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# INDUCTION OF OVULATION WITH GnRH AND $PGF_{2\alpha}$ IN LACTATING *BOS TAURUS* × *BOS INDICUS* COWS

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Induction of ovulation for timed artificial insemination (TAI) with the Ovsynch protocol was evaluated in 49 anoestrous and lactating *Bos taurus* × *Bos indicus* cows. Palpation per rectum and transrectal ultrasonography were used on Days –30, –20, –10 and 0 (start of treatment) to confirm anoestrus but with the presence of follicles ≥ 10 mm, and every other day during treatment to determine ovarian activity. Cows were randomly assigned to: (1) Ovsynch (n = 24; Day 0, 200 µg GnRH; Day 7, 150 µg $PGF_{2\alpha}$; Day 9, 200 µg GnRH + TAI 16 to 20 h later) and (2) control (n = 25; no treatment). Rates of ovulation for the first GnRH injection, detection of a corpus luteum (CL) at $PGF_{2\alpha}$ injection, pregnancy and induction of cyclicity were greater ($P < 0.05$) with Ovsynch. There was no effect of body condition score ($P > 0.05$). In conclusion, the Ovsynch protocol was not effective in obtaining acceptable pregnancy rate for TAI, but it was effective for induction of cyclicity in anoestrous and lactating *Bos taurus* × *Bos indicus* cows under tropical conditions.

**Key words:** Cattle, GnRH, induction of ovulation, Ovsynch, timed artificial insemination

Tropical cattle show poor reproductive efficiency. In the Mexican tropic, the interval from calving to first oestrus averages 78 ± 34.6 days, the interval from calving to conception 149 ± 46.1 days, and the intercalving period 447 ± 57.8 days, with only 3.4 calvings throughout the productive life of the cow (Anta et al., 1989). Furthermore, oestrus detection is particularly difficult in *Bos indicus* breeds, because of their peculiar behavioural traits (Gutierrez et al., 1993) and the extensive management system (Vaca et al., 1985), limiting the spread of AI in the tropics.

With the aim to reduce the dependence on oestrus detection for improving reproductive efficiency in lactating dairy cows, TAI was combined with a protocol for synchronisation of ovulation called Ovsynch. The Ovsynch (injections of GnRH given 7 days before and 48 h after $PGF_{2\alpha}$ injection and AI 16 to 20 h after

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the second GnRH) synchronises follicle maturation with luteal regression before GnRH-induced ovulation, which occurs 24 to 32 h after the second GnRH injection, allowing TAI without the need for oestrus detection (Pursley et al., 1995). Conception rates are not different when dairy (Burke et al., 1996; Pursley et al., 1997) or beef (Geary et al., 1998*a*) cows are artificially inseminated according to detected oestrus versus TAI with the Ovsynch protocol.

The use of Ovsynch in lactating dairy cows has been widely documented, but there is little information about its use in tropical cattle. Therefore, the objective of this study was to evaluate the effectiveness of the Ovsynch protocol in the induction of ovulation for TAI in anoestrous and lactating *Bos taurus* × *Bos indicus* cows in commercial herds from the Mexican humid tropic.

## Materials and methods

*Study characteristics and location*

This study was carried out from March to November 2002 in four *Bos taurus* × *Bos indicus* commercial herds, three located at 19º 03´ N. Lat., 96º 09´ W. Long., and one at 19º 03´ N. Lat., 96º 14´ W. Long., in Veracruz State, Mexico, at 10 m altitude, with sub-humid tropical climate, annual temperature 24.7 to 25.5 °C and annual rainfall 1510.6 to 1677 mm (García, 1981).

*Experimental animals*

Forty-nine anoestrous and lactating *Bos taurus* × *Bos indicus* multiparous cows at 60 to 120 days postpartum and with ovarian follicles ≥ 10 mm in diameter were selected. Cows averaged 48 ± 7 months of age and weighed 410 ± 57.9 kg. At the beginning of the study, body condition score (BCS) was assessed using a scale of 1 to 5 (1 = thin, 5 = obese; Edmonson et al., 1989). Cows included in the study ranged from BCS 1.5 to 2.5. All cows grazed in fields of *Cynodon plectostachyus, Digitaria decumbens, Hyparrhenia rufa, Panicum maximum, Brachiaria brizantha, Paspalum* spp. and *Axonopus* spp. Calves were reared under a restricted suckling management program and cows were milked once a day in the morning.

*Determination of ovarian activity*

Every 10 days, from Day –30 until Day 0 (start of treatment), palpation per rectum (PR) and transrectal ultrasonography (US) were performed on all cows (n = 49) to confirm anoestrus but with the presence of ovarian follicles ≥ 10 mm in diameter. Thereafter, in order to determine ovarian activity and to detect a corpus luteum (CL), PR and US were also performed every other day during the treatment, including the days of hormone injections (n = 41). A portable ultrasound scanner Aloka SSD 500 (Japan Ltd.) with a 5.0 MHz transrectal transducer was used. Ovulation and cyclicity induction were determined when a CL was found by PR and US.

*Induction of ovulation*

Treatment was initiated on Day 0 when all cows (n = 49) showed ovarian follicles ≥ 10 mm. Cows were randomly assigned to the Ovsynch protocol (n = 24), receiving 200 µg of GnRH i.m. (Gonadorelin, Fertagyl, Lab. Intervet Mexico) (Day 0), followed by 150 µg of $PGF_{2\alpha}$ i.m. (D-Cloprostenol, Prosolvin C, Lab. Intervet Mexico) 7 days later (Day 7) and by 200 µg of GnRH 48 h later (Day 9), with TAI 16 to 20 h after the second GnRH. The remaining cows (n = 25) were control group with no treatment, being observed for oestrus continuously from Day 0 until Day 30 post-AI of Ovsynch cows, and receiving AI 12 h after detected oestrus. All cows were artificially inseminated by the same inseminator. After each GnRH injection, calves of Ovsynch and control cows were removed for 48 h following recommendations of the hormone manufacturer. Pregnancy was diagnosed by US 30 days after AI.

*Statistical analyses*

Ovarian structures and rates of ovulation, pregnancy and cyclicity induction were statistically analysed by the chi-squared and the Fisher exact tests, available in the statistical package SAS (Gody and Smith, 1991).

## Results

*Ovarian structures*

On the day of the first GnRH injection all cows showed follicles ≥ 10 mm ($P > 0.05$) (Table 1). Thereafter, ovarian structures were US scanned in 16 Ovsynch and in all control cows. On the day of $PGF_{2\alpha}$ injection 100% of Ovsynch and 0% of control cows had a CL ($P < 0.05$) (Table 1), and 50% of Ovsynch cows showed a dominant follicle, too ($P < 0.05$) (Table 1). $PGF_{2\alpha}$ induced luteolysis in 100% of the Ovsynch cows ($P > 0.05$). On the day of the second GnRH injection, follicles ≥ 10 and < 10 mm were detected in 31.2 and 68.8% of Ovsynch cows, respectively, and in 0 and 100% of control cows, respectively ($P < 0.05$) (Table 1). No effect of BCS was found in any case ($P > 0.05$).

**Table 1**

Ovarian structures ultrasonographically detected during induction of ovulation with the Ovsynch protocol in anoestrous *Bos taurus* × *Bos indicus* cows

| Treatment | Day 0: 1st GnRH injection | Day 7: $PGF_{2\alpha}$ injection | | Day 9: 2nd GnRH injection | |
|---|---|---|---|---|---|
| | Follicle ≥ 10 mm | Corpus luteum | Large follicle ≥ 10 mm | Follicle ≥ 10 mm | Follicle < 10 mm |
| Ovsynch (%) | 24/24 (100)[a] | 16/16 (100)[a] | 8/16 (50)[a] | 5/16 (31.2)[a] | 11/16 (68.8)[a] |
| Control (%) | 25/25 (100)[a] | 0/25 (0)[b] | 0/25 (0)[b] | 0/25 (0)[b] | 25/25 (100)[b] |

Data are presented as cows that showed such structures/number of cows (%); [a, b]Columns with different superscripts differ significantly (P < 0.05)

## *Ovulation rate*

Ovulation rate was determined only after the first GnRH injection. This was done by US in the same cows in which ovarian structures were scanned after the first GnRH. The synchronised ovulation rate (after the second GnRH injection) was not determined in order to avoid manipulation of the genital tract after AI. Ovulation rate was 100% for Ovsynch and 0% for control cows (P < 0.05) (Table 2). Within Ovsynch, the ovulation rate of the largest follicle (≥ 10 mm) present on the day treatment was initiated, was higher than that of any other largest follicle (P < 0.05; Table 2). There was no effect of BCS in any case (P > 0.05).

**Table 2**

Ovulation rates for the first GnRH injection and of the largest follicle present at the start of treatment in anoestrous *Bos taurus* × *Bos indicus* cows receiving the Ovsynch protocol

| Treatment | Ovulation rate | | |
|---|---|---|---|
| | Overall (%) | Largest follicle present at the start of treatment (%) | Any other largest follicle (%) |
| Ovsynch | 16/16 (100)[a] | 15/16 (93.8)[c] | 1/16 (6.2)[e] |
| Control | 0/25 (0)[b] | 0/25 (0)[d] | 0/25 (0)[d] |

Data are presented as cows that ovulated/number of cows (%); [a, b]Different superscripts mean statistical difference (P < 0.05); [c, d, e]Different superscripts mean statistical difference (P < 0.05)

## *Pregnancy rate*

Pregnancy rate for the first AI service was 20.8% for Ovsynch and 0% for the control group (P < 0.05). (Table 3). BCS did not affect the number of pregnant cows for Ovsynch (P > 0.05).

**Table 3**

Pregnancy rate for the first artificial insemination service in anoestrous *Bos taurus* × *Bos indicus* cows treated with the Ovsynch protocol

| Treatment | Cows inseminated (%) | Pregnant cows (%) |
|---|---|---|
| Ovsynch | 24/24 (100) | 5/24 (20.8)$^a$ |
| Control | 0/25 (0) | 0/25 (0)$^b$ |

Data are shown as pregnant cows/number of cows (%); $^{a,\ b}$Different superscripts mean statistically significant difference ($P < 0.05$)

*Cyclicity induction rate*

The cyclicity induction rate based on the presence of a CL by US scanning was 62.5% for Ovsynch and 0% for the control group ($P < 0.05$) (Table 4). BCS did not affect the number of induced cows for Ovsynch ($P > 0.05$).

**Table 4**

Cyclicity induction rate with the Ovsynch protocol in anoestrous *Bos taurus* × *Bos indicus* cows

| Treatment | Induced cows (%) |
|---|---|
| Ovsynch | 15/24 (62.5)$^a$ |
| Control | 0/25 (0)$^b$ |

Data are shown as induced cows/number of cows (%); $^{a,\ b}$Different superscripts mean statistically significant difference ($P < 0.05$)

## Discussion

In this study, rates of ovulation for the first GnRH injection and induction of a CL in the Ovsynch cows were similar to those reported for cyclic beef cows synchronised with 8 µg buserelin on Day 0 (Twagiramungu et al., 1994). Likewise, ovulation rate of the largest follicle present on the day when treatment was started, was higher than the 85% value described for dairy cows in an earlier report by Pursley et al. (1995). In the present study, the proportion of cows with a dominant follicle, besides a CL on the day of $PGF_{2\alpha}$ injection, was lower than the 83.3% value reported in cyclic Nelore cows (Barros et al., 2000), and the luteolysis induction rate following $PGF_{2\alpha}$ was comparable to the 93% rate previously reported for dairy cows (Vasconcelos et al., 1999). Although it has been demonstrated that suckling, BCS and heat stress affect ovarian activity, the results obtained in the present trial were acceptable, despite the fact that the cows were suckling their calves, had a low BCS and experienced heat stress due to high ambient temperatures. Although heat stress diminishes particularly the number of small follicles (Wolfenson et al., 1995) and delays luteolysis in cows (Wilson et al., 1998), this was not observed in the present study; on the contrary, $PGF_{2\alpha}$ induced luteolysis in all cows that had a CL, and the follicles that consequently developed were small (3 to 5 mm) but great in number.

In the present study, pregnancy rate for the first AI service in the Ovsynch cows was lower than in previous studies using 100 µg GnRH per injection, such as 42.4% in $F_1$ Brahman × Hereford cows (Williams et al., 2002), 59% in cyclic Angus cows (Geary et al., 1998*b*), 31% in suckled beef cows (Thompson et al., 1999) and 35% in anoestrous dairy cows during the hot season (Cartmill et al., 2001). However, pregnancy rate in the present study was higher than in two previous studies using 8 µg buserelin, such as 14.9% in anoestrous and lactating Nelore cows (Fernandes et al., 2001), and 14% in dairy cows during the hot season (De la Sota et al., 1998), and was comparable to the value (22%) reported for dairy cows during the hot season (Burke et al., 1996). In lactating dairy cows receiving 200 µg GnRH per injection, pregnancy rate was 34% (Gábor et al., 2002). The pregnancy rate obtained in the present study was unacceptable when trying to get a large percentage of cows pregnant with TAI during the breeding season. Several factors could be responsible for this low pregnancy rate, including genotype and BCS of the females, calf presence and the season of the year. In this respect, tropical cattle show an overall poor reproductive efficiency (Madalena and Hinojosa, 1976), and early weaning (2 to 3 months of age) increases pregnancy rates in some Zebu-cross herds (Schlink et al., 1994). Although in the present study BCS did not affect the number of pregnant Ovsynch cows, it can be mentioned that their BCS was low. In grazing *Bos indicus* and *Bos taurus* × *Bos indicus* cows, pregnancy rate was 0, 3 and 11% in cows with BCS 1, 2 and 3 (on a scale of 1 to 9), respectively, and 85% in cows with BCS 7, 8 and 9 (Martínez and Castillo, 1995). The low pregnancy rate obtained in the present study during the hot months (March to August) could be a result of embryonic loss due to high ambient temperatures, as heat stress affects the ovum quality during the periovulatory period and increases early embryonic losses (Hansen et al., 1992). Likewise, high ambient temperatures after TAI and throughout the breeding period can affect the overall pregnancy rate in grazing dairy cows (Cordoba and Fricke, 2001). As about 40 days are required for the bovine follicles to grow from the antral stage (Lussier et al., 1983), when small follicles are damaged by heat stress during the summer, they may ovulate an infertile ovum or develop a subfunctional CL at the end of summer or during autumn (Howell et al., 1994). This could explain the low pregnancy rate obtained at the end of summer or during autumn (September to November) in the present study.

Most of the studies concerning synchronisation of ovulation with GnRH + $PGF_{2\alpha}$ have been carried out in cyclic cows, with little information available about induction of cyclicity in anoestrous cows. However, an acceptable induction rate was obtained in this study with the Ovsynch protocol.

In conclusion, the Ovsynch protocol was not effective in obtaining acceptable pregnancy rate for TAI, but it was effective for induction of cyclicity. Therefore, further research is needed to evaluate the response in ovulation, pregnancy and cyclicity induction rates that could be obtained with the Ovsynch protocol in anoestrous and cyclic *Bos taurus* × *Bos indicus* cows, to learn if this protocol is suitable to be used for induction and synchronisation of ovulation for TAI in tropical cattle, as most of the research has been carried out in European cattle.

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