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Oestrous Synchronization of Ewes, Using Norgestomet Combined with $PGF_2\alpha$ and hCG in the Reproductive Season

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Abstract: To determine if with doses of norgestomet (N) at lower (3 mg) and shorter treatment time to that normally used (9 d), estrous can be induced, with the aim to reduce synchronization costs without altering main reproductive variables, 24 Dorset ewes were randomly assigned to four treatments (T, n = 6): T_1) 3 mg of N for 9 d; T_2) 2 mg of N for 9 d; T_3) 3 mg of N for 7 d and T_4) 2 mg of N for 7 d, all groups received an intramuscular (i.m.) $PGF_{2\alpha}$ (15 mg) injection 2 d before implant removal and on the day of the removal received 1000 U.I. of human Chorionic Gonadotrophin (hCG) i.m. There was a 66% of estrous onset in T_1, 83% in T_2 and 33% in T_4. Average progesterone (ng mL^{-1}) concentration was higher in T_2 ($p<0.05$), although life span of the corpus luteum was similar between treatments. Lambing rate were higher in T_2 (60%) compared to T_1 (50%). Under the conditions of the present experiment a dose of N lower than 3 mg does not affect main reproductive variables in the reproductive season.

Key words: Synchronization, ewes, norgestomet, oestrous, reproductive season

INTRODUCTION

The main objective of the ovine reproductive technology is to increase reproductive efficiency of females, controlling or increasing their fertility; the more effective techniques are related with ovulation induction (Lopez, 1991). Synchronization of estrous and ovulation allows to increase efficiency reducing the periods of reproductive inactivity allowing to have three parturitions every two years or two parturitions every year (McDonald, 1989; Larson and Kiracafe, 1995).

When estrous is synchronized, a group of females is in the same phase of the cycle and show estrous at the same time in a short period of time, for which luteal regression is induced or procedures that mimic the corpus luteum function (Beck *et al*., 1996; Mendez *et al*., 2000) are applied.

Treatments with Norgestomet (N) implants are used to synchronize estrous in bovine: 6 mg of N in the top of the ear for 11 d plus an intramuscular (i.m.) injection of 3 mg of N and 5 mg of estradiol valerate. In ovine, half of the dose of bovine has been used: ½ N implant (3 mg of N) during 11 d and an i.m. injection of 1.5 mg of N plus 2.5 mg of estradiol valerate or without the i.m. injection (Cuevas *et al*., 1993). Experiments have been performed in which the implant remained for 9 d (Mendez *et al*., 2000) obtaining different results.

The cost of estrous synchronization is high and duration of the ovine estrous cycle is 17 d; with this in mind, the objectives of this research were to determine if with lower doses of N (3 mg) and a shorter treatment time to that normally used (9 d), estrous can be induced, without altering gestation rate.

MATERIALS AND METHODOS

Experimental location: Research was conducted in the Experimental Ovine Unit of the Colegio de Postgraduados, located in Montecillo, Texcoco, State of México, México with an altitude of 2241 m and medium annual rainfall of 632.5 mm (Garcia, 1988).

Experimental animals: Twenty four 10 month age Dorset ewes with an average weight of 45.46±3.97 kg and a body condition of 3-3.5 in a scale of 1 to 5 (Cottle, 1991). Ewes were confined and received a diet with 68.18% of alfalfa, 22.73% of corn silage and 9.09% of concentrate (16% CP).

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Estrous synchronization: Ewes were randomly assigned to four (n = 6) treatments: T_1 (control) ½ N implant (Synchromate-B, Rhone Merieux, México) with theoretically 3 mg of the active hormone subcutaneously in the top of the ear for 9 d; T_2⅓ implant with theoretically 2 mg of the active hormone for 9 d; T_3 ½ N implant for 7 d; T_4 ⅓ implant with 2 mg of N for 7 d. All groups received an intramuscular (i.m.) $PGF_{2\alpha}$ (15 mg Lutalyse, Pharmacia and Upjohn, México) injection 2 d before implant removal and the day of the removal received 1000 U.I. of human Chorionic Gonadotrophin (hCG, Chorulon-Intervet, México) i.m.

Estrous detection and natural breeding: At implant removal started estrous detection and natural breeding with presentation of the females to the male and allowing the service to the females in estrous, with a second service 12 h later. Females that did not showed estrous were followed every 4 h for 96 h. Detection of return to estrous was performed from d 15 to 23 after implant removal, checking for estrous in the morning and in the evening. Gestation was confirmed 45 d after service using an ultrasound (Sonovet 600) with a transrectal linear transducer (7.5 Mhz), to detect the head, trunk and members and checking the beginning of ossification and cardiac beat.

Blood sampling for Progesterone (P_4) and Luteinizing Hormone (LH): After N implant insertion blood samples were collected though jugular vein every 48 h, to determine P_4 concentrations in a period of 30 d. To determine LH pulse, samples were collected via jugular vein beginning 24 h after implant removal and every 4 h during 96 h. Samples were separated by centrifugation at 2500 × g for 15 min and serum was stored at -20°C until assayed for P_4 and LH.

Hormonal assays: Progesterone concentrations were determined by Radioimmunoassay (RIA) of solid phase (Srinkandakumar *et al*., 1986) with a commercial kit (Coat-A-Count Progesterone®, Diagnostic Products Co, U.S.A.). Sensibility of the assay was 0.07 ng mL^{-1} with intra and interassay coefficients of variation of 2.2 and 10.01%. LH assays were performed by double antibody RIA (Niswender *et al*., 1969) with sensibility of 0.77 ng mL^{-1}, and intra and interassay coefficients of variation of 9.4 and 13%, respectively. Onset of estrous and duration of preovulatory LH pulse were obtained with the technique of Van Cleeff *et al*. (1988).

Variables to measure:

- Estrus onset, considered as a regular but limited period of sexual receptivity, associated with the release of ovules capable of being fertilized (Galina *et al*., 1986).
- Onset of LH pulse, alter implant removal, considering a pulse the increase in LH 2 standard deviation over the mean (Evans *et al*., 1996).
- Life span of a corpus luteum, if P_4 concentration is >1.0 ng mL^{-1} for 11 d (Garverick and Smith, 1986).
- Gestation rate, number of females served/number of pregnant females.
- Lambing rate, number of pregnant females/number of lambing females.

Statistical analysis: Data of the females that showed estrous and became pregnant were analyzed with the Fisher (Daniel, 2002) test. Data of estrous onset, LH pulse and P_4 are mean values±standard Error of the Mean (EEM), using the GLM procedure. Differences in mean of treatments (p = 0.05) were analyzed with the Tukey test (SAS, 1982). Progesterone concentration was analyzed with the MIXED procedure (SAS, 1982) for a randomly design array with factorial arrangement 2×2 (Little *et al*., 1998).

RESULTS AND DISCUSSION

Estrous onset: Synchronized estrous occurred in 66% (4/6) of the ewes in T_1, 83% (5/6) of T_2 and T_3 and 33% (2/6) of the T_4. Fitzgerald *et al*. (1985) gave N (3 mg) subcutaneously (s.c.) during 10 d and 1.5 mg of N plus 0.5 mg of estradiol valerate i.m. at implant insertion; estrous was synchronized in 93% of the ewes within 5 d post treatment. In other study 10 ewes received ½ N implant (3 mg) s.c. for 9 d plus 1 mL of a mix with 1.5 mg of N and 2.5 mg of estradiol valerate at implant insertion, there was a 88.8% of estrous synchronization (Mendez *et al*., 2000). Authors mentioned that there could have been residues of the estrogen injection following implant removal, which would enhance a higher presentation of estrous, since they administered half implant to other group and only observed 10.5% of synchronized estrous.

Onset of preovulatory LH pulse: In this research 100% of the ewes showed a preovulatory LH pulse, with no differences between all treatments. Average time of the pulse following implant removal was at 50.66±5.2, 50.83±4.1, 48.01±3.4 and 47.65±4.4 h, for T_1, T_2, T_3 and T_4,

Table 1: Average values for progesterone concentration in the synchronized estrous progesterone (ng mL^{-1})

Group	Treatment	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
T_1	3 mg / 9 d	0.63	1.35	2.77	4.77	5.67	4.57	3.42	1.79	1.49
T_2	2 mg / 9 d	0.64	1.97_a	↑ 5.51_b	6.91	4.59	4.57	3.45	2.27	2.41
T_3	3 mg / 7 d	0.54	1.44	3.05	5.11	4.65	4.15_c	↓ 1.69_d	0.35	0.16
T_4	2 mg / 7 d	0.76	1.78	3.17	4.63	4.28	3.16_c	↓ 0.67_d	0.34	0.61

a,b,c,d. Different superscripts in the same column indicate difference for a day of the estrous cycle ($p = 0.05$), There were no differences between treatments for one same day ($p>0.05$), Values preceded with ↑ or ↓ are different ($p = 0.05$) with an increase or decrease in P_4, compared to the previous value

Table 2: Rate of service in the different groups at the synchronized estrous and rate of lambings

Group[a]	Treatment	Service at the synchronized estrous%	Lambings at the syncrhonized estrous
T_1	3 mg/9 d	66% (4/6)	50% (2/4)
T_2	2 mg/9 d	83% (5/6)	60% (3/5)
T_3	3 mg/7 d	83% (5/6)	0% (0/5)
T_4	2 mg/7 d	33% (2/6)	0% (0/2)

[a]All groups received 15 mg of $PGF_{2\alpha}$ 2 d before implant removal and 1000 U.I. of hCG the day of implant removal

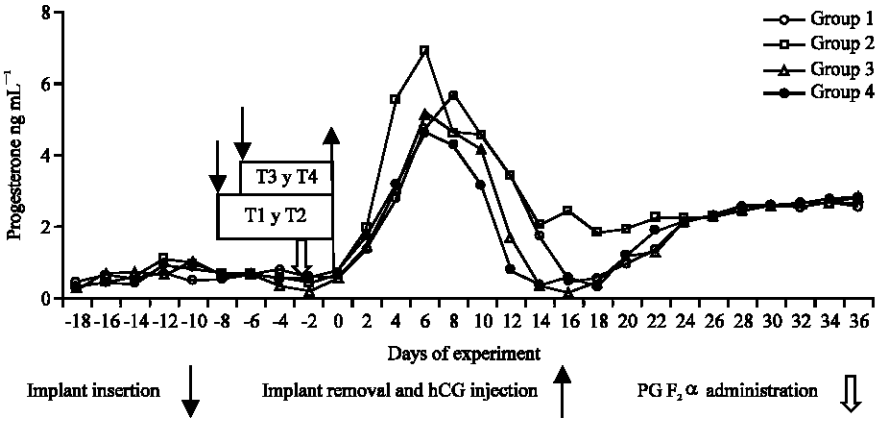

Fig. 1: Average progesterone concentration during the experiment. In ewes from T_1 (3mg N) and T_2 (2 mg N) the implant remained in place during 9 d; in ewes from T_3 (3 mg N) and T_4 (2 mg N) the implant remained in place during 7 d. All ewes received 15 mg $PGF_2\alpha$ 2 d before implant removal and 1000 U.I. of hGC the day of implant removal. Arrows indicate the day of implant removal and day 0 was the day of estrous

respectively. These results differ from those of Sanchez *et al.* (1995) which observed that onset of preovulatory surge was delayed in heifers with the highest dose of N (48 mg) at the same time, compared to those with lower doses (6, 12, 18, 24, 30, 36 and 42 mg) and explain that the delay could have been due to the higher doses of the progestogen inhibiting the pulse frequency of LH, altering follicular development and delaying onset of the preovulatory surge. In other research using hCG, a higher percentage of ewes ovulated in any phase of the estrous cycle, since receptors for LH are present very early in follicles and are not limited to the follicular phase (Driancourt *et al.*, 1990) which could explain the presence of a preovulatory pulse in all treatments.

Pattern of P_4 secretion: Progesterone concentrations from day 0 to day 17 are shown in Table 1. There were no differences between treatments in the same day, with an increase in P_4 concentration in all treatments in day 5 due to the normal function of the corpus luteum (Bartlewski *et al.*, 1999), however on day 5, there was a difference ($p\leq0.05$) in T_2 with respect to the previous sample, probably for a higher P_4 support of the corpus luteum of gestation; however, on day 13 of the estrous cycle, there was a decrease in P_4 concentration ($p\leq0.05$) in T_3 and T_4 with respect to the previous sample, because females did not got pregnant at this time and in an absence of an embryonic signal, pulsatile release of $PGF_{2\alpha}$ from days 12 trough 15 of the cycle cause corpus luteum regression and a decrease in P_4 concentration (Abecia *et al.*, 1994). Average P_4 concentration in T_2 was 4.24 ± 1.67 ng mL^{-1} and it was due to the luteal phase, this results are similar to the concentrations of ewes in the first month of gestation (Sanchez *et al.*, 1992). Ewes from T_3 and T_4 showed a normal life span corpus luteum with an average of 12.5 d (Fig. 1), because injection of hCG stimulate luteinization of follicles, which is coincident to Driancourt *et al.* (1990).

Gestation and lambing rate: Lambings from the synchronized estrous were present only in ewes from $_{T1}$ and T_2 (Table 2), with a lambing rate of 49%, which is similar to that reported in literature (Folch, 1990). Ewes from $_{T3}$ and $_{T4}$ did not got pregnant and did not lamb as a result of the synchronized estrous, which could be due to the short time of presence of the progestogen, which caused defects in the follicle who became unable for fertilization (Garverick *et al*., 1992; Sanchez *et al*., 1993; Smith *et al*., 1994). In the following estrous cycle, ewes not pregnant at the synchronized estrous, became pregnant.

CONCLUSION

Based on results obtained in the present research, it was concluded that application of 2 mg of N for 9 d combined with hCG have similar results to the control treatment (3 mg N for 9 d) in estrous synchronization and gestation rate, allowing to use a lower dose of N than the normally used to synchronized estrous in ewes during the reproductive cycle.

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