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Effects of GnRH or HCG on Ovarian Response in PMSG-Superovulated Ouled Djellal Ewes (Algeria)

[1,2]*Farida Afri-Bouzebda,* [1,3]*Ramzi Lamraoui,* [1,2]*Zoubir Bouzebda,*
[1]*Fayçal Chacha and* [1,2]*Djellal-Eddine Gherissi*

[1]Laboratoire des Productions Animales, Biotechnologies et Santé,
Université de Mohammed Chérif Messaadia Souk Ahras, Algérie
[2]Institut des sciences Agro-Vétérinaire,
Université de Mohammed Chérif Messaadia Souk Ahras, Algérie
[3]Institut de Biologie, Université d'El-Hadjar Lakhdar Batna, Algérie

Abstract: Superovulation plays an important role in the embryo transfer (ET) programs. The objective of this study was to evaluate the effects of Gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) treatment on ovarian response in PMSG-superovulated ewes. Intravaginal pessaries containing 40 mg fluorogestone acetate (FGA) were inserted in all ewes (n=14) and remained *in situ* for 14 days. Two days prior to pessary removal, all ewes were treated with 1000 IU of PMSG. On the day of sponge removal (day 0), the females were randomly assigned to 3 treatments. The first group (T1; n=3) received no further treatment, while second group (T2; n=5) treated inter-muscular with GnRH (100 ug) at day 1, finally the third group (T3; n=6) treated inter-muscular with hCG (500 IU) during days 0-2. On day 8, laparotomy was performed to assess numbers of corpora lutea (CL) and anovulatory follicles (AF). Blood samples were collected for analysis of serum progesterone (P_4) using radioimmunoassay (RIA) method. The results obtained for first, second and third group were in number of CL (6.33±1.15, 9.00± 1.58 and 10.50± 5.54), number of AF (2.00 ± 3.46, 3.60 ± 6.50 and 4.16 ± 5.70), then the levels of P_4 (5.75 ± 4.45, 13.97 ± 4.67 and 13.22 ± 6.80 ng/ml), respectively. In conclusion GnRH or hCG treatment post sponge removal increases number of CL and improves luteal function after PMSG-superovulatory treatment. However, it didn't reduce the number of AF.

Key words: Algeria · Anovulatory Follicle · Corpus Luteum · Ouled Djellal ewe · GnRH · hCG · Progesterone · Superovulation

INTRODUCTION

In Algeria, sheep raising is concentrated in the steppe and the mutton is the most favorable meat. Ouled Djellal (OD) breed is the most dominant in this region representing nearly 60% of the 22.868 million heads [1]. This is the true sheep of the steppe, the most suitable to nomadism. This is an all-white sheep. Wool covers the entire body to the knees and hocks; his head is white and the spiral horns. The long and strong legs support walking for long distances [2].

Multiple ovulation and embryo transfer (MOET) technologies remain the fastest way for genetic improvement in small ruminants. However, the response to superovulation, one of the most important steps in MOET, is usually unpredictable [3-6].Superovulation in ewes can generally be performed utilizing PMSG. It is usually administered as a single injection, due to its long half-life, approximately 48 h before progestagen removal [3, 7, 6].

One of the more problematic aspects of the ET procedure is the variable response by the donor to superovulatory treatment and the percentage of embryos available for transfer from each donor [8, 9, 10, 6].

Corresponding Author: Ramzi Lamraoui, Laboratoire des Productions Animales, Biotechnologies et Santé, Université de Mohammed Chérif Messaadia Souk Ahras, Souk Ahras, Algerie.
Tel: (213) 663460825.

The human chorionic gonadotropin (hCG) is secreted by the trophoblast as soon as the blastocyst leaves its zona pellucida. It is first found in the maternal blood from day 8-12 after fertilization, to achieve maximal concentrations during the first trimester of pregnancy. Afterwards, the levels decrease, but are still detectable till parturition. hCG produces an effect similar to LH on luteal cells since it managed by the same receptors as hypophyseal gonadotropin [11]. hCG is used in domestic animals because its LH-like activity lasts longer than the activity of LH [12].

The possibility to induce additional corpus luteum (CL) formation and increase circulating progesterone by means of hCG administration at different times during the estrous cycle in goats [13-16], ewes [17-20, 6] and cattle [21] have been reported.

Gonadotropin-releasing hormone (GnRH) belongs to a group of neuropeptides originally discovered and successfully isolated as factors of hypothalamic origin that control secretions of the anterior pituitary gland. GnRH influences reproductive processes, mainly by regulating pituitary gonadotropin synthesis and release [22-24].

The use of GnRH was examined by Walker *et al*. [25] as a means of increasing the efficacy of embryo collection, treatment consistently improved fertilization rates and the number of embryos collected per ewe was enhanced when compared with untreated controls. In the timing of GnRH, 24h after progestagen treatment was the preferred time in ewes treated with PMSG; 36h was preferred in ewes treated with FSH.

GnRH through LH release may provide luteotrophic stimulation to CL. This luteotrophic stimulation may either be in the form of conversion of small luteal cells to large luteal cells which then secrete higher concentrations of progesterone [17] or may even be due to an increase in the size of large luteal cells [26].

Human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH) treatments have similar effects on the ovary [27, 28], inducing ovulation [29] and the formation of accessory corpora lutea [27, 30], with a significant increase in plasma progesterone concentrations achieved 7 d after treatment [31].

The aim of the present study was to evaluate if the administration of GnRH or repeated administration of hCG after sponge removal, reduces the number of anovulatory follicles, enhances CL's number and serum P_4 concentration at the time of embryo collection in Ouled Djellal ewes that are superovulated with PMSG.

MATERIAL AND METHODS

Animals and Treatments: This trial was carried out in Ras El Aioun town, Department of Batna, Algeria. The present study was performed during the reproductive season (September) for ewes. Adult non-lactating, non-pregnant and clinically healthy Ouled Djellal ewes (n = 14) were used. They averaged 25 months old, 55kg mean body weight and raised in a semi-intensive system under a natural lighting. None of these females has previously received a superovulation treatment.

Estrus was synchronized (during the breeding season) using intravaginal sponges that contained 40 mg fluorogestone acetate (*Synchropart*), which was inserted for 14 days. The ewes were superovulated using an administration of a single dose of 1000 IU PMSG (*Folligon®, Intervet International, Pays-Bas*), 2 days before sponge withdrawal. On the day of sponge removal (day 0) females were randomly assigned to three treatments, the first group (T1; n = 3) did not receive further treatment. The second group (T2; n = 3) received intramuscular injection of GnRH (100µg) (*Fertagyl®, Intervet International, Pays-Bas*) at day 1. While, third group (T3; n = 6) received a double injection of hCG (500 IU) (*Chorulon®, Intervet International, Boxmeer, Pays-Bas*) at days 0 and 2 (Fig 1).

Control of Ovarian Response

Laparotomy: Ovarian response was performed through an anterior mid-ventral laparotomy, on day 8 post-sponge removal. After the reproductive tract was exposed, the superovulatory response was assessed, by counting CL and AF. The AF diameters were recorded on the surface of the ovary with the aid of a caliper.

Hormone Analysis: Blood samples (10 ml) were taken by jugular venipuncture into vacutainers on day 8 post-sponge removal. The samples were centrifuged for 10 min at 2000×g, the serum was aspirated and frozen at -20°C, until assayed. Concentrations of P_4 were measured by radioimmunoassay analysis (RIA) [32]. The sensitivity of the assay was 0.05 - 60 ng/ml and the intra and inter assays coefficient of variation were 5.8 and 9.0%, respectively.

Statistical Analyses: Statistical differences between the treatment groups, in CL and AF numbers per animal, were analyzed by student *t* test. Correlation analyses were used to determine the correlations between the number of CL, AF and serum P_4 concentrations on day 8.

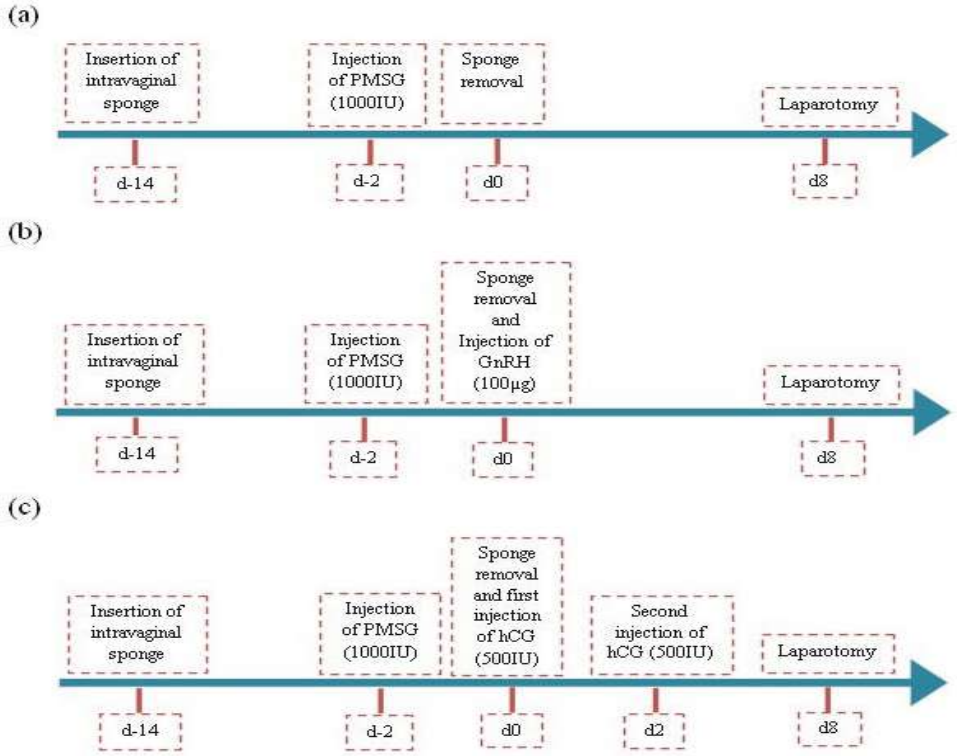

Fig. 1: Schematic representation of the synchronization and superovulatory treatment in the control group (a) treatment GnRH group (b) and treatment hCG group (c).

RESULTS AND DISCUSSION

The data of the luteal and follicular characteristics on day 8th are set out in Table 1.

Percentage of ewes responding to synchronization of estrus treatment with fluorogestone acetate (40 mg) was 100% (Table 1). A proportion of ewes (21.43 %) did not respond to PMSG induction for superovulation, which was manifested by the presence of 1 to 4 corpus luteum (CL). In agreement with our data, Windorski *et al*. [32] reported that about 20-30 % of ewes did not respond to superovulatory treatment. However, the percentage of superovulated ewes was 78.57% which observed 8 days post-vaginal sponge removal by laparotomy (Fig. 2, 3 and 4). Yields are decreased by the presence of females not bearing any ovulation and ewes with very low ovulatory responses after the exogenous hormone supply [33]. The superovulated ewes have more than 4 CL [5, 32].

Administration of GnRH 24h after PMSG injection increases the number of CL, this is confirmed by significant increase (P <0.05) of the number of CL, if we compare the number of CL in T1 with T2 groups. Indeed, treatment with GnRH at a dose of 100 µg improves the response to superovulation with PMSG by increasing proportions of follicles that ovulate [25, 34, 35].

The numbers of CL induced by the hCG treatment were significantly greater (P<0.05) in T3 than T1groups. However, no significant differences noticed between the numbers of CL in T3 and T2 groups. hCG increases the number of CL, this is confirmed in sheep [17, 18, 19, 36, 20], which indicate that the activity of hCG is to induce additional CL.

In this study, it was not possible to reduce the number of AF present in the ovaries. Indeed, no significant differences noticed between the numbers of AF in different groups. Armstrong *et al*. [37] when administering either hCG or GnRH at the onset of estrus in goats superovulated with eCG, was unable to reduce the number of follicles present in the ovaries during the subsequent luteal phase. Furthermore, the continued presence of eCG in the circulation, due to its long half-life, could induce the growth of new estradiol-producing follicles, even after the first wave of superovulation has taken place [38].Thus suggesting that the problem was not necessarily an inadequate preovulatory LH surge, but rather a lack of response of some follicles to the LH secreted at that time [16, 20].

Table 1: The mean effects of GnRH or hCG on luteal and follicular characteristics in PMSG-superovulated Ouled Djellal ewes

Variable	T1*	T2*	T3*
Number of ewes	3	5	6
Ewes in estrus (%)	(3/3)100	(5/5) 100	(6/6)100
Average number of CL*	6.33±1.15	9.00± 1.58	10.50± 5.54
Average number of AF*	2.00±3.46	3.60± 6.50	4.16±5.70
Average of TR*	8.33±4.16	12.60± 6.34	14.66±2.33
Average of AF rate	15.38±26.64	17.32± 28.32	27.46±37.87
Averageof ovulation rate	84.61±26.65	82.67± 28.33	72.53±37.87
Average of AF diameter (mm)	12.5±2.16	9.66± 1.75	12.85±4.92
Average of P_4* level (ng/ml)	5.75±4.45	13.97± 4.67	13.22±6.80

T1* (PMSG 1000IU);T2* (PMSG 1000IU + GnRH 100µg); T3* (PMSG 1000IU + 2 injections of hCG 500IU); CL* (Corpus Luteum); AF* (Anovulatory Follicles); TR*(Total Response); P_4*(Progesterone).

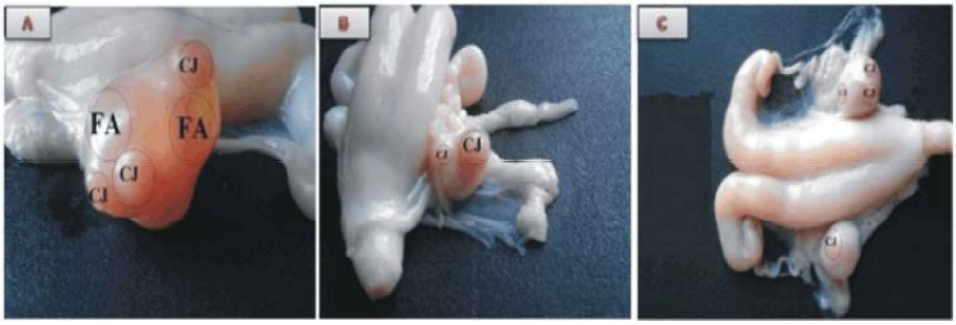

Fig. 2: Ewes' ovary in T1*
T1*(PMSG 1000IU); CL (Corpora Lutea); AF (Anovulatory Follicles); A and C (superovulated ewes); B (non-superovulated ewe)

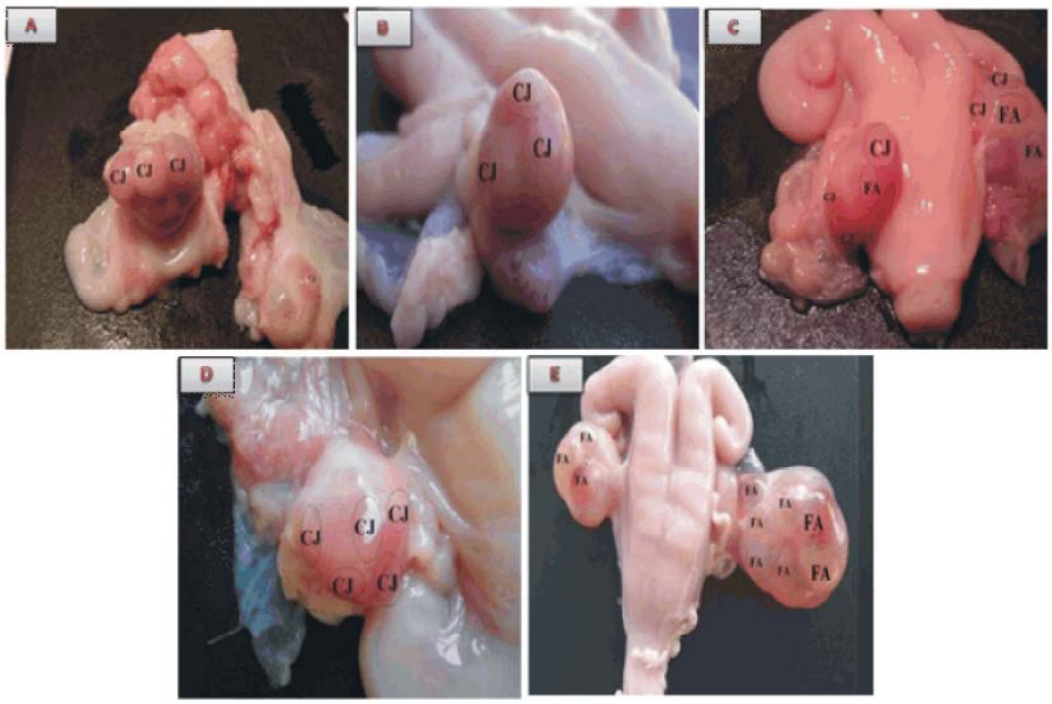

Fig. 3: Ewes' ovary in T2*
T2*(PMSG 1000IU + GnRH 100µg); CL (Corpora Lutea); AF (Anovulatory Follicles); A, B, C and D (superovulated ewes); E (non-superovulated ewe).

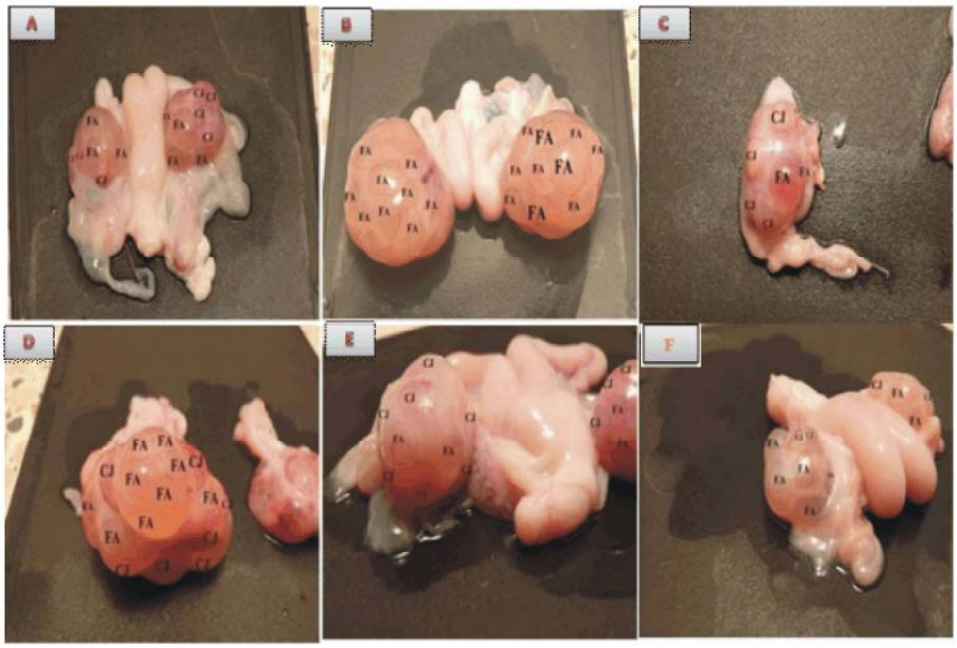

Fig. 4: Ewes' ovary in T3*.
T2*(PMSG 1000IU + 2 injections of hCG); CL (Corpora Lutea); AF (Anovulatory Follicles); A, C, D and E (superovulated ewes); B and F (non-superovulated ewe).

Results obtained in this study show that the mean AF diameter increased in T3 group (12.85±4.92mm), compared to T1 and T2 groups (12.5± 2.16 and 9.66 ± 1.75mm). No data were available in the literature regarding the effects of repeated administration of hCG or GnRH on follicle diameter in ewes.

GnRH or hCG treatment increased (P<0.05) mean serum P_4 concentrations on the 8th day post-sponge removal, this is confirmed, when compared T1 with T2 and T1 with T3 groups. GnRH through LH release and hCG by its LH like activity may provide luteotrophic stimulation to CL[19]. hCG treatment in sheep has been linked to elevated numbers of large luteal cells and a concomitant reduction in the number of small luteal cells, accompanied by increased serum P_4 concentrations. The administration of hCG in the early luteal stage induces the formation of accessory corpora lutea, increases the surface area and the volume of the CL and may, or may not increase the diameter of CL. It also encourages luteal cells to become larger and rise serum P_4 concentrations. This rise mainly due to secretion by accessory CL besides the stimulation of the spontaneous CL[39].The mechanism by which GnRH and hCG stimulate luteal function may be similar [19]. hCG and GnRH have similar effects on the ovary, but hCG acts independent of the pituitary gland and has a longer half-life than natural LH [39].

In the present study, the number of CL was positively correlated to the serum P_4 concentrations (r=0.69). Windorski *et al*. [32] reported a positive relationship between number of CL and serum P_4 level. According to Amiridis *et al*. [40], the serum P_4 concentrations cannot predict exact number of CL trained. While, A negative correlation between the number of AF and the serum P_4 concentrations was recorded (r= - 0.40). According to Veiga-Lopez *et al*. [33], most of the AF showed signs of functionality failures, either immaturity or atresia, as indicated by a low intrafollicular estradiol concentration. However, 22.4% of them were highly estrogenic (>200 ng/ml) and their permanence beyond the occurrence of ovulation was related to a drop in the fertilization rate, leading to decreased final superovulatory yields.

The presence of sheep without or with very few ovulations still remains as one of the main causes of the high variability in multiple ovulation and embryo transfer (MOET) yields. Evidence exists that the large variation in superovulatory response may be due in part to the differences in the developmental stage of follicles present in the ovary at the beginning of treatment [8,41, 42]. The response to superovulation is considered related to the presence of a large (dominant) follicle or the presence/absence of corpora lutea at the start of or during

superovulation treatments. Dominant follicles impair the development of smaller gonadotrophin-dependent follicles by suppressing FSH and inducing their atresia [43]. Possible causes may be related to a deficient or inexistent preovulatory LH surge, or to the presence of non-responsive follicles, due to a down-regulation of the granulosa and theca LH receptors [33].

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