

## Use of oxytocin on luteal function and fertility of beef cows

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### Abstract

The aim of this study was to evaluate the application of oxytocin (OT) on the daily serum progesterone (P<sub>4</sub>) levels, and fertility of estrus synchronized Angus x Hereford cows. The cows in estrus were mated with bulls and assigned to the following treatments: 20 IU/d of OT (n=7, 10 IU a.m., 10 IU p.m.), saline solution (SS, n=7) and no management (NM, n=6). The cows with OT and SS were flushed on day 16 post-mating and no embryos were recovered. The corpus luteum (CL) diameters and P<sub>4</sub> levels were similar in OT and SS treatments in the early, intermediate and late luteal phases. The fertility (confirmed on day 35) in cows of NM (5/6) was better (P<0.01) than that of OT and SS treatments (0/14). The P<sub>4</sub> levels were compared for the OT, SS and NM groups on days 5, 10 and 15 post-mating. They were not different (P>0.05) on day 5 (0.80±0.6, 1.21±0.6 and 1.52±0.7 ng/mL for OT, SS and NM, respectively), but they were higher in the NM group (P<0.01) on days 10 (4.90±0.6, 5.05±0.6 and 7.10±0.7 ng/mL for OT, SS and NM, respectively) and 15 (6.10±0.7, 5.97±0.6 and 9.20±0.7 ng/mL for OT, SS and NM, respectively). It is possible that the daily handling caused stress and the biochemical messengers associated with the use and application of OT were responsible for changes in the serum P<sub>4</sub> levels and in the uterine environment, and interrupted the embryo's viability in the early or intermediate luteal phases, before it had sent out an antiluteolytic signal.

**Keywords:** Fertility, Oxytocin, Progesterone, Luteal Phase

### Introduction

The establishment of gestation involves the maternal recognition of gestation, implantation and homeostatic equilibrium. Reproductive success is insured by factors that include the uterine environment favoured by adequate levels of P<sub>4</sub> and the anti-luteolytic signals generated by the embryo (Spencer and Bazer, 2004).

The growth and development of the conceptus (embryo, fetus and associated embryonic membranes) in mammals require the action of the P<sub>4</sub> and placental hormones that regulate the differentiation and endometrial function in the uterus, as well as signals of the maternal recognition of gestation, uterine receptivity for the implantation of the blastocist, and the uterus-conceptus interaction (Carson et al., 2000; Paria et al., 2000; Gray et al., 2001). Thus, the hormones of the

conceptus act in the uterus in a paracrine manner to establish and maintain gestation.

In many mammals, production of P<sub>4</sub> by the CL is required for gestation to continue. The P<sub>4</sub> acts in the uterus to stimulate and maintain the uterine function that is necessary for the early development of the embryo, the implantation, placental growth and successful fetal development up to the end of gestation (Spencer and Bazer, 2002; Spencer and Bazer, 2004).

Dual purpose systems in the Mexican tropics have suffered failures in the area of reproductive behaviour through estrous repetition, and in some cases early abortions (Luna et al., 2007). They added that this is possibly due to a decrease in the level of P<sub>4</sub> during the intermediate luteal stage associated with the application of OT to milk ejection, or to stress factors generated by the change from manual or mechanical milking.

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These Changes in the daily management cows may cause acute or chronic stress, and generate responses that alter growth, reproductive functions or even cause death (Friend, 1991; Broom, 1993). Reproduction is directly at risk when important levels of stress are present. Although evidence is not yet sufficient, the effect of stress has been related to the physiologic and hormonal responses that result in reproductive events. This indicates that folliculogenesis, ovulation and embryo implantation are the reproductive stages that are most vulnerable and at risk under conditions of stress (Moberg, 1985; Smith et al., 2003).

The aim of this study was to evaluate the daily morning and evening application of 10 IU/day of oxytocin in the serum P<sub>4</sub> levels and fertility in cows.

## Materials and Methods

The study was carried in the Teseachic ranch of the Universidad Autonoma de Chihuahua, located at the longitude 107° 26' 00" W, the latitude 28° 56' 00" N, and the altitude 1910 m above sea level, in the municipality of Namiquipa, Chihuahua, during the fall of 2005 (Medina et al., 2006).

The experiment used not pregnant multiparous Angus cows and their crosses with Hereford (401.14±12.28 kg). They were selected considering their corporal condition (CC) and reproductive health, after being synchronized with a dose of injectable PGF<sub>2</sub>α. The cows in estrus were mated three times with Angus and Hereford fertile bulls. They were then assigned to a treatment, kept in corrals and provided a maintenance diet based on hay oats, eventually supplemented with flaked maize and cottonseed meal.

The cows received 20 IU/day of oxytocin (OT, n=7; 10 IU am, 10 IU pm) or 2 mL of physiologic saline solution (SS, n=7) daily, from day 2 to day 16 post-mating (day 0 is the day of start of estrus signs). On day 14 the reproductive tracts were flushed with phosphate buffered saline (PBS) in order to recover the embryos. The CL's were measured longitudinally. A third treatment consisted in no management (NM, n=6). After mating with bulls, the cows were placed in another corral (no daily management). Gestation was confirmed after 35 days, using transrectal ultrasonography.

Daily blood samples were collected from the cows in the OT and SS groups to compare the serum P<sub>4</sub> levels. Blood samples were taken from the NM cows on days 5, 10 and 15 post-mating. The serum samples were frozen at -20°C and analyzed in the radioimmuno analysis laboratory of the New Mexico State University, Las Cruces, NM, USA. The kit used for P<sub>4</sub> was the Diagnostic Products Corp. (DPC) Los Angeles, CA, following a procedure validated by Schneider and Hallford (1996) in the same laboratory. The within- and

between-essay coefficients of variation were 2.97% and 0.4%, respectively.

## Statistical Analysis

A random block statistical design was used based on the body condition (BC). The variable reproductive success was analyzed using Fisher's exact test and the PROC FREQ procedure (SAS, 2001). The counts were grouped based on whether the animals were handled daily (n=14) or not (n=6). The variable sizes of CL and P<sub>4</sub> levels were analyzed using the PROC MIXED procedure (SAS, 2001). These sizes were compared for the early (days 4-8), intermediate (days 9-13) and late (days 14-15) luteal phases (Nishimura et al., 2004) in the OT and SS treatments. The fixed effects were the treatments, and the random effects included cows nested in a treatment and a block effect. Another comparison included P<sub>4</sub> levels among treatments in the early (day 5), intermediate (day 10) and late (day 15) phases (Milvae and Hansel, 1983).

## Results and Discussion

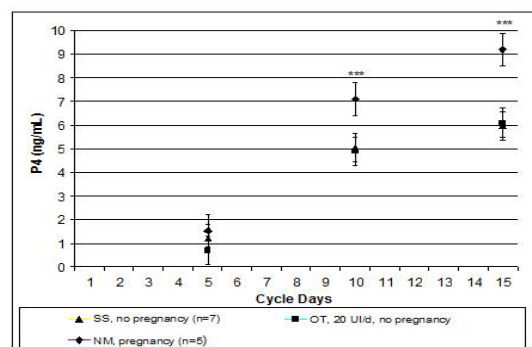
No embryos were recuperated after flushing of the uteri of the cows in treatments OT (0/7) and SS (0/7). No differences ( $P=0.21$ ) were observed for the variable CL diameter in the animals treated with 20 UI/d of OT (25.39±0.99) and SS (24.24±0.99). Equally, no differences ( $P=0.25$ ) were observed in P<sub>4</sub> levels during the early (1.6±0.22 vs. 2.24±0.24 ng/mL), intermediate (5.09±0.24 vs. 5.24±0.26 ng/mL) and late (6.11±0.36 vs. 5.86±0.38 ng/mL) luteal phases in the OT and SS groups, respectively.

According to Fisher's exact test and the information recorded for all the cows that were handled daily, including those with OT and SS no pregnancies (0/14) were observed and those with NM (5/6), pregnancies were observed ( $P<0.01$ ).

The P<sub>4</sub> levels recorded on day 5 for the cows treated with 20 UI/d of OT, SS and NM (Table 1) were not different ( $P>0.05$ ). However, they were different ( $P<0.01$ ) on day 10 and day 15 (Table 1) for the OT, SS and NM groups, respectively (Figure 1). According to the ortogonals contrasts, this difference was detected in favour of the cows in the NM group, in comparison with those in the OT and SS groups.

**Table 1: The recorded P<sub>4</sub> levels (ng/mL) on the examined days post mating**

Cows treatment	OT	SS	NM	Level of significance
Number of cows	7	7	6	
Day 5	0.8 ± 0.6	1.21 ± 0.6	1.52 ± 0.7	P>0.05
Day 10	4.9 ± 0.6 <sup>b</sup>	5.05 ± 0.6 <sup>b</sup>	7.1 ± 0.7 <sup>a</sup>	P<0.01
Day 15	6.1 ± 0.7 <sup>b</sup>	5.97 ± 0.6 <sup>b</sup>	9.2 ± 0.7 <sup>a</sup>	P<0.01



\*\*\*P&lt;0.01

Figure 1. P<sub>4</sub> serum concentration in pregnancy and no pregnancy cows in the oxytocin (20 IU/d), saline solution (SS) and no management (NM) groups.

The cows in the OT and SS groups were handled daily to apply the OT and to obtain samples, and this was associated with failure in fertility and embryo recuperation. These results of were suggested to be related to the uterine environment that was established by the lower P<sub>4</sub> levels in comparison with that in the NM cows, as a close relationship between P<sub>4</sub> levels and fertility has been recorded (Vanroose et al., 2000).

Despite the P<sub>4</sub> levels, the development and the CL diameters observed in the OT and AS groups were normal and statistically similar. No physical evidence to indicate the presence of embryos. According to information generated by Dunne et al. (2003), 32% of embryo losses occur before day 14 or between days 8 and 16 post-mating (Roche et al., 1981) due to unknown factors.

The comparison of the reproductive success of the OT and SS cows, with that of the NM cows, presented statistical evidence in favour of the last group. This difference is possibly due to greater P<sub>4</sub> levels in the intermediate (day 10) and late (day 15) luteal phases, that were greater in the NM cows (Figure 1).

The observed results allowed thinking that under these conditions, the greater levels of P<sub>4</sub> created a better uterine environment for the best fertility and for the development of the embryo during the early stages (Varoos et al., 2000; Mann and Lamming, 2001; Spencer and Bazer, 2002; Spencer and Bazer, 2004; Bazer et al., 2008). This is also observed in the expression of genes associated with changes in the composition of the histotroph and the later establishment of gestation (Spencer et al., 2008; Forde et al. 2009). In addition, this contributes to prevent the secretion and action of the PGF<sub>2</sub>α at the level of the luteal cells (Niswender et al., 2000; Spencer and Bazer, 2002).

The second reason for the obtained results lies in the fact that all the cows used in all the treatments came from extensive grazing systems, and the change and lack of custom to a confined environment and to daily handling for the application of treatments and sampling

may have generated stress (Smith et al., 2003; Dobson et al., 2003). Several organisms may inhibit or block reproductive activities under non-optimal conditions in response to stress until the time there is a more favourable environment (Dobson et al., 2003). This is attained by suppressing GnRH-LH or through a control system that is activated under conditions of stress (Dobson et al., 2003). They added that this system involves neuro-transmitters such as noradrenaline and neuropeptid Y. Corticotropin-releasing hormone (CRH), the principal regulator of the hypothalamic-pituitary-adrenal axis, as well as its receptors, have been identified in most female reproductive tissues, including uterus, placenta, and ovary (Kalantaridou et al., 2003).

On the other hand, adenocorticotrophic hormone (ACTH) may induce a reduction of LH pulses in the follicular phase (Dobson et al., 2000; Vitoratos et al., 2006), the luteinization of the CL, and the concentration of P<sub>4</sub> at the level of small luteal cells (Vanroose et al., 2000; Berisha and Schams, 2005). This physiologic mechanism could modify the uterine environment and induce failure in early embryo's viability, and this might explain the results recorded in this study.

## Conclusions

The current failure in embryo's viability may possibly be due to factors associated with handling, as this caused stress and the negative response in embryo's viability during the early or intermediate luteal phases. The uterine environment established by different P<sub>4</sub> levels resulting from the possible changes in the synthesis of ovarian steroids, may explain the differences in the reproductive success of the OT and SS cows that were handled daily, compared with the NM cows. These explanations may be valid as the cows used in this study were accustomed to an extensive grazing system in which they were not familiar with daily handling.

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