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Ovulation induction in ewes using GnRH in long and short-term synchronization protocols

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Abstract

This study was done to evaluate the efficiency of GnRH along with long-term and short-term synchronization protocols on ovulation induction and corpus luteum development. Ewes underwent four protocols: Long+GnRH (n = 11) with vaginal sponge (60 mg MAP) for 12 days along with 300 IU of eCG on day 12 and 0.025 mg of GnRH 27 h after sponge removal; Long (n = 10) with vaginal sponge for 12 days along with 300 IU of eCG on day 12; Short+GnRH (n = 10) with vaginal sponge for 7 days along with 37.5 µg of D-cloprostenol on day 5 and 300 IU of eCG on day 7, plus 0.025 mg of GnRH used 27 h after sponge removal; and Short (n = 10) with vaginal sponge for 7 days. D-cloprostenol (37.5 µg) was administered on day 5 and eCG (300 IU) was administered on day 7. Ovulation was evaluated 52, 56, 60, 66, 72, 76 h after sponge removal. Blood was collected twelve days after sponge removal to measure progesterone concentration. On this same day, the corpus luteum was measure and counted. When GnRH was used, all ewes ovulated, while 70 and 80% of ewes ovulated in protocols that had not received GnRH (Long and Short, respectively). The GnRH accelerated ovulation ($P < 0.05$) in relation to sponge removal in both protocols and induced ovulation in approximately 28 h. The GnRH was effective in inducing ovulation without decreasing the corpus luteum volume and progesterone concentration.

Keywords: artificial insemination, estrous synchronization, progesterone, reproduction, sheep.

Introduction

A short-term treatment in ewes has been as effective as long-term treatment in inducing estrus and has produced high fertility (Robinson *et al*., 1970; Fitzgerald *et al*., 1985; Scaramuzzi *et al*., 2006; Ustuner *et al*., 2007; Özyurtlu *et al*., 2011). Ultrasonographic evaluations show that follicular turnover is slower during the last period of treatment and the ovulatory follicle has a prolonged development in the long-term-treated ewes resulting in lower pregnancy rate. The short-term treatment has resulted in a higher pregnancy rate probably due to the ovulation of newly recruited growing follicles (Viñoles *et al*., 2001).

It is established that an increase in the estradiol level stimulates the neurosecretory system to increase GnRH secretion. Consequently, GnRH induces a LH surge, ovulation, and the subsequent luteal phase (Ben Saïd *et al*., 2007). In most species, the development and final maturation of antral follicles after luteolysis is dependent upon increase in LH pulsatile secretion, as FSH concentration is decreased due to the high estradiol and inhibin A concentration secreted by the ovulatory follicle (Campbell *et al*., 2007). It´s known that LH is an essential requirement for normal ovulatory follicle development and subsequent luteal function. The response to the use of exogenous GnRH will depend on the endocrine environment preceding an induced LH rise, which is more important than the size of the follicle to determine the ovulatory response (Rubianes *et al*., 1997).

The use of GnRH in estrus synchronization protocols seeks to induce and synchronize the ovulation to artificial insemination in a set time frame. The proximity between the time of AI (Artificial Insemination) and the ovulation time could improve fertility, especially when frozen semen is used. The timing of insemination and the use of precise and effective ovulation control protocols in ewes, in particular the use of GnRH, may have contributed to improve fertilization rates (Beilby *et al*., 2009; Menchaca *et al*., 2010).

The purpose of this study was to evaluate the efficiency of GnRH along with long-termand short-term synchronization protocols on ovulation induction and corpus luteum development.

Material and Methods

This study was developed during February, in Brasília, Distrito Federal, located in the Central-West region of Brazil (latitude 15°52'S, longitude 48°00'W), with an altitude ranging from 1050 to 1250 m. This region presents a tropical rainy climate, with marked dry winters and rainy summers. All procedures were approved by the Ethics Committee on Animal Use of the Institute of Biological Sciences, University of Brasília, Brazil.

In a factorial 2 x 2 design, 41 Santa Inês ewes, a non-prolific (1.2 prolificacy - number of lambs born from ewes pregnant at first service) breed from the

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Northeast of Brazil, with cyclical behavior throughout the year, between 18 and 36 months old, and with body condition score above 2.5 (scale 1-5) underwent four estrus synchronization protocols: Long+GnRH (n = 11) with vaginal sponge (60 mg MAP, Progespon® Intervet Schering-Plough, BR) for 12 days along with 300 IU of eCG (i.m., Novormon®) on day 12 and 0.025 mg of GnRH (gonadorelin acetate, i.m., Gestran®) 27 h after sponge removal; Long (n = 10) with vaginal sponge for 12 days along with 300 IU of eCG on day 12; Short+GnRH (n = 10) with vaginal sponge for 7 days along with 37.5 µg of PGF2α (D-cloprostenol, i.m., Veteglan Luteolítico®, Hertape Calier, Minas Gerais, Brazil) on day 5 and 300 IU of eCG on day 7, plus 0.025 mg of GnRH used 27 h after sponge removal; and Short (n = 10) with vaginal sponge for 7 days. PGF2α (37.5 µg) was administered on day 5 and eCG (300 IU) was administered on day 7.

The occurrence of oestrus was observed at four-hour intervals between 12 and 66 h after sponge withdraw, by the use of teaser rams, which remained with the sheep for the entire oestrus evaluation period. Ewes marked by the rams were also recorded. Oestrus was defined as the moment when the ewe stood to be mounted by the ram. Ovulation was evaluated 52, 56, 60, 66, 72 and 76 h after sponge removal by transrectal ultrasonography with a linear transducer of 6MHz (Aquila Pie Medical®). Ovulation was to have occurred when the largest follicle in the ovary disappeared. The time of ovulation was considered to be when the first follicle ovulated.

Blood was collected from the jugular vein twelve days after sponge removal, centrifuged and the serum frozen at -20°C for future measurement of progesterone concentration by radioimmunoassay. On the same day, the number and diameter of corpus luteum was monitored by ultrasonography. Measurements were done for the major and minor axes of the best-fitted ellipsis for each corpora lutea. The diameters of the corpus luteum were determined as a mean of the two axes measured. Corpus luteum volume (Rodgers *et al*., 1984) was measured by cylinder formula ($V = \pi r^2 h$), where π is Pi, approximately 3.142, *r* is the radius of the circular end of the cylinder and *h* is the height of the cylinder. The luteinized mass volume was determined as a sum of the volumes of all corpus luteum that was present in the ovaries.

The data were analyzed using the Sistema para análises estatísticas e genéticas (SAEG, 1997). The variables were tested for normality by Lilliefors test and for homoscedasticity by Barttlet test. Estrus and ovulation rate were analyzed by Fisher's exact test. The time of ovulation after onset of estrus and the volume of the largest corpus luteum was compared using ANOVA and the Duncan test. The time of onset of estrus in relation to removal of MAP; the time of ovulation in relation to removal of MAP, the number of corpus luteum, the average volume of corpus luteum; luteinized mass volume, and progesterone concentration did not show normal distribution and homoscedasticity, then those variables were analyzed by nonparametric Kruskal-Wallis test. The time of ovulation in relation to the administration of GnRH was analyzed using the Wilcoxon test. The data are presented as Mean ± Standard Deviation or in percentage, and the differences were considered significant when $P < 0.05$.

Results

In the protocols that used GnRH all the ewes ovulated, while 70 and 80% of the ewes in protocols that had not received GnRH ovulated (long-term and short-term protocols, respectively). The GnRH decreased the estrus manifestation in the long and short-term protocols compared to short-term without GnRH ($P < 0.05$) and compared to long-term without GnRH tended to decrease, whereas Long+GnRH had a $P = 0.08$ and Short+GnRH had a $P = 0.06$. The interval between sponge removal and estrus manifestation was similar among the treatments ($P > 0.05$). The GnRH anticipated the ovulation ($P < 0.05$) in relation to sponge removal in ewes submitted to the Long+GnRH and Short+GnRH protocols. The use of GnRH induced ovulation approximately 28 h after application, on average (Table 1).

Corpus luteum numbers, average volume, luteinized mass volume, and progesterone concentrations were similar among treatments (Table 2; $P > 0.05$).

Table 1. Estrus manifestation and ovulation, time of estrus manifestation to sponge removal, time of ovulation to sponge removal, time to estrus after GnRH injection in ewes subjected to synchronization protocols with progestagen, short and long term, eCG, and with or without GnRH.

	Long + GnRH	Long	Short + GnRH	Short
Estrus manifestation % (n)	36.4% (4/11)^{b}	80% (8/10)^{ab}	30% (3/10)^{b}	90% (9/10)^{a}
Animals ovulated % (n)	100% (11/11)	70% (7/10)	100% (10/10)	80% (8/10)
Duration from sponge removal to estrus manifestation (h)	37.0 ± 7.0	44.2 ± 6.2	42.0 ± 6.0	45.6 ± 11.6
Duration from sponge removal to ovulation (h)	54.5 ± 2.7^{b}	71.4 ± 4.1^{a}	57.0 ± 6.7^{b}	71.5 ± 5.0^{a}
Duration from estrus to ovulation(h)	19.0 ± 9.6^{b}	28.9 ± 2.3^{a}	26.0 ± 9.2^{ab}	29.3 ± 4.3^{a}
Duration from GnRH to ovulation (h)	27.5 ± 2.7	---	30.0 ± 6.7	---

Values with different superscript letters (^{a} and ^{b}) within the same row differ statistically with $P < 0.05$.

Table 2. Corpus luteum, corpus luteum average volume, luteinized mass volume and progesterone concentration in ewes subjected to synchronization protocols with progestagen, short and long term, eCG, and with or without GnRH.

	Long + GnRH	Long	Short + GnRH	Short
Corpus luteum (n)	2.4 ± 1.1	1.5 ± 0.5	1.4 ± 0.7	1.7 ± 0.5
Corpus luteum average volume (cm^3)	0.20 ± 0.14	0.14 ± 0.05	0.15 ± 0.06	0.18 ± 0.11
Luteinized mass volume (cm^3)	0.80 ± 0.56	0.57 ± 0.29	0.58 ± 0.26	0.74 ± 0.43
Progesterone concentration (ng/ml)	5.2 ± 5.7	5.3 ± 2.2	4.1 ± 2.9	5.7 ± 2.4

There was no difference among treatments (P > 0.05).

Discussion

Reproduction researches have sought to increase reproductive efficiency and this requires the manipulation of the estrous cycle. One challenge of assisted reproduction techniques in ewes is to increase the fertility rate using frozen/thawed semen. In this study, the use of GnRH was able to induce ovulation in 100% of the ewes treated, both in long and in short term protocols, demonstrating that inducing ovulation can increase the efficiency of the FTAI. Cavalcanti *et al*. (2012), using short-term protocols associated with GnRH, reached 90% of ovulation rate, but does not verify improved ovulation or pregnancy rate.

Studies using GnRH to induce ovulation have shown that the interval between GnRH and LH peak is 2 hand ovulation occurs between 21 and 26 h after GnRH is administered (Cumming *et al*., 1973; Cahill *et al*., 1974; Symons *et al*., 1974; Quirke *et al*., 1979). In the present study, the average interval between GnRH administration and ovulation was approximately 28 h, similar to the results observed in other studies. In addition, the GnRH accelerated the time of ovulation by approximately 17 h in relation to animals in which ovulation was not induced, suggesting that adjustments to the timing of fixed time artificial insemination are necessary to improve the results depending upon whether synchronization protocols for inducing ovulation in ewes are utilized or not. These results become more important if considering the FTAI using frozen semen with a limited life span needing the perfect synchrony between ovulation and insemination time.

Most animals did not show estrus when GnRH was used in long-term and in short-term protocols, probably due to the acceleration of the LH surge, which did not allow the dominant follicle present in the ovary to produce enough estradiol to induce the onset of estrus. According to Murdoch and Kirk (1998), estradiol secretion of the preovulatory follicle is interrupted by high LH levels and the follicle begins the progesterone secretion. Thus, the use of GnRH in synchronization protocols renders the observation of estrus irrelevant in artificial insemination programs.

Mann (2009) noted that the size of mature corpus luteum has no influence on the serum progesterone concentration, and progesterone production can be increased without a corresponding increase in size.The volume of the corpus luteum and the progesterone concentrationin this study were measured on day 10 of the estrous cycle, considering the day of estrusas day 0. During this period the corpus luteum reached maturity.

Murdoch and Kirk (1998) and Vasconcelos *et al*. (2001) found that in ewes and cows respectively, small follicles in which ovulation was induced, produced smaller corpus luteum with lower progesterone production. Although this study has not evaluated ovulatory follicle size, it was observed that the inducing of ovulation using GnRH did not alter the volume of the corpus luteum or the level of progesterone. The administering of GnRH27 hours after MAP withdrawal may have contributed to this result, considering that the dominant follicle did not have its follicular phase abbreviated, having time to mature and secrete estradiol. Murdoch and Kirk (1998) showed that the number of corpus luteum and the progesterone levels in ewes which received GnRH12 hafter PGF2α were lower than in those which received GnRH36 hours later.

In conclusion, GnRH was effective in inducing ovulation and accelerated the time of ovulation, both in long-termand short-term estrus synchronization protocols, when compared with the protocols in which ovulation was not induced. Furthermore, the use of GnRH did not reduce the corpus luteum development and progesterone production.

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