	2/20 (10.0)[a]
CIDR+EB	21/21 (100)	2/21 (9.5)[a]
Control	3/20 (15)	0/20 (0.0)[a]

[a]No statistical difference (p>0.05)

progesterone concentrations achieved by effect of the CIDR in order to suppress basal LH levels and improve follicular recruitment (Sanchez *et al*., 1995). The ECP and EB were administered to suppress circulating FSH concentrations and the growth of the largest follicles to synchronize the emergence of a new follicular wave (Caccia and Bo, 1998; Colazo *et al*., 2007). Pregnancy rates for the different treatments were low and not affected by the treatment. However although low, these results can be considered acceptable, taking into account that the cows were anestrous and with low body condition and that the study was carried out during the hot season.

Similar observations were previously made by Ahuja *et al*. (2005) in a study with anestrous *Bos taurus* x *Bos indicus* cows in poor body condition in a tropical environment. Heat stress may have contributed to the low pregnancy rate obtained since, it affects the oocyte quality during the periovulatory period increasing early embryonic losses (Hansen *et al*., 1992) and reduces pregnancy rate after TAI (Cordoba and Fricke, 2001).

Pregnancy rates obtained in the present study with the CIDR+ECP+P4 and CIDR+ECP treatments were comparable to those reported by Ahuja *et al*. (2005) in a study carried out in the Mexican tropics with similar environmental conditions in anestrous *Bos taurus* x *Bos indicus* cows with low body condition treated with GnRH and $PGF_{2\alpha}$. Pregnancy rates in the study were >11.8% reported by Diaz in cattle from the tropics but were lower than those of other studies.

To this respect, Balla *et al*. (2004) in nonlactating multiparous crossbred zebu cows receiving 2 mg EB plus a progesterone device, $PGF_{2\alpha}$ at the device removal, 1 mg EB 24 h later and TAI 28 a 32 h after EB, obtained pregnancy rates of 50.4, 48.9 and 43.2% for cows that had the device during 7, 8 or 9 days, respectively; Bo *et al*. (2004) reported pregnancy rates of 42.9 and 56.3% in lactating and non-lactating crossbred zebu cows treated with a CIDR device and 2 mg EB on day 0, plus $PGF_{2\alpha}$ at CIDR removal (day 8th) and 1 mg EB 24 h later with TAI 52-56 h after CIDR removal. In the present study, the addition of 100 mg of synthetic progesterone at CIDR insertion either with ECP or EB did not improve pregnancy rate which was coincident with the reports by Rhodes *et al*. (2001) in anestrous dairy cows and Colazo *et al*. (2004) in beef cattle. In suckled crossbred beef cows that received a CIDR device for 7 days together with 100 mg of synthetic progesterone and either 1 mg EB or 5 mg E-17β on day 0, plus $PGF_{2\alpha}$ at CIDR removal and either 1 mg EB or 1 mg E-17β 24 h after CIDR removal with TAI 54 h after CIDR withdrawal, Martinez *et al*. (2000) obtained pregnancy rates from 52-71%. Colazo *et al*. (2004) in lactating beef cows receiving a CIDR device and 1 mg EB with or without 100 mg of synthetic progesterone at CIDR insertion, plus $PGF_{2\alpha}$ at CIDR removal (day 7th) and 1 mg EB 24 h later with TAI 52-56 h after CIDR removal, reported 64.5 and 60.2% of pregnancy rate for cows that received or did not receive synthetic progesterone at CIDR insertion, respectively.

Nonetheless, it is worth mentioning that in all these studies, the cows were in good body condition, contrary to the study where the cows had a low body condition. The high concentrations of BE administered in the present study were those recommended by the manufacturer of the product for use in Mexico but they could have influenced on the low pregnancy rate since, although there was no statistical difference in the results obtained with the use of either ECP or EB, pregnancy rates for the treatments that included ECP were numerically higher than those for treatments with EB.

To this respect, MacMillan and Burke (1996) reported higher estrus manifestation rate in dairy cows treated with a CIDR device and no EB compared to cows that received a gelatin capsule with 10 mg EB attached to the CIDR device while Caccia and Bo (1998) reported a more synchronous follicular wave emergence when CIDR-treated beef cows were administered 2.5 mg EB than 5 mg EB.

CONCLUSION

In this study, treatment with different combinations of natural and synthetic progesterone with estradiol for TAI in anestrous lactating *Bos taurus* x *Bos indicus* crossbred cows with poor body condition resulted in acceptable pregnancy rates.

Nonetheless, neither the addition of synthetic progesterone to the treatment protocols nor the use of ECP or EB as the estradiol source had an effect on the results. Because of the difficulties for estrus detection in *Bos indicus* influenced cattle, the use of the protocols for TAI included in this study may provide better opportunities for achieving pregnancies than AI after detected estrus in cows with *Bos indicus* breeding in a tropical environment.

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