

Effect of Repeated Administration of hCG on Ovarian Response in PMSG-superovulated Ouled Djellal Ewes (Algeria)

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Summary

The objective of this study was to evaluate the effect of repeated administration of hCG on ovarian response in PMSG-superovulated ewes. Intravaginal pessaries containing 40 mg fluorogestone acetate (FGA) were inserted in all ewes (n=9) 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 2 treatments. The first group (n=3) did not receive any hCG, while the second group (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 (P4) using radioimmunoassay (RIA) method. The results obtained for first and second group was in number of CL (6.33 ± 1.15 and 10.50 ± 5.54), number of AF (2 ± 3.46 and 4.16 ± 5.70), then the levels of P4 (5.75 ± 4.45 and 13.22 ± 6.80 ng/ml), respectively. These results indicate that the repeated administration of hCG post-sponge removal increases number of CL and improves luteal function in ewes after PMSG-superovulatory treatment.

Résumé

Effet de l'administration répétée de l'hCG sur la réponse ovarienne chez la brebis « Ouled Djellal » superovulée avec de la PMSG (Algérie)

L'objectif de cette étude est de déterminer l'effet de l'administration répétée de l'hCG sur la réponse ovarienne chez la brebis « Ouled Djellal » superovulée avec de la PMSG. Après synchronisation des chaleurs, par des éponges vaginales de 40 mg de FGA pendant 14 jours, 2 jours avant le retrait des éponges toutes les brebis (n=9) reçoivent une injection de PMSG (1000 UI). Au jour de retrait des éponges (J0), les brebis ont été réparties en 2 lots, le premier (n=3) n'a reçu aucune injection de l'hCG, cependant le deuxième lot (n=6) a reçu une double injections de l'hCG (500 UI) au jour 0-2. Le contrôle de la réponse ovarienne est effectué au 8^e jour, par comptage des structures ovariennes par laparotomie : Corps Jaune (CJ), Follicules Anovulatoires (FA) et par un dosage radio immunologique de Progestérone (P4). Les résultats obtenus dans le 1^{er} et le 2^e lot sont respectivement en nombre de CJ ($6,33 \pm 1,15$ et $10,50 \pm 5,54$), en nombre de FA ($2 \pm 3,46$ et $4,16 \pm 5,70$), et les niveaux moyens de P4 ($5,75 \pm 4,45$ et $13,22 \pm 6,80$ ng/ml). Les résultats obtenus dans cette étude montre que l'administration répétée de l'hCG après le retrait des éponges vaginales augmentent le nombre de CJ et améliore la fonction lutéale chez la brebis superovulée avec de la PMSG.

Introduction

Superovulation plays an important role in the embryo transfer (ET) programs. It aims at inducing a high number of ovulations and a high yield of embryos of good quality (4). Any treatment regimen designed to induce multiple follicular development

must override a physiologic system operating to permit only a single follicle to mature for ovulation. Thus, treatment must be initiated prior to the emergence of the dominant follicle. Additionally, the stimulation must not only promote follicular development but must also provide normal

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development of granulosa and thecal cells so that luteal function being normal (12).

Superovulation protocols allow taking advantage of the relatively short gestation length of sheep and utilize the ewe to her fullest potential (5). 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 (4).

The pregnant mare's serum gonadotrophin (PMSG) act as analog to the hypophyseal gonadotropin FSH (10).

Human chorionic gonadotropin (hCG), which is similar to LH in function, causes ovulation in female animals. Due to its high content of sialic acid, the half life of hCG (about 8 hours) is much longer than that of LH, ranged between 12 to 50 min. hCG produces an effect similar to LH on luteal cells since it managed by the same receptors as hypophyseal gonadotropin (10). Thus, possibility of utilizing hCG associated with PMSG for inducing superovulation has to be verified.

Four gonadotropic preparations (human menopausal gonadotropin, human follicle stimulating hormone, pregnant mare's serum gonadotropin, and porcine FSH), in combination with human chorionic gonadotropin, are successful in inducing multiple follicular develop. Treatment with these gonadotropin preparations, in combination with hCG, results in multiple follicular development (12).

The aim of the present study was to test the hypothesis that the repeated administration of hCG at a dose level of 500 IU after sponge removal, in PMSG-superovulated Ouled Djellal ewes, may improve ovarian response and enhance serum P4 concentration at the time of embryo collection.

Materials 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=9) were used. They averaged 25 months old, 55 kg 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, Netherlands), two days before sponge withdrawal (1). On the day of sponge removal (day 0) females were randomly assigned to two treatments, the first group (n=3) did not receive any hCG treatment, while the second group (n=6) received a double injection of hCG (500 IU) (Chorulon®, Intervet International, Boxmeer, Pays-Bas) at days 0 and 2 (Figure 1) (7).

Control of ovarian response

a. 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.

b. 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 2000xg, the serum was aspirated and frozen at -20°C, until assayed. Concentrations of P4 were measured by radioimmunoassay analysis (RIA) (13). 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 P4 concentrations on day 8.

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 (11.11%) did not respond to PMSG induction for superovulation, which was manifested by the presence of 1 to 4 corpus luteum (CL) after PMSG treatment. However, the percentage of superovulated ewes was 88.88% which observed 8 days post-vaginal sponge removal by laparotomy (Figures 2 and 3).

Yields are decreased by the presence of females not bearing any ovulation and ewes with very low ovulatory responses after the exogenous hormone supply (11). The superovulated ewes has more than 4 CL (5, 13).

The treatment of ewes with double injection of Human chorionic gonadotrophin (hCG) increases The mean number of CL in T2 group (PMSG 1000 IU+2 injections of hCG) (10.50 ± 5.54) than in T1 (PMSG 1000 IU) (6.33 ± 1.15). The mean number of anovulatory follicles (AF) tended to be greater in hCG treated group (T2) (4.16 ± 5.70) than in control

ewes (T1) (2.00 ± 3.46) (Table 1).

The number of CL induced by the hCG treatment were significantly greater ($P < 0.05$) in the T2 group than the T1. However, no significant differences noticed between the number of AF in T1 and T2 groups (Table 1).

The presence of AF was early described by Grant *et al.* (6). Its incidence can reach 50% of the preovulatory follicles with some treatments. According to Veiga-Lopez *et al.* (11) the incidence of anovulation in superovulated sheep can reach up

Table 1

The mean effect of repeated administration of hCG on luteal and follicular characteristics in PMSG-superovulated Ouled Djellal ewes.

Variable	T1*	T2*
Number of ewes	3	6
Ewes in oestrus (%)	(3/3)100	(6/6)100
Average number of CL*	6.33 ± 1.15	10.50 ± 5.54
Average number of AF*	2.00 ± 3.46	4.16 ± 5.70
Average of TR*	8.33 ± 4.16	14.66 ± 2.33
Average of AF rate	15.38 ± 26.64	27.46 ± 37.87
Average of ovulation rate	84.61 ± 26.65	72.53 ± 37.87
Average of AF diameter (mm)	12.5 ± 2.16	12.85 ± 4.92
Average of P4* level (ng/ml)	5.75 ± 4.45	13.22 ± 6.80

T1* (PMSG 1000IU); T2* (PMSG 1000IU + 2 injections of hCG); CL* (Corpus Luteum); AF* (Anovulatory Follicles); TR*(Total Response); P4*(Progesterone).

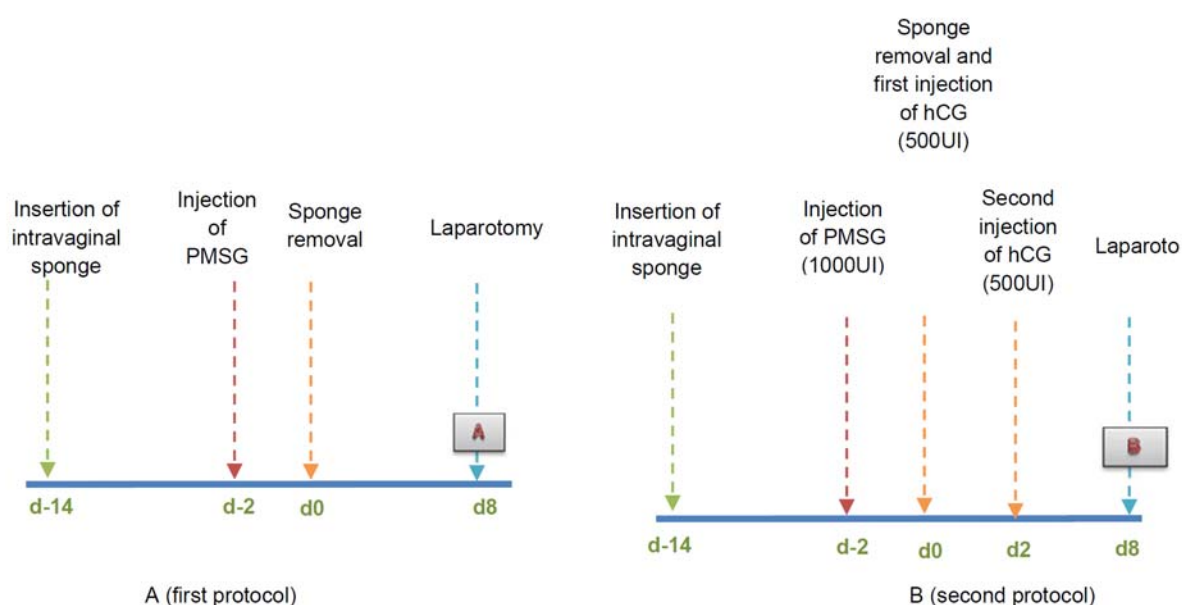


Figure 1: First and second protocol.



Figure 2: Ewes' ovary in T1*.

T1*(PMSG 1000IU); CL (Corpora Lutea); AF (Anovulatory Follicles); A, B and C (superovulated ewes).



Figure 3: Ewes' ovary in T2*.

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

to 34.6% of the preovulatory follicles. However, in the present study, the incidence of AF was 15.38% and 27.46% in both treatments T1 and T2, respectively.

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

The serum P4 concentrations were significantly higher ($P < 0.05$) in T2 group, compared to T1 group on day 8 after intravaginal sponge removal (13.22 ± 6.80 vs. 5.75 ± 4.45 ng/ml), respectively (Table 1). Human chorionic gonadotrophin (hCG), with its LH-like activity, may provide luteotrophic stimulation to the CL. This luteotrophic stimulation may either be in the form of conversion of small luteal cells to large luteal cells, or may even be ascribed to an increase in the size of large luteal cells, hCG has been shown to increase luteal weight and endogenous synthesis of progesterone (P4) from the CL in sheep (7). 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 plasma P4 concentrations (9). Although the luteal cell ratio and luteal size were not assessed in the present study.

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 plasma P4 concentrations. This rise was mainly due to secretion by accessory CL besides the stimulation of the spontaneous CL (9).

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.

The number of CL was positively correlated to the serum P4 concentrations ($r = 0.75$). Windorski *et al.* (13) reported a positive relationship between number of CL and serum P4 level.

In our results, a negative correlation between the number of AF and the serum P4 concentrations was recorded ($r = -0.37$). According to Veiga-Lopez *et al.* (11), 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.

In this study, approximately 11.11% of ewes did not respond to the superovulation treatment. In agreement with our data, Windorski *et al.* (13) report that about 20% of ewes did not respond to superovulatory treatment.

There is evidence that the superstimulatory ovarian response in ewes may be increased when eCG is administrated several days prior to progesteragen sponge withdrawal. According to Ali (1), administration of eCG 2 days before intravaginal sponge removal in ewes induced an increase in the number of small follicles (< 3 mm) on day 3 after sponge removal.

In this study, it was not possible to reduce the number of AF. The administration of hCG at the onset of estrus in ewes superovulated with PMSG, was unable to reduce the number of AF. 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 (7).

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. 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 (11). Ovulatory failure may be ascribed to asynchronous follicular growth, which could ultimately result in a lack of LH receptors in those follicles that are in the earlier stages of development at the time of the endogenous preovulatory LH peak (8).

The causes of these partial ovulation failures may be also related to altered patterns of the LH secretion, since high LH concentrations for extended periods before LH surge has been found to disturb the ovulatory ability of follicles. However, AF might appear even if the LH surge follows a normal pattern, and alterations in the follicular status at the onset of the superovulatory treatment and/or their growth would be the main causes. Follicles in early atresia can be rescued and stimulated to growth by exogenous gonadotrophins; however, their development has been associated with a lower ability to ovulate (3). 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 (8).

It has been hypothesized that a lack of superovulatory response by some ewes is due to heterogeneity in the morphological feature of the ovulatory follicles or to the number of small antral follicles present in the ovaries when superovulatory treatment was initiated (5). 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 (2). Multiple ovulation output is also decreased by the presence of females with

ovaries joining ovulations and anovulatory follicles.

Conclusion

In conclusion, the present data obtained on PMSG-superovulated Ouled Djellal ewes confirm that, we can improve superovulation response by repeat administration of hCG. However it was not possible to reduce the number of AF. Further detailed studies are needed to clarify the effect of repeated hCG administration on not only the plasma P4 production and CL characteristics, but also on the quality and quantity of embryos produced.

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