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The Effect of hCG Treatment on Follicular Characteristics and Luteal Function in Seasonally Anestrous Ewes Lambs

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Abstract: The objective of this research is to study the effect of hCG on follicular and luteal characteristics in anestrous ewe lambs. About 91 Corriedale ewe lambs of 8 months of age were used during non breeding season. Three experiments were performed: hCG was administered 36 h after sponge removal (Experiment 1) at day 13.5 after sponge removal (Experiment 2) and in both time (Experiment 3). Follicular characteristics, ovulation day, Corpus Luteum area (CLa) and Plasmatic Concentration of Progesterone (PCP) were not affected by 150 or 300 IU of hCG administered at the beginning of the estrous cycle ($p>0.05$). The CLa after the administration of 300 IU of hCG at day 13.5 was significantly higher than that in h150 and control groups ($p<0.05$). Furthermore, there was an effect of treatment by day interaction in the PCP ($p<0.05$). When the hCG was administered at the beginning of the estrus cycle and during the luteal phase, treatment by day interactions for the CLa and PCP were observed ($p<0.05$). The effect of hCG on luteal function of saeasonally anestrous ewes lambs is dose and time of administration dependent.

Key words: hCG, ewe lambs, non breeding season, follicular characteristics, luteal function

INTRODUCTION

Inadequate luteal function is an important cause of reduced reproductive efficiency due to preimplantation losses (Wilmut *et al*., 1986; Ashworth *et al*., 1989; Nancarrow, 1994). Such condition could be associated to poor ovary stimulation by gonadotrophins during the periovulatory period (Davies and Beck, 1993) or in the first days post conception (Nephew *et al*., 1994; Khan *et al*., 2003). In order to reduce embryo mortality, supplementation with exogenous progesterone, GnRH analogues and human Chorionic Gonadotrophin (hCG) during early pregnancy have been used in sheep and cattle (Nephew *et al*., 1994; Sreenan *et al*., 1996; Thatcher *et al*., 2001; Cam *et al*., 2002; Mehmet and Kuran, 2004; Khan *et al*., 2007, 2009).

Human chorionic gonadotrophin has been employed with the objective of increasing plasma progesterone levels, to improve lambing rate, litter size and fetal growth (Kittot *et al*., 1983; Ishida *et al*., 1999; Khan *et al*., 2003, 2007, 2009). In cyclic ewes, Khan *et al*. (2003) demonstrated that hCG administrated at the time of mating improves conceptus growth, placentation and number of lambs born the same workers observed an increase in plasma progesterone and oestradiol concentrations, weight of corpora lutea, conceptus growth and placental attachment in ewes treated on day 12 post-mating. However, the observed positive effects of hCG administration in adult ewe were not observed or were less effective in cyclic ewe lambs (Khan *et al*., 2007, 2009). During the non-breeding season and with different mating methods the lambing rate of estrus-induced ewes is lower (40-70%) than during the breeding season (Fukui *et al*., 1989, 1993a, b; Ishida *et al*., 1999). Ishida *et al*. (1999) and Fukui *et al*. (2001) attempted to improve fertility in the non-breeding season by administering hCG during the luteal phase of estrus-induced ewes and reported that the treatment increases progesterone concentrations but does not enhance fertility of treated ewes. Also, treatment of ewes with hCG at the time of mating during the non-breeding season did not increase significantly pregnancy rate though the treated group showed an increase of 19% compared to the untreated group (Catalano *et al*., 2006).

It is not known whether administration of hCG in ewe lambs during the non-breeding season affects reproductive performance. Consequently, the objective of the present study was to assess whether administration of hCG at the beginning of the estrous cycle during the luteal phase or at both stages has any effect on luteal function in ewe lambs induced estrus during the non-

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breeding season. In addition, it was further studied whether administration of hCG at the beginning of the estrous cycle affects follicular characteristics and their possible relationship with luteal function.

MATERIALS AND METHODS

Animals and management: The experiments were conducted at Field Station of Faculty of Veterinary Sciences UNCPBA, (37°S, 60°W) Tandil, Argentina during the non-breeding season (spring season). About 99 ewe lambs 8 months old were continuously exposed to natural photoperiod and handled according to the Animal Welfare Act (Resol. 087/02, Faculty of Veterinary Sciences, UNCPBA).

Previous to the ovulation induction treatment, ovaries were examined by transrectal ultrasonography using a SonoVet 900 (Medison, Co, Korea), real-time linear-array scanner equipped with 7.5 MHz transducer (two times separated by 7 days) to confirm absence of luteal structures.

To induce ovulation, all ewe lambs were pretreated with an intravaginal sponge impregnated with 60 mg of medroxyprogesterone acetate (Progespon®, Laboratorios Syntex S.A., Argentina) for 10 days and 300 IU of equine chorionic gonadotropin (eCG-Novormon®, Laboratorios Syntex S.A., Argentina) were injected intramuscularly upon withdrawal of sponges. The day of sponge withdrawal was designated as day 0. Ninety one ewe lambs ovulated after ovulation induction treatment and were used in the three experiments.

Experiment 1: About 25 ewe lambs with live weight of 39.9±0.7 kg (mean±SEM) and body condition score of 3.3±0.1 (mean±SEM) according to Russel *et al.* (1969) were used. The ewe lambs were allocated to three groups on the basis of body weight and condition score using a system of randomized stratification. About 36 h after sponge removal, animals were given a single intramuscular injection of either physiological saline (control group, n = 9) or the same dose used by Khan *et al.* (2003) in cycling ewe lambs (150 IU hCG. Ovusyn®, Laboratorios Syntex S.A., Argentina); Group h150 (n = 8) or twice the dose used by these researchers (300 IU hCG); Group h300 (n = 8).

Follicular characteristics were determined according to Rubianes. Daily ovarian ultrasonography continued until the ovulation day upon removal of the sponges. Ovarian data were combined for both ovaries of each ewe lambs. Maximum follicle diameter; rate of growth (difference between the maximum follicle diameter and the diameter registered at the day of withdrawal of sponges) durations of static phase (number of days when follicle maintains the maximum follicular diameter) and the day of ovulation (defined as the day on which follicles ≥4 mm in size disappeared) were determined. Ewe lambs with two more corpora lutea were considered with Multiple Ovulations (MOEL).

To determine the effect of hCG treatment on Corpus Luteum area (CLa), transrectal ultrasonography was performed every 48 h from day 7.5-19.5 (day 0: day of sponge withdrawal). Jugular venous blood was collected for determination of progesterone concentration in all ewe lambs every 48 h from day 3.5-13.5. From day 13.5-19.5, blood samples were collected every 24 h in order to determine progesterone concentration at luteolysis time. Immediately, plasma was obtained and stored at -20°C until assayed for progesterone. A Radioimmunoassay (RIA) kit (Diagnostic Product Corporation, Los Angeles, CA, USA) was used. The sensitivity of the assay was 0.01 ng mL^{-1}, the intra-assay coefficient of variationwas <7% for concentrations between 0.1 and 40.0 ng mL^{-1} and the inter-assay coefficient of variation was 3.5%.

Experiment 2: Twenty eight ewe lambs (live weight = 40.6±0.6 kg; body condition score = 3.4±0.1) were used. Percentage of MOEL was determined as in Experiment 1. On day 13.5 upon withdrawal of sponges, the ewe lambs were allocated into three groups on the basis of condition score using a system of randomized stratification. The ewe lambs with multiple ovulations were distributed between groups. About 9 ewe lambs that presented a 22.2% of MOEL were given a single intramuscular injection of physiological saline (control group); ten ewe lambs with 10.0% of MOEL were treated by an intramuscular injection of 150 IU hCG (Group h150) and nine ewe lambs with 11.1% of MOEL received an intramuscular injection of 300 IU hCG (Group h300).

To determine the effect of hCG treatment on CLa, transrectal ultrasonography was performed every 48 h from day 13.5-19.5 after sponges removal. From day 13.5-19.5, blood samples were collected every 24 h and processed for determining progesterone concentration as in Experiment 1. The sensitivity, the intra-assay coefficient of variation and the inter-assay coefficient of variation were the same as in Experiment 1.

Experiment 3: Thirty eight ewe lambs (live weight = 44.9±0.7 kg; body condition score = 3.5±0.1) were used. Ovulation day and percentage of MOEL were determined as in Experiment 1. The ewe lambs were allocated into four groups on the basis of body weight and condition score using a system of randomized stratification. The control

group consisted of eight ewe lambs that were given an intramuscular injection of physiological saline 36 h after sponge removal and on day 13.5; ten ewe lambs were treated by an intramuscular injection of physiological saline 36 h after sponge removal and 300 IU hCG on day 13.5 (Group h0-300), ten ewe lambs were treated by an intramuscular injection of 150 IU hCG 36 h after sponge removal and 300 IU hCG on day 13.5 (Group h150-300) and 10 ewe lambs received an intramuscular injection of 300 IU hCG 36 h after sponge removal and 300 IU hCG on day 13.5 (Group h300-300).

Follicular characteristics were determined by ovarian ultrasonography studies every 12 h from removal of the sponges until ovulation day. To determine the effect of hCG treatment on CLa, transrectal ultrasonography was performed every 48 h from day 5.5-19.5. Blood samples were collected and processed for determining progesterone concentration as in Experiment 1. The sensitivity, the intra-assay coefficient of variation and the inter-assay coefficient of variation were as in Experiment 1.

Statistical analysis: CLa and plasma concentrations of progesterone were analyzed by ANOVA for repeated measures in SAS (1989), PROC mixed. The statistical model included the effects of the treatment, day and their interaction. The covariance structure was autorregressive (1).

Maximum follicle diameter, rate of growth, duration of static phase of ovulatory follicle and days with progesterone level ≥1 ng mL^{-1} after day 13.5 were analysed as a completely randomised design (the treatments were characterized by the different dose of hCG administrated at different days of estrous cycle).

The percentage of ewe lambs with multiple ovulations was analyzed using CATMOD from SAS (1989). If significant differences were determined, tests of Tuckey were also performed. The level of significance was set at 0.05. If the effect of treatment was significant, tests of Tuckey were also performed. Results are presented as mean±SEM.

RESULTS

Follicular characteristics and ovulation day: Since, no differences were observed between Experiments 1 and 3 respect to the follicular characteristics and ovulation with day, consolidated data from both experiments of ewe lambs treated with saline, 150 IU or 300 IU of hCG 36 h after sponge removal are shown in Table 1 (p>0.05).

Luteal characteristics: The administration of 150 or 300 IU of hCG at the initial phase of the estrous cycle (Experiment 1) did not modify the luteal area nor the plasmatic concentration of progesterone as compared with the administration of saline (p>0.05) (Fig. 1a and b).

In Experiment 2, the luteal area in the hCG treated group with 300 IU at day 13.5 was significantly higher than for ewe lambs in h150 and control groups (p<0.05, Fig. 2a). Furthermore, there was an effect of treatment by day interaction in the plasma progesterone concentration (p<0.05, Fig. 2b). The number of days with progesterone levels ≥1 ng mL^{-1} after day 13.5

Table 1: Ovary characteristics, ovulation day and percentage of multiple ovulations in seasonally anestrous ewe lambs given saline or hCG 36 h after sponge removal

Characteristics	Saline	150 IU hCG	300 IU hCG
Ewe lambs (n)	27	18	18
Maximum follicular diameter (mm±SEM)	5.3±0.1	5.1±0.1	5.1±0.1
Rate of growing (mm day^{-1}±SEM)	0.6±0.1	0.6±0.1	0.6±0.1
Static phase (days±SEM)	1.5±0.1	1.3±0.1	1.4±0.1
Ovulation day (mean±SEM)*	2.5±0.1	2.4±0.1	2.4±0.1
Multiple ovulation (%)	29.6	38.9	33.3

Saline: ewe lambs of control groups (Experiments 1 and 3) and h0-300 group (Experiment 3); 150 IU hCG: ewe lambs of h150 group (Experiment 1) and h150-300 group (Experiment 3); 300 IU hCG: ewe lambs of h300 group (Experiment 1) and h300-300 group (Experiment 3). *Day 0: day of sponge removal

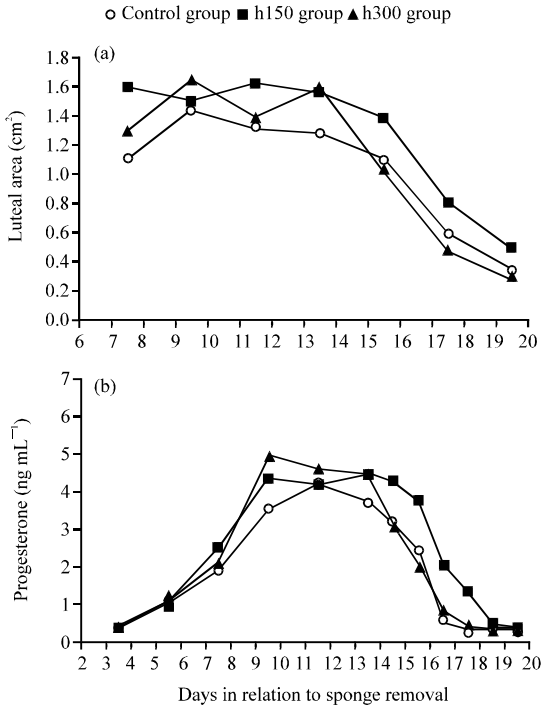

Fig. 1: a) Luteal area and b) plasma progesterone concentration in seasonally anestrous ewe lambs given hCG or saline 36 h after sponge removal

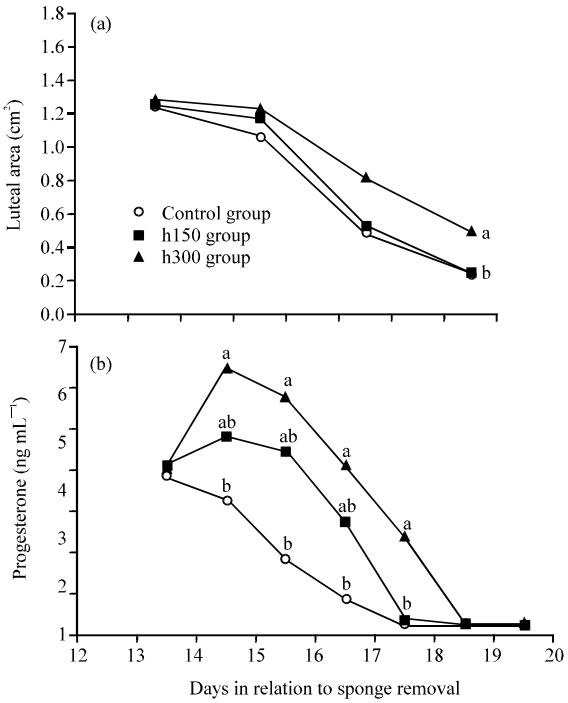

Fig. 2: a) Luteal area and b) plasma progesterone concentration in seasonally anestrous ewe lambs given hCG or saline at day 13.5 after sponge removal. Different letters indicate differences between groups ($p<0.05$)

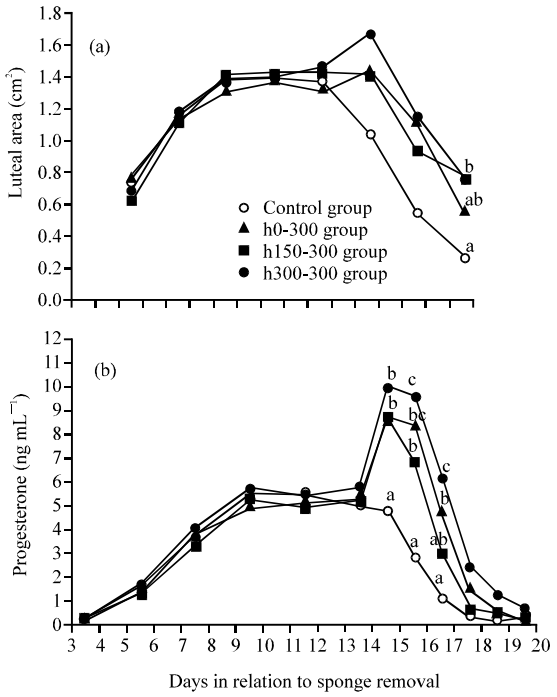

Fig. 3: a) Luteal area and b) plasma progesterone concentration in seasonally anestrous ewe lambs given hCG or saline 36 h after sponge removal and 13.5 days post sponge removal. Different letters indicate differences between groups ($p<0.05$)

was higher ($p<0.05$) in h300 group (3.4±0.1 days) than those in the h150 and control groups (2.8±0.1 days and 2.0±0.1 days, respectively).

When the hCG was administered at the beginning of the estrus cycle and during the luteal phase (Experiment 3), treatment by day interactions for the luteal area and plasma progesterone concentration were observed ($p<0.05$; Fig. 3a and b). On day 19.5, the Groups h150-300 and h300-300 showed higher luteal area than the control group ($p<0.05$) while the h0-300 group did not show differences with any group ($p>0.05$; Fig. 3a).

With respect to plasma progesterone concentration, all groups treated with 300 IU of hCG at day 13.5 showed significant differences with the control groups at days 14.5 and 15.5. On day 16.5, only the groups h0-300 and h300-300 were different from the control group ($p<0.05$; Fig. 3b). In addition, the number of days with progesterone levels ≥1 ng mL^{-1} after day 13.5 was significantly ($p<0.05$) higher in h0-300, h150-300 and h300-300 groups (3.5±0.1, 3.1±0.1 and 3.8±0.3 days, respectively) than in the control group (2.4±0.1 days).

DISCUSSION

The results of this study show that 8 months old autumn-born ewe lambs ovulated during non breeding season in response to progestagen and eCG treatment. Similar results were obtained by Gonzalez *et al.* (2000) in Corriedale x Milchschaf ewe lambs that received the same hormonal treatment. This response could be due to the fact that the animals were older than 7 months and weighted >60% of the adult weight which is necessary for response to hormonal treatment as suggested by Gordon (1967).

The administration of different doses of hCG at the beginning of the induced estrous cycle did not modify the follicular characteristics and the ovulation day. These results are coincident with previous findings where 400 or 800 IU of hCG were injected within 3 h of the onset of the oestrus and had no effect upon ovulation time (Killeen and Moore, 1970). However, in the reported study, the proportion of follicles which ruptured was higher in ewes treated with hCG; a response not observed in the current research. Killeen and Moore (1970) used cyclic, adult ewes and this could explain differences between studies.

Khan *et al*. (2003) reported that hCG administration at mating time in cyclic ewe lambs didn't increase plasma progesterone concentration. The results of the present study are in agreement with this finding. The reason why in the Experiment 1 changes on luteal area and plasma progesterone concentration were not observed could be due to the absence of effects on follicular characteristics. Davies and Beck (1993) suggest that the low response in ewe lambs would be related with a small preovulatory LH surge at the onset of estrus however, the results of the current work are in agreement with the previous finding that indicate that the ovaries of ewe lambs are less responsive to GnRH or hCG treatments compared with the response in ewes (Khan *et al*., 1998).

Current study demonstrated (Experiments 2 and 3) that all treatments that included 300 IU of hCG on day 13.5 after sponge removal had effect on corpora lutea functionality. These results are in agreement with previous findings with cyclic (Nephew *et al*., 1994) and non cyclic adult ewes (Kittok *et al*., 1983; Ishida *et al*., 1999; Fukui *et al*., 2001) and cyclic ewe lambs (Khan *et al*., 2007) treated with hCG during the luteal phase. Although, the highest plasma progesterone levels on day 16 after sponge removal were achieved in the group treated with 300 IU of hCG at the beginning of estrous cycle and during the luteal phase (h300-300 group), the cause of this response has not been determined. However, the first injection of hCG may have sensitized the luteal tissue at the action of the second injection of this hormone.

The mechanism by which hCG increased the luteal area and the plasma progesterone concentration in ewe lambs in the non breeding season remains to be determined. However, there is some evidence that the effect of this gondatropin on luteal function is probably due to a direct effect on weight of corpora lutea, the conversion of small luteal cells to large luteal cells (Farin *et al*., 1988) and/or an increase in the blood flow to the corpora lutea (Niswender *et al*., 1976). On the other hand, it has been suggested that administration of hCG causes ovulation of a dominant follicle, emergence of a accessory corpus luteum and increase in plasma progesterone levels however, this response was observed in cyclic adult ewes but not in cyclic ewe lambs (Khan *et al*., 2009). In the present study, researchers could not determine whether hCG administered at day 13.5 caused such response because the ewe lambs initiated the luteolytic process and new luteal structures not were observed.

CONCLUSION

On the basis of this study it is concluded that the effect of hCG on luteal area and plasma progesterone concentration of ewe lambs during the non breeding season with oestrus induction treatment is dose and time of administration dependent. Furthermore, no relationship was found between follicular characteristics and luteal function.

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