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Comparison of Estradiol Cypionate and Estradiol Benzoate Effects on Ovaric Activity, Estrus and Ovulation on Anestrus *Bos indicus* Cows

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Abstract: The objective was to compare the effect of Estradiol Cypionate (EC) vs. Estradiol Benzoate (EB) on follicular population, ovulation rate, percentage of animals in estrus and Estrus-Ovulation knterval (E-OI) in non-cycling *Bos indicus* cows. Three groups of eleven cows each and similar Body Condition Score (BCS) were implanted with a CIDR cattle device at day 0 and given I mg of EB and after nine days CIDR was removed. EC group (BCS = 5.1) received 0.5 mg of EC + 25 mg of $PGF_{2\alpha}$; EB group (BCS = 5.0) received 25 mg of $PGF_{2\alpha}$ and 24 h after CIDR withdrawal were given 0.5 mg de EB; Control group (CC = 5.2) received 25 mg of $PGF_{2\alpha}$ + 1 mL of saline solution. From CIDR removal day, ovary activity was monitored by ultrasonography (US) at 0, 24, 48 and 60 h and later every 12 h until ovulation. Heat detection was conducted three times a day and Corpus Luteum presence (CL) was confirmed after 13 days of estrus with US. No statistical differences ($p>0.05$) were found for any of the variables. After CIDR removal the small and medium follicle populations were the same and one large follicle per cow developed. The ovulation rate was of 73% for EC, 100% for EB and 100% for control group. The percentage of cows in heat was of 73% for EC, 82% for EB and 54% for control group. The E-OI was between 30 and 32 h. It was concluded that using EC or EB had no effect over the variables studied.

Key words: Anestrus, *Bos indicus*, CIDR, estrus, ovulation, follicle population

INTRODUCTION

In tropical countries, a high percentage of reproductive programs still rely on natural service. Over the last few years, estrus synchronization and Artificial Insemination (AI) has been used in meat and dairy cattle; however, the results concerning to fertility rate after estrus synchronization have been low especially on zebu cattle (Galina and Arthur, 1990; Ross *et al*., 2004). In general and mainly in *Bos indicus* cows, one of the major constraints to obtain good fertility rates in AI programs is the failure of adequate heat detection, due to its very short estrus periods (about 10 h and with 1.3-20 h variations) (Pinheiro *et al*., 1998), the signs of heat are less intense (Galina and Arthur, 1990) and a great proportion of cows become in estrous during the night (Membrive, 2000).

There are several products available in the market to synchronize both estrus and ovulation in cattle, such as those based on the use of GnRH and $PGF_{2\alpha}$ (Ovsynch protocol), combined with progestin (Cosynch protocol), or treatment with progesterone alone (CIDR or Crestar). At present, the method mores used for estrus synchronization is the CIDR cattle device combined with hormones such as GnRH, eCG and estradiol in different formulas (estradiol benzoate-EB; estradiol cypionate-EC; estradiol valerate-EV), with satisfactory results in ovary response and percentage of cows in heat. It also reduces the time between estrus and ovulation (Colazo *et al*., 2003; Kim *et al*., 2005).

Depending to the application time, the use of different estradiol formulas (EB, CE) in synchronizing programs with progesterone has two main functions. When is applied at the begging of the treatment its function is to suppress the largest follicles growth, leading the follicle wave to atresia and generating a new, more synchronized follicular wave, generally, at 5 days upon the application of EB (Bo *et al*., 1996) and at 4 days upon application of EC (Colazo *et al*., 2007). The second function of estradiol (EB, EC) serves at the moment of progesterone withdrawal. When estradiol is administered at this moment, it has a direct effect over Luteinizing Hormone (LH), arousing the preovulatory peak of LH (Lammoglia *et al*., 1998), increasing thus the percentage of ovulating cows (Martínez *et al*., 2005) and higher proportion of cows and heifers in heat (86-100%) can also be observed. The estrus-ovulation time is also synchronized and time to ovulation is reduced

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(Taniguchi *et al*., 2007), allowing to set a fixed time for insemination (AIFT) in order to increase fertility, when estrus is synchronized (Diskin *et al*., 2002).

BE is the most frequently used hormone for synchronizing and inducing heat. However, it has the disadvantage of being administered 24 h after progesterone withdrawal, which requires additional management, forcing cows to pass four times through the handling chute in an AI Fixed Time protocol (AIFT). The later has led to search for alternatives that reduce the handling of animals; one of them is the use of EC at the progesterone (CIDR) removal time. However, research on this field is scarce and more information is required, especially on zebu cattle under tropical conditions.

The objective of this research is to compare the effect of EC vs. BE over the follicle population, ovulation rate, percentage of animals in heat and estrus-ovulation interval in non estrus *Bos indicus* cows under tropical conditions.

MATERIALS AND METHODS

The experiment was carried out in Tabasco state sited in the humid tropic region of Mexico, located between 18°20'North parallels and 93°15'West with an altitude of 10 m above sea level. The weather is hot and humid with abundant summer rainfalls (Aw1). The annual mean temperature is 33.6°C and the annual mean rainfall is 2,237 mm (Enciclopedia de los Municipios de México, 2005). A total of 33 *Bos indicus* cows were used. The cows were multiparous (4-5 calvings) non-lactating, anestrous (no corpus luteum detected through palpation), with a body condition of 5.09±0.88, in a 1-9 scale (where 1 is emaciated and 9 is obese) (Ayala *et al*., 1990) and from 6-8 months from last calving. Animals were kept in grazing conditions 24 h a day with African Star Grass (*Cynodon nlemfuensis*) and receiving nutritional supplement with one kg of commercial balanced meal (14% crude protein). The following groups were arranged:

Treatment I: Estradiol cypionate group (EC; n = 11), this group of cows had 5.09±1.13 CC. A CIDR® cattle insert was implanted (Pfizer Labs, New Zeland) along with 1 mg of estradiol benzoate (Syntex Labs., Argentina) on the day 0; upon CIDR removal (day 9), 25 mg of PGF2α (Lutalyse®, Pharmacia and Upjohn, USA) and 0.5 mg EC (ECP®, Pharmacia and Upjohn, USA); were both administered intramuscularly (Colazo *et al*., 2003).

Treatment II: Estradiol benzoate group (EB; n = 11), this group of cows had 5.00±0.63 CC. A CIDR was implanted along with 1 mg EB (day 0); upon insert withdrawal (day 9) 25 mg of PGF2α was injected and 24 h later, 0.5 mg EB was administered in the same way (Zavaleta *et al*., 2006).

Treatment III: Control group (n = 11) this group of cows presented 5.18±0.87 CC. A CIDR was implanted along with 1 mg EB (day 0); upon insert withdrawal (day 9) 25 mg of PGF2α was injected with 1 mL of saline solution, both intramuscularly.

Follicular activity was monitored by rectal palpation using a real time ultrasound scanning (Pie Medical, Falcon 100, with a 6/8 MHz transducer) upon insert removal (0 h) and as 24, 48 and 60 h after withdrawal. Afterwards, follicular development was followed every 12 h to determine ovulation time (vanishing of the largest diameter follicle). Number and diameter of follicles was recorded on an ovary map. From these values, maximum follicular diameter prior to ovulation was obtained (Colazo *et al*., 2003). Follicular population was estimated according to Bo *et al*. (2003) classification. Small: ≤4 mm; Medium: 4.1-8 mm; Large: >8 mm.

Estrus was detected trough visual observation three times a day (06:00, 12:00 and 18:00 h) with 1 h observation periods, starting 24 h after CIDR removal and ending five days later.

Corpus Luteum (CL) presence was detected with ultrasound scanning 13 days after observing estrus for all cows in treatment.

Statistical analysis: The effects of treatments over the time from CIDR removal to ovulation, time of estrus occurrence to ovulation and follicular diameter were analyzed with ANOVA (SAS, 2002). The percentage of cows in estrus and ovulating cows were analyzed by Chi-square. Follicular population from CIDR removal to 60 h later and dominant follicle growth was analyzed by a GLM procedure with repeated measures, which included treatment effects, day and interaction of both and experimental error.

RESULTS AND DISCUSSION

Follicle population: It can be observed that 24 h after CIDR removal there were around 55% of small follicles (8-9) and 38% of medium follicles (5-6), whereas the large follicle population averaged 7% (one per cow) (Table 1). At 48 h, the follicle population was the same for small and medium follicles (46 and 47%, respectively), whereas large follicles remained one per cow. At the end of the observations (60 h after CIDR removal) the follicle population was the same (6-7 follicles) with 46% for small follicles and 47% for the large ones. The large follicle

Table 1: Mean±SD values of follicle populations from 24-60 h after CIDR removal

	EC (n = 11)			EB (n = 11)			Control (n = 11)		
Time (h)	≤4	4.1-7.9	>8	≤4	4.1-7.9	>8	≤4	4.1-7.9	>8
24	9.18 (3.71)[a]	5.82 (3.09)[a]	1 (0.63)[a]	7.90 (3.24)[a]	5.36 (2.66)[a]	1.09 (0.30)[a]	8 (3.61)[a]	5.82 (3.68)[a]	1.27 (0.79)[a]
48	7.45 (3.01)[a]	7.18 (2.86)[a]	1 (0.45)[a]	5.82 (2.71)[a]	6.73 (3.00)[a]	1.09 (0.30)[a]	7.91 (2.88)[a]	6.64 (4.10)[a]	1.09 (0.30)[a]
60	8.09 (3.83)[a]	7 (3.90)[a]	0.91 (0.83)[a]	8.27 (2.65)[a]	7.91 (4.08)[a]	0.91 (0.30)[a]	6.64 (4.70)[a]	8.54 (4.95)[a]	0.91 (0.30)[a]

Different letters between the lines indicate statistical differences (p<0.05). Numbers in parentheses are the standard deviations

populations began to decrease because as some cows had already ovulated (p>0.05). The characteristics on the largest diameter follicles are the same as those observed by Ginther *et al.* (1997), Martínez *et al.* (2000a) and Calvalho *et al.* (2008) after CIDR removal. The reduction of the small follicle population and the presence of one large follicle per cow after CIDR removal are mainly caused by the decrease in exogenous progesterone thus increasing the estrogen levels, triggering the LH release and causing the dominant follicle to suppress the other follicles growth leading them to atresia.

Dominant follicle growth and maximum follicle diameter: Figure 1 shows the dominant follicle growth (mm) at the moment of CIDR removal to 60 h after. There is an increase in the size of the dominant follicle from 0 to 24-48 h, reaching at this point its maximum development, which was between 11-12 mm until ovulation. This growth was similar for all groups (p>0.05). The maximum follicle diameter observed before ovulation were similar for the three groups, averaging 1.26±0.18 mm for EC group, 1.25±0.19 mm for EB group and 1.20±0.19 mm for control group (p>0.05). These results are similar to those reported by Burke *et al.* (2001) and Calvalho *et al.* (2008), who worked with *Bos indicus* cows and found moments before ovulation, the follicles reached average sizes >11 mm diameter. The LSD agrees with results by Figueiredo *et al.* (1997) which range from 11.3-12.05 mm in *Bos indicus* cows. Both results are independent and treatments don't modify growth and follicle diameters.

Percentage of estrous cows and CIDR removal to estrus interval: From the total of cows in the experiment, 70% (23/33) of them were observed in estrus; from this percentage, 87% (20/23) was observed between 36-48 h after CDRI removal, whereas the rest of the cows became estrous between 54-72 h after removal. The percentage of estrous cows observed was low compared to results by Martínez *et al.* (2000b) and Garcia and Jarnette (2003), who reported 87 and 93.3% of estrous cows, respectively. Similarly, Lammoglia *et al.* (1998) established that the percentage of cows in estrus with CIDR based synchronization protocols vary between 86 and 100%.

Table 2 shows that the percentage of cows in heat was higher for EB and EC groups compared to control

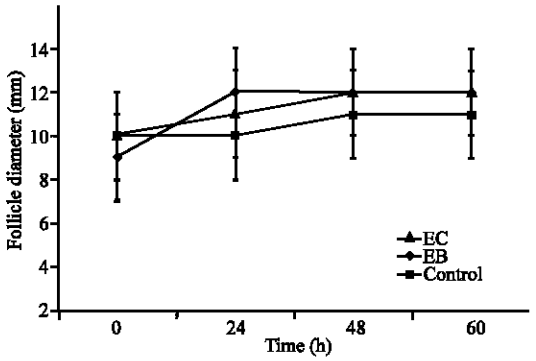

Fig. 1: Dominant follicle growth (mm) from the time of CIDR removal to 60 h later

Table 2: Percentage of estrous cows, CIDR removal to estrus interval, ovulation rate, CIDR removal to ovulation interval and beginning of estrus to ovulation interval

Parameters	CE	BE	Control
Number of cows	11	11	11
Estrous cows (%)	73[a]	82[a]	54[a]
CIDR removal to estrus (h)	43±8.33[a]	43±6.32[a]	50±11.80[a]
Cows that ovulated (%)	73[a]	100[a]	100[a]
CIDR removal to ovulation (h)	70±7.69[a]	73±6.47[a]	88±22.30[a]
Beginning of estrus to ovulation (h)	30±6.0[a]	31±8.71[a]	32±12.39[a]

Different letters between the lines indicate statistical differences (p<0.05)

group (p>0.05). No previous works comparing these two treatments have been found, so the results were compared with studies where one or another treatment alone (EC or EB) was used. García and De Jarnette (2003) reported that Angus and F1 heifers treated with EC showed similar results to those of control group (93.3 vs. 95.6%); these results differ to those found on the present work. Martínez *et al.* (2000b) found significant differences in F1 heifers treated with EB compared to the control group (100 vs. 83%), the EB treatment result is similar to the one on this study but different to the observed on the control group. The average time for the beginning of estrus was similar for all treatments and ranged from 43-50 h after CIDR removal (p>0.05). García and De Jarnette (2003) observed that estrus in heifers treated with EC began after 51.4 h compared to 48.8 h on the control group, which was similar to the observed on the present research. Lemaster *et al.* (1999) observed in Brahman heifers treated with EB that the estrus began after 45.5 h compared to 59.2 h on the control group; which was similar to the observed on the present study. The absence of differences between the two treatments indicate that the

use of EC or EB don't affect the CIDR removal to estrus interval. These results are contradictory because it was expected that the use of estradiol would reduce the time for the beginning of estrus, since one of the reported effects of estradiol is to induce heat (Sumano and Ocampo, 1997). There was a reduction in time but not significant, which could be explained by the number of animals used on the experiment.

Ovulation percentage and estrus-ovulation interval: The ovulation percentage was of 91%. In the EC treated group 73% of the cows ovulated 70 h after CIDR removal, whereas in EB and control groups 100% of the cows ovulated at 73 and 88 h, respectively ($p>0.05$). The general average of ovulation was similar to the observed by Martínez *et al.* (2005) in Hereford cows using EB and 17β estradiol. Stevenson *et al.* (2004) obtained 79 vs. 91% of ovulation using EC, compared to a control group. These results are similar to the ones of the present study. Ambrose *et al.* (2005) stated that the use of EC reduces the time to ovulation after CDRI removal in an average of 66 h. Martínez *et al.* (2005) obtained a 100% ovulation rate in Hereford cows using EB, which was similar to the results obtained on the present research. The time to ovulation is similar to those reported by Bo *et al.* (2003) and Martínez *et al.* (2005), which averages 72 h. Lemaster *et al.* (1999) reported that time to ovulation with EB was of 74.5 vs. 93.5 h of a control group, in accordance to present research. There were no significant differences between treatments regarding time to ovulation after CIDR removal however, there is a tendency to reduce the time (15 h) in groups treated with EC and EB. This reduction could be related to the use of estradiol which exerts a positive feedback effect on the hypothalamus and the pituitary gland, triggering the increase of the pulse and frequency of LH (Lammoglia *et al.*, 1998).

Regarding the estrus-ovulation interval, the results obtained were similar for the three groups and range from 30-32 h (Table 2). Stevenson *et al.* (2004) observed that the ovulation time using EC and a control group was of 30 and 28 h, respectively for Holstein cows. Pancarci *et al.* (2002) stated that this event occurred at 27.5 h. Lemaster *et al.* (1999) observed that the time from the heat to ovulation on Brahman heifers using 0.5 mg of EB was of 29.3 h as opposed to 26.1 h on a control group. The interval observed on the present study was constant, as also found in other studies. This is explained by Larsson (1987), who states that once the heat begins there is a preovulatory peak of LH which keeps a constant relation to the time of ovulation, which normally happens between 26 and 36 h after the LH peak. Gustafsson *et al.* (1986) reported that there could be a variation in the time from the beginning of the estrus to the LH peak which begins within the first 6 h of the estrus, which might explain the slight variation between the groups.

CONCLUSION

The use of estradiol cypionate or estradiol benzoate didn't have a significant effect on the studied variables.

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