Hormonal and Pregnancy Rate of Nelore Cows Artificially Inseminated at a Fixed Time (AIFT) Associating Melengestrol Acetate, Gonadorelin and Prostaglandin

Jose Eduardo Jardim *Murta*[(1)], Venício Jose de *Andrade*[(2)], Vicente Ribeiro do *Vale Filho*[(2)]

State Universite of Montes Claros - UNIMONTES- Av. Reinaldo Viana, 2630, Mailbox 91, Bico da Pedra, CEP: 39440-000 – Janaúba, MG.

[(2)]Veterinary School of UFMG. Av. Antônio Carlos 6627, Mailbox 567, CEP: 30123-970 Belo Horizonte, MG

Abstract

Pregnancy rates and follicular diameter of Nellore cows submitted to AIFT were evaluated in two groups: In G1, n = 30 (dose of 100 mg gonadorelin on day one - d1; 75 mg D-cloprostenol on day seven - d7, and a second dose of gonadorelin on day nine - d9); in G2, n = 30 it was applied treatment similar to G1, plus 0.05 mg MGA / cow / day, in the mineral supplement, from d1 to d7. Mean follicular diameter, by ultrasonic measures, on d7 and d9 were, respectively, 0.94 ± 0.32 and 1.09 ± 0.30 cm in cows not pregnant; and 1.14 ± 0.35 and 1.32 ± 0.27 cm (P>0,01) in those pregnant. Pregnancy rates registered after AIFT were 26.6% and 26.6% and after using roundup bulls 86.36% and 81.81%, respectively, for G1 and G2 (P>0.05). The mean MGA serum concentration was 0.12 ± 0.20 ng/mL in animals that received MGA (G1, n = 8) and did not differ (P>0.05) from the control group (G2, n = 8). It was concluded that treatment with MGA associated or not with GnRH and Prostaglandin analogues does not affect the follicular diameter mean neither the gestation rate of Nellore cows.

Keywords: Follicles, artificial insemination, bovine, progestagens

Introduction

The gonadotropins are important to the final development of dominant follicles. Thus, any factor that interferes with its secretion limits the reproductive performance. Some studies have reported that sheep under poor nutrition have lower ovulation rate, associated with a decreased frequency of LH pulses due to a lower hypothalamic GnRH secretion. It is evident that the return of follicular waves without ovulation in postpartum cows with low body condition, promotes increased calving interval to first ovulation and to conception (Boland, O'Callaghan and Lonergan, 2001).

The melengestrol acetate (MGA) was used to enhance the estrous response and reproductive performance of beef cows after calving. The addition of gonadorelin to the protocol that uses MGA improves the synchrony of estrus, increasing fertility in beef cows (Patterson et al., 2002)

The protocol that uses vaginal implant (CIDR ®) seems to have better application compared to MGA due to lower treatment period (10 days vs 36 days). The best results in CIDR ® protocol can be attributed to the constant release of progestin by vaginal implant, when compared to the variable patterns of consumption of MGA (Patterson, 2006).

The objective of the present study was to evaluate the pregnancy rate, follicular diameter, follicular growth rate and serum using or not associated to MGA, prostaglandin and gonadorelin protocols in AIFT, and record spending on drugs and its relation to pregnancy the cost.

Material and Methods

A total of 60 Nelore multiparous, lactating Nellore cows, equally divided in two groups, with average age (years), postpartum period (days), condition score (scale 1 – 5), respectively, of 8.02±1.19 and 7.79±1.61; 106.17±12.31 and 102.41±12.72; 2.83±0.29 e 2.84±0.27, for G1 where each cow received intramuscular injection of 100 µg of GnRH analogue (gonadorelin) in day 1 of the experimental phase (d1), subcutaneous injection of 75 µg of D-cloprostenol in the vulvar mucosa in day 7 (d7) and a second dose of 100 µg of gonadorelin in day 9 (d9) and G2 that received similar treatment plus 0.05 mg Melengestrol Acetate (MGA) along with the mineral supplement from d1 to d7.

Artificial inseminations, by a single inseminator, at a fixed time (AIFT) in d9, using semen from Nellore bulls of the same collection, previously evaluated according to Brazilian College of Animal Reproduction Manual (1998) were performed after ultrasound evaluation, at the time of the second dose of GnRH analogue.

Ultrasound evaluations (Aloka SSD 500, 5.0 MHz rectal transducer) of the cows reproductive tract, carried out in d1, d7 and d9 aimed to identify and to measure the structures present in the ovaries. The generated images were stored and the measurements of maximum follicular diameter (MFD) and presence or absence of the corpus luteum in d7 were performed later using the computer program (Image J ®), using processor and image analysis system of Java of public domain.

Eight blood samples per group (jugular or coccygeal vein) for hormone dosage (MGA) was performed in d1, d7 and d9. Once collected, the blood samples were stored on ice and later centrifuged, and serum conditioned (Eppendorf) and frozen at -20° C until determination of hormone concentrations. Hormone concentrations (MGA) were determined in the laboratory of retroviruses, Department of Veterinary Preventive Medicine, School of Veterinary Medicine, Federal University of Minas Gerais (UFMG) by enzyme immunoassay technique (ELISA - Enzyme-Linked Immuno Sorbent Assay) using commercial specific kits (RIDASCREEN melengestrolacetat - Biopharm ®).

In order to separate pregnancies resulting from AIFT from those of natural matings, 15 days after AIFT, two bulls andrologycally evaluated (CBRA, 1998), were introduced in each group of cows.

After 30 days of AIFT it was carried out ultrasonic pregnancy diagnosis (Aloka SSD 500, rectal transducer of 5.0 MHz. Another pregnancy diagnosis by rectal palpation was performed 60 days later, for checking for bulls pregnancies.

For the statistical analysis (mean, coefficient of variation and standard error of mean) of follicular diameter the procedures PROC univariate computational resources of the SAS statistical package (SAS Institute, 2002) was used and for the dispersion of frequencies (presence of corpus luteum and pregnant cows), it was used the chi-square test at 5% level, according to Sampaio (1998). For comparison the means of the animals age, body condition score and service period, it was used the Student t or Tukey test as they shall have been subject to evaluation the characteristic or the index in all or only those pregnant females. For comparison of hormone concentration means (MGA), it was utilized the nonparametric Mann-Whitney test, as recommended by Sampaio (1998).

Results and Discussion

The average diameters of the larger follicles present in ovaries (Table 1) were of 1.04 ± 0.37 and 0.98 ± 0.35 cm at d1; 1.00 ± 0.28 and 1,01 ± 0.40 at d7, and 1.19 ± 0.32 and 1.12 ± 0.30 cm in d9, respectively, for G1 and G2 (P> 0.05). Among the pregnant cows (n = 16) the maximum follicular diameter, in the d9 measured 1.31 ± 0.28 and 1.35 ± 0.29 cm, while for non-pregnant females (n = 44), the same measures were 1.13 ± 0.32 and 1.04 ± 0.27 cm (P> 0.05), respectively for G1 and G2.

The results reported are comparable to those described by Perry et al. (2002), that reported average diameters of dominant follicles of 13 ± 2.3, 11.4 ± 1.5 and 12.2 ± 1.5 mm, respectively, in females in anoestrus, in anoestrus but supplemented with MGA and cyclic supplemented with MGA in the first year and 13.6 ± 1.03; 11.5 ± 1.03 and 11.1 ± 1.3 mm, and in the second year (P> 0.05). Similarly, Bossis et al. (1999) concluded that, in heifers with feed restriction, ovulatory and anovulatory follicles are similar in size but smaller than those of heifers in nutritional maintenance.

The follicular diameters observed in the evaluated animals confirmed the literature findings that follicles acquire ovulatory capacity with approximately 10 mm in diameter, which corresponds to 1 day after the start of follicular deviation, but require higher doses of LH to induce ovulation. It is assumed that the acquisition of ovulatory capacity involves increased expression of LH receptors in granulose cells (Sartori et al., 2001).

Pregnancy rates observed after AIFT (Table 2) were 26.6% (8 / 30) and 26.6% (8 / 30) and after using bulls of 86.36% (19 / 22) and 81.81% (18 / 22), respectively, for G1 and G2 (P> 0.05).

Recorded pregnancy rates, after AIFT, are comparable with those reported by Ambrose et al. (2010), which registered a variation of 18.2% to 39.0%, respectively, in anestrous and cyclic cows. Ahuja et al. (2005), working with anestrus lactating cows with low body condition, found pregnancy rates of 21.0%, using the Ovsynch protocol.

In G2, when progestin (MGA) was administered, there was no change in pregnancy rate or in the follicle ovulatory capability, possibly due to lack of uniformity in the intake of the product administered along with the mineral supplement, due to the lack of control of the consumption, suggesting there is no change in plasma LH, and therefore not changing the follicular growth, as described by Peters & Pursley (2003).
By enzyme immunoassay technique (ELISA - Enzyme-Linked Immuno Sorbent Assay) using commercial kits (RIDASCREEN melengestrolacetat - Biopharm ®) in blood serum it was revealed detectable concentrations of MGA in three animals.

The mean serum MGA concentration of 0.12 ± 0.20 ng / mL was detected in animals that received MGA (N = 8) not differing (p>0.05) from those that did not receive MGA (N = 8). The reported results showed that the intake of MGA along with the mineral supplement was not sufficient to raise MGA serum levels in those animals sampled, not affecting the follicular diameter and pregnancy rate. However, there seems to be needed of even consumption for longer period, when animals are raised under pasture condition, to obtain significant results in reproductive parameters, using MGA associated with GnRH and with prostaglandin.

Serum MGA levels found in the present study differ from those reported by Daxenberger et al. (1999) using heifers consuming different doses of MGA (0, 0.5, 1.5 and 5.0 mg / day) for eight weeks. They recorded medium plasma level after the initial period (3.5 to 29.5 days) of MGA intake, observing a decrease in plasma levels during treatment. Plasma MGA levels were of 0.44 ± 0.12 and 0.51 ± 0.09 ng / ml, recorded in two animals that received 0.5 mg of MGA daily for a period of 3.5 to 29.5 days. Samples were collected at intervals of 48 hours in two animals per group for 56 days, showing residual effect of the product in the plasma of animals during the study period (Daxenberger et al., 1999). Possibly the form of MGA administration (mineral supplement) and the short period of ingestion have affected the results registered.

Conclusions

Consumption of MGA for seven days, interspersed with gonadorelin and prostaglandin did not affect the follicular diameter or the pregnancy rate.

Melengestrol Acetate serum level was detected in a few animals, not affecting the larger follicular diameter or the pregnancy rate.

References

AHUJA C.; MONTIEL F.; CANSECO R.; SILVA E.; MAPES G. (2005). Pregnancy rate following GnRH + $PGF_{2\alpha}$ treatment of low body condition, anestrous *Bos taurus* by *Bos indicus* crossbred cows during the summer months in a tropical environment. **Animal Reproduction Science**, v. 83, p. 203-213.

BOSSIS, I.; WETMANN, R. P.; WELTY, S. D.; VIZCARRA, J. A.; SPICER, L. J.; DISKIN, M. G. (1999). Nutritionally induced anovulation in beef cattle: Ovarian and endocrine function preceding cessation of ovulation. **Journal Animal Science**, v. 77, p. 1536-1546.

MANUAL para exame andrológico e avaliação de sêmen animal. (1998). Colégio Brasileiro de Reprodução Animal. Belo Horizonte: CBRA, 28p.

PATTERSON. D. J. (2006). Revisão de sistema de sincronização do estro utilizando a progesterona oral Acetato de Melengestrol. In: NOVOS ENFOQUES NA PRODUÇÃO E REPRODUÇÃO DE BOVINOS. 10. 2006. Uberlândia, MG. **ANAIS**... Uberlândia, CONAPEC Jr., p. 140-174.

PATTERSON, D. J.; STEGNER, J. E.; KOJIMA, F. N.; SMITH, M. F. (2002). MGA Select improves estrus response in postpartum beef cows in situations accompanied with high rates of anestrous. **Proceedings, Western Section, American Society of Animal Science**. v. 53, p. 418-420.

PERRY, G. A.; BADER, J. F.; SMITH, M. F.; PATTERSON, D. J. (2002). Evaluation of a fixed-time artificial insemination for beef cows. **Journal Animal Science**, v. 80, p.1932-1938.

PETERS, M. W. and PURSLEY, J. R. (2003). Timing of final GnRH of the Ovsynch protocol affects ovulatory follicles size, subsequent luteal function, and fertility in dairy cows. **Theriogenology,** v. 60, n.6, p. 1197-1204.

SAMPAIO, I. B. M. (1998). **Estatística aplicada à experimentação animal**, Belo Horizonte, FEP-MVZ, 87 p.

SARTORI, R.; FRICKE, P. M.; FERREIRA, J. C. P.; GINTER, O. J.; WILTBANK, M. C. (2001). Follicular deviation and acquisition of ovulatory capacity in bovine follicles. **Biology of Reproduction**, n. 65, p. 1403-1409.

SAS INSTITUTE. (2002). **SAS: user's guide**. Versão 9.1.3. Cary: SAS Institute.

Table 1. Mean and standard errors of the maximum follicular diameter of Nellore cows during hormone treatment.

Treatment	N	d1	dD7	d9
G1 (Cosynch)	30	1,04±0,37 cm	1,00±0,28 cm	1,19±0,32 cm
G2 (MGA)	30	0,98±0,35cm	1,01±0,40 cm	1,12±0,30 cm
Pregnant cows				
G1 (Cosynch)	8			1,31±0,28 cm
G2 (MGA)	8			1,12±0,30 cm
Non pregnant cows				
G1 (Cosynch)	22			1,13±0,32 cm
G2 (MGA)	22			1,04±0,27 cm

(P>0,05)

Table 2. Presence of corpus luteum (CL) and pregnancy rate of Nellore cows in G1 and G2

	G1	G2
CL (d7)	8/30 (26.66%)[a]	9/30 (30.0%)[a]
CL (d7) and pregnancy after AIFT	3/8 (37.50%)[b]	1/9 (11.11%)[b]
Pregnancy rate (AIFT)	8/30 (26.66%)[a]	8/30 (26.66%)[a]
Pregnancy rate (Bulls)	19/22 (86.36%)[a]	18/22 (81.81%)[a]

(P>0,05)