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Effects of Repeated Use of PMSG on Reproductive Performances of the Ouled Djellal Ewes

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Abstract: The objective of this research was to study the effects of repeated use of oestrous induction/ synchronisation hormones (FGA+PMSG) on oestrous cycles and reproductive performances of Ouled Djellal ewes. A total of 56 adult ewes were divided into 4 groups where: the first group included 15 ewes that have not previously received hormonal Treatments (T1: used as control ewes), the second group included 20 ewes that received two successive Treatments (T2), the third group included 16 ewes that received three times successive Treatments (T3) and the last group included 5 ewes and that received four successive Treatments (T4). Groups were homogenous with respect to mean age, live weight and body condition score. Consecutive PMSG administrations were 8 months apart. Measures of anti-PMSG antibody concentrations were obtained using radio-immuno-assay on plasma samples taken before treatments begun (FGA deposit corresponds to j = 1) and at 7, 14, 18, 25, 30, 35 and 40 days after PMSG administration. Gestation diagnosis was realised by dosing PAGo. Fractions of individual binding following PMSG injections (day 7 to day 40) were small and reached rarely 12%. There were no significant differences among treatments ($p>0.05$) with respect to anti-PMSG antibody concentrations. Furthermore, regardless of the treatment number, accumulated results for classes (<3-7%) of anti-PMSG antibody treatments showed no depressing effects of residual antibodies on oestrus activities of ewes compared to the control treatment. However, negative effects of residual anti-PMSG antibodies on ewes' reproductive behaviour were observed for the 3-7% class. Repeated use of PMSG at 8 month intervals seemed not to affect reproductive performances of ewes in natural breeding mode. It should be possible to induce synchronised oestrus cycles of Ouled Djellal ewes by repeated use of PMSG without compromising ewes' reproductive performances.

Key words: Ouled Djellal ewes, synchronisation, radio-immuno-assay, anti-PMSG antibody, reproductive performances

INTRODUCTION

The Pregnant Mare Serum Gonadotrophin (PMSG), known actually as Equine Chorionic Gonadotrophin (ECG), is a glycoprotein from the placenta (Moore *et al*., 1980). This hormone is involved in sustaining gestation in mares. It is found in the mare's serum between the 37th and the 40th day after conception. The use of PMSG with and without Artificial Insemination (AI) is common in sheep (Clarke, 1973; Gherardi and Martin, 1978; Jabour and Evans, 1991). When used, PMSG is usually preceded by applying a progesterone treatment during a period equivalent to that of corpus luteum persistence in a regular oestrous cycle. This treatment consists of depositing a sponge imbedded with Fluorogestone Acetate (FGA) in the ewe's vagina. Reasons for the use of exogenous gonadotrophins on farm species are induction and synchronisation of oestrous and ovulation, super ovulation, stimulation of follicle growth and maturity and consequently the improvement of fertility. PMSG is widely used for oestrous synchronisation because of its lasting effects and reduced costs (Drion *et al*., 1998). However, in recent years, repeated use of PMSG was interrogated in some species. Multiple PMSG treatments may produce anti-PMSG antibodies that may compromise its effectiveness (Beckers *et al*., 1990; Bodin *et al*., 1995).

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Possible causes for reduced responses to hormonal treatments in sheep and goats (Remy *et al*., 1991), cattle (Jainudeen *et al*., 1966; Alwan *et al*., 1988) and guenon (Bavister *et al*., 1986) may be acquired immunity against exogenous gonadotropins (Clarke, 1973; Gherardi and Martin, 1978; Boland and Gordon, 1982). These residual anti-PMSG antibodies, resulting from repeated use of gonadotrophins (Maurel *et al*., 1994; Roy *et al*., 1999) are a means of resistance to similar treatments in the future (Bodin *et al*., 1995). Late oestrous and ovulations may be caused by reduced biological activities of PMSG following the surge of anti-PMSG antibodies from preceding treatments that females have undergone (Baril *et al*., 1996). These depressive anti-PMSG antibody effects will deteriorate reproductive performances especially when associated with AI. These depressive effects (anoestrous females, late oestrous) become important when the rate of anti-PMSG antibody liaisons is higher than 5% (Baril *et al*., 1996).

The aim of this research was to study the effects of the repeated use (every 8 months) of FGA associated with PMSG on Ouled Djellal ewes' reproductive performances.

MATERIALS AND METHODS

Animals and management conditions: A total of 56 multiparous Ouled Djellal ewes were used in this study. Ewes were dry in the beginning of the experiment. They were partitioned into four groups homogeneous with respect to mean age: = 4.1±0.13-5.1±0.29 year; mean live weight: 46.19±1.59 to 47.6±2.52 kg and mean body condition score: 2.7±0.22 to 2.79±0.08. The first group (T1) included 15 ewes that have not previously received hormonal Treatment (T1: used as control). The second group of 20 ewes that received 2 identical successive hormonal Treatments (T2), the third group included 16 others that received three times successive Treatments (T3) and the last group included 5 ewes and received four successive Treatments (T4). All groups of ewes received the same hormonal treatment. Ewes were isolated from rams and received an anti-parasite treatment at least 45 days before the breeding period. Animals were logged in the experimental station at the Baba Ali institute «Institut Technique des Elevages de Baba Ali », in the Mitidja region almost 30 km south-west of Algiers. Rations, formulated to meet animals' feed requirements were based on vetch-oat hay and a commercial concentrate. Pasture was also available on summer (straw) and spring (green roughages) seasons. Live weights and body condition scores were recorded right before depositing the imbedded sponge into ewes' vagina. Weights were determined with a 100 kg balance (precision =0.5 kg) and body scores were attributed on a 0-5 scale (Russel *et al*., 1969).

Oestrous synchronisation and breeding: Oestrus cycles were induced simultaneously every 8 months using grey sponges with 40 mg FGA during thirteen days. Right after the sponge withdrawal, each ewe was injected with a unique dose of PMSG (500 UI). The onset of oestrus was detected 48 h after PMSG injection using breeding rams. Breeding was controlled and occurred in February (1st, 2nd and 3rd injections) and in October (2nd, 3rd and 4th injections) in 2005, 48-56 h following PMSG injection.

Anti-PMSG antibody and pregnancy associated glycoprotein determination

Anti-PMSG antibodies: Duplicate blood samples were collected using disposable needles on 5 mL EDTA tubes when sponges were deposited and at 7, 14, 18, 25, 30, 35 and 40 days following PMSG injection for the 4 treatments. Blood samples were centrifuged (3.000 tours min^{-1}) during 12 min. Then, plasma samples were kept in a freezer at -20°C.

Radio-Immunologic dosage of Anti-PMSG antibodies (RIA) and gestation proteins (PAGo) were determined on congealed plasma samples at the University of veterinary medicine, Liège, Belgium. Plasma samples were diluted and a coloured antigen was added. At equilibrium, an antiserum anti-immunoglobulin was added in addition to PEG in order to have a complete precipitation of plasma antibodies. Free hormones were separated from linked ones by centrifugation and washing. Radioactive bases, proportionate to plasma gonadotrophin antibody concentrations, were determined using a gamma counter. Finally, results were expressed in % binding with the marked hormone introduced in each tube.

Dosage of antibody concentrations was conducted as follows: Duplicate plasma samples were diluted to the tenth in a Tris-BSA buffer: The buffer was a Tris- HCL (300 g of Tris; 20 g of $Mgcl_2$ and 2 g of sodium in 10 L of distilled water) with a pH of 7.5 and was added 2 g of bovine serum albumin (BSA). The same buffer was also used for the antiserum and PEG dilution. Only samples of 500 μL were incubated. Each sample was obtained adding reagent as follows: firstly 300 μL of Tris-BSA; followed by 100 μL of diluted plasma and finally 100 μL of ^{125}I eCG equivalent to 10.000 CPM. Sample incubation last a whole night at room temperature, then 100 μL of a solution of goat anti –immunoglobulin was added to each sample: it's the first antibody. Incubation was then carried out for 3 h at the same conditions (20°C). Therafter, 500 μL of PEG (PEG Mw 10.000 Merck Inc; Darmstadt and F.R. Germany) was added to enhance Ag-Ac precipitation. PEG was used at 4% (weight/volume) in a Tris-BSA buffer. Two hours following the addition of PEG, the mixture was centrifuged at 2.500 g during 15 min and floating bases were delicately collected. Collected bases were washed out using 3 mL of buffer and centrifuged

again for 15 min. Following the elimination of surface (floating), radioactivity was measured using Multigamma counter (LKB Wallac 126 Multigamma counter, Turku, Finlande).

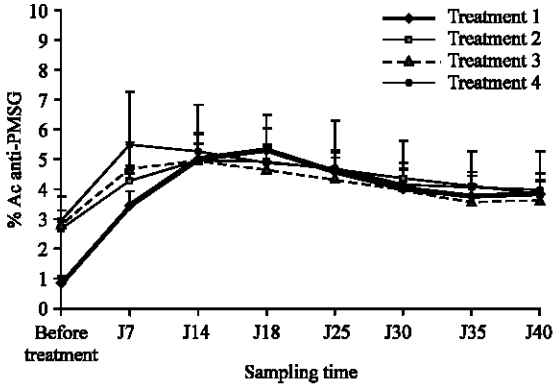
Pregnancy associated glycoprotein: The dosage of PAGo was realised using semi-homologous RIA (Benyounes *et al*., 2006). This dosage was done on the 23rd day post breeding for gestation diagnosis. PGAo concentration was determined using bovine PAG ($boPAG_{67kDa}$) (Zoli *et al*., 1991) as a tracer and a primary ovine anti PAG antibody ($ovPAG_{57+59kDa}$; AS#780, initial dilution was 1: 40.000). Results were expressed in ng mL^{-1}.

Gestation diagnosis and lambing follow-up: Diagnosis of gestation was realized 23 days post breeding by the dosage of PAGo. Any ewe with PGAo concentration in the plasma greater than 1.2 ng mL^{-1}, was considered pregnant (Verberckmoes *et al*., 2004; Benyounes *et al*., 2006). Gestation was monitored for possible abortion and lambing dates, litter size, lamb deaths and lamb sex were recorded. PAGo concentration, abortions and lambing served for the determination of conception dates.

Statistical analysis: Means of anti-PMSG antibodies at various times within each treatment were compared using the Student t test for dependant samples, while between treatment means were compared by the Duncan multiple range test (Minitab, 2000). On the other hand, frequencies (reproductive measures and proportions of elimination and accumulation of anti-PMSG antibodies) were tested using the chi square (χ^2) test.

RESULTS

Levels of temporary binding of anti-body anti PMSG: The evolution of anti-PMSG antibodies of ewes receiving 1, 2, 3, or 4 500 UI PMSG doses is illustrated in Fig. 1. The mean base level of anti- PMSG antibodies for T1 ewes was 0.85±0.23% varying from 0-2.17%. Almost 77% of ewes had a -1.5% level. Two ewes of this control group had unacceptable levels and were excluded from the analysis. Except for the base level, there were no differences ($p>0.05$) among antibody means of various treatments in all sampling times. Furthermore, individual binding levels between time intervals from PMSG injection (d7-d40) were low. These linkage levels reached 9-12% for the various treatments, which is equivalent to a 1-3% presence of this threshold. Mean antibody levels at various times following PMSG injection were statistically similar varied from 3.45±0.48% to 5.29±0.76%; 3.82±0.47% to 4.95±0.55%; 3.59±0.55% to 4.95±0.85% and 3.98±1.28% to 5.48±1.78% for T1, T2, T3 and T4, respectively, (Fig. 1).



***Treatment 1: A single hormonal treatment, control ewes; Treatment 2: Two successive hormonal treatments; Treatment3: three successive hormonal treatments and Treatment 4: four successive hormonal treatments. Each two consecutive administrations are 8 months apart**

Fig. 1: Evolution of liaison level (%) of anti body anti-PMSG for the 4 treatments

Trends of temporary evolution of antibody anti PMSG binding levels were similar for all treatments. These trends showed an increasing phase from base (control ewes) or residual levels (0.85±0.23%) till that developed at day 7 (4.67±0.91%) following PMSG injection. Then antibody mean rates picked between day 7 and day 18 to reach values between 4.95±0.55% and 5.48±1.78%, suggesting high liaison levels. Peak levels were followed by a lengthy decreasing phase between day 18 and day 40 (antibody means were between 3.59%±0.55 and 4.93±0.56%) (Fig. 1 and Table 1).

Anti-PMSG antibody peak levels were higher ($p<0.0001$) for all treatments compared to that recorded at base or residual levels except for T4 ($p = 0.059$). Antibody levels at day 40 for all treatments were similar ($p>0.05$) to residual levels but significantly lower than those recorded in the peak ($p<0.05$) (Fig. 1 and Table 1).

Residual antibody mean levels (Fig. 1 and Table 1) obtained after treatments T1, T2 and T3 were comparable ($p>0.05$). They ranged between 2.66%±0.31% and 2.92%±0.84%. These levels were however higher ($p<0.01$) than those recorded for control ewes which have not previously been injected by PMSG. On the other hand, antibody binding levels at day 40 were identical for all treatments (varied between 3.64±0.61% to 3.98±1.28%) (Fig. 1 and Table 1).

Accumulation antibody rates, compared to residual levels before each PMSG injection, were similar for treatments T2, T3 and T4 ($p>0.05$); while that accumulated following T1 was higher ($p<0.001$) than the base level (Table 1). The accumulation was important following the first PMSG injection and remained practically unchanged as the number of treatments increased.

Table 1: Accumulation and elimination rates of anti-PMSG antibodies for the 4 treatments

	Anti-PMSG antibodies (%)		Accumulated (%)		Elimination (%)		
Treatment (ewes)	Residual	peak	d40	Residual-peak	Peak-d40	d40-residual	Peak-residual
T1 (15)	0.85^{*a1}	5.29^{2} (18j)	3.85^{1}	522.35^{a}	27.22 (22j)	30.91 (45j)	49.72 (45j)
T2 (20)	2.66^{b1}	4.95^{2} (14j)	3.82^{1}	86.09^{b}	22.83 (26j)	26.96 (45j)	43.64 (45j)
T3 (16)	2.79^{b1}	4.95^{2} (14j)	3.64^{1}	77.42^{b}	26.47 (26j)	19.78 (45j)	41.01 (45j)
T4 (05)	2.92^{b12}	5.48^{1} (7j)	3.98^{2}	87.67^{b}	27.37 (33j)	26.63^{**} (45j)	46.72^{**} (45j)

Means on the same colon with the same letter and means on the same line with the same superscript are not significantly different ($p<0.05$), (d) Day of the peak; Peak-d40 interval; d40-residual interval; Peak-residual interval, * Base level before treatment, ** Expressed by comparison to treatment 3 residual, Residual:% of antibodies in the blood from the preceding treatment right before the following treatment was applied, *Treatment 1: A single hormonal treatment, control ewes; Treatment 2: Two successive hormonal treatments; Treatment3: three successive hormonal treatments and Treatment 4: four successive hormonal treatments. Each two consecutive administrations are 8 months apart

Table 2: Reproductive parameters in relation to residual antibodies following repeated use of PMSG on Ouled Djellal ewes

		Treatment*			
Parameter (%)	Residual antibodies (%)	T1	T2	T3	T4
Oestrus	<3	80^{a}	92.86^{b1}	80^{a1}	75^{a1}
	3 -7	-	66.67^{a2}	66.67^{a2}	100^{b2}
	<3 -7	80^{a}	85^{a}	75^{a}	80^{a}
Fertility	<3	66.67^{ab}	85.71^{c1}	60^{b1}	75^{ac1}
	3 -7	-	33.33^{a2}	66.67^{b1}	100^{c2}
	<3 -7	66.67^{b}	70^{ab}	62.5^{b}	80^{a}
Litter size	<3	130^{a}	125^{a1}	167^{b1}	133.3^{a1}
	3 -7	-	100^{a2}	175^{b1}	100^{a2}
	<3 -7	130^{a}	121.43^{a}	170^{b}	125^{a}

Means on the same colon with the same letter and means on the same line with the same superscript are not significantly different ($p<0.05$), *Treatment 1: A single hormonal treatment, control ewes; Treatment 2: Two successive hormonal treatments; Treatment3: three successive hormonal treatments and Treatment 4: four successive hormonal treatments. Each two consecutive administrations are 8 months apart

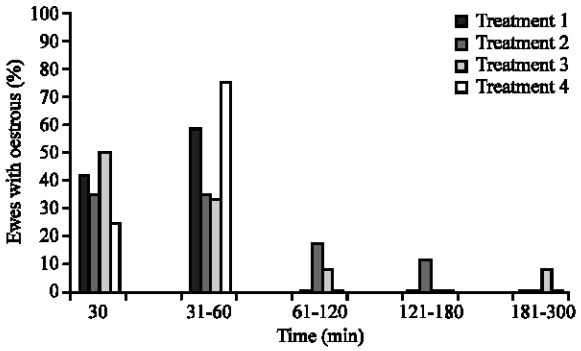
Table 3: Delay (in % of ewes) in oestrus the incidence beyond 48 h following four PMSG treatments* for <3 and 3-7 classes of residual antibodies

	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
Time (mn)	<3	3-7	<3	3-7	<3	3-7	<3	3-7
≤30	40	-	38.46	25	50	50	-	100
31-60	60	-	38.46	25	37.5	25	100	-
61-120	-	-	15.38	25	-	25	-	-
121-180	-	-	7.69	-	-	-	-	-
181-300	-	-	-	25	12.5	-	-	-

*Treatment 1: A single hormonal treatment, control ewes; Treatment 2: Two successive hormonal treatments; Treatment3: three successive hormonal treatments and Treatment 4: four successive hormonal treatments. Each two consecutive administrations are 8 months apart

Anti-PMSG antibody peak levels occurred late for T1 (d18), were medium for T2 and T3 (d14) and were early for T4 (d7). Nevertheless, elimination rates of antibodies recorded at d40 (peak-d40), right before the PMSG injection (d40-residual and peak-residual) were similar ($p>0.05$). They ranged between 22.83 and 27.22%; 19.78 and 30.91% and 41.01 and 49.72% for peak-d40; d40-residual and peak-residual, respectively (Table 1).

Effects of repeated usage of PMSG on reproductive parameters: Reproductive performances recorded for ewes following PMSG treatments are given in Table 2. Oestrus occurrences following all PMSG treatments were statistically similar (Table 2) ranging from 75-85%. Comparison among treatments within the same anti-PMSG antibody class revealed that T3 and T4 resulted in responses (oestrus occurrence) comparable to that of the control treatment for the <3% class (Table 2). Responses following T2 (92.9%) were higher than those following T1 ($p<0.01$). In the 3-7% class, T4 was followed by ewe responses higher ($p<0.0001$) than those generated by T2 and T3. Comparison between effects of high and low antibody classes within the same treatment showed that the <3% class was associated with high oestrus rates. This class rates were 92.86 and 80% and 66.67 and 66.67%, for T2 and T3, respectively. However, the opposite was observed for T4, for which high ($p<0.0001$) oestrus rates were found in the 3-7% class. On the other hand, the distribution of oestrus appearance seemed to be similar for all treatments at 60 mn (Fig. 2), except for T2 and T3 where some ewes showed oestrus between 61 and 120 mn (17.7%), 121 and 180 mn (11.8%), 61 and 120 mn (8.3%) and 181 and 300 mn (8.3). Similar distributions of oestrus were observed for cumulative frequencies and by classe of anti-PMSG residual antibodies, particularly at 60 mn intervals, except for T2 and with lesser degree T3 (Fig. 2 and Table 3). In fact, regardless of the residual antibody class, 100% of ewes had oestrus at 60 mn



*Treatment 1: A single hormonal treatment, control ewes; Treatment 2: Two successive hormonal treatments; Treatment3: three successive hormonal treatments; and Treatment 4: four successive hormonal treatments. Each two consecutive administrations are 8 months apart

Fig. 2: Delay in oestrus incidence beyond 48 h following PMSG injection for the four treatments

intervals for the control and those having been treated three times before. Only 77 and 87.5% and 50 and 75% of ewes had oestrus at similar times for T3 and T4 in the <3% and 3-7% residual antibody classes, respectively. That is equivalent to 27 and 12.5% in favour of low residual antibody levels (Table 3).

There were no depressing effects of residual antibodies on fertility measures. Similar results ($p>0.05$) were obtained for treatments T1, T2 and T3; while T4 has resulted in higher ($p<0.05$) fertility compared to T1 and T3 (Table 2). Comparison of fertility among treatments within antibody classes showed that T3 and T4 had similar effects compared to control in the <3% class ($p>0.05$). On the other hand, T2 had effects higher than those of T3 and control treatments on fertility ($p<0.001$). Inversely, T4 and T3 effects (Table 2) were higher than those of the rest of treatments in the 3-7% residual antibody class ($p<0.0001$). Comparisons among different class levels of residual antibodies within treatments showed no depressing effects on fertility. Fertility measures were in general comparable among various antibody classes for all treatments with those corresponding to the 3-7% being the highest ($p<0.001$), except those recorded following T2 for the same antibody class, which were the lowest (Table 2).

For the prolificacy, its rates for the treatments 2 and 4 were clearly similar to the control ($p>0.05$); while T3 resulted in the highest prolificacy rate ($p<0.0001$) (ranged between 121.43 and 130% vs 170%). It seems that residual antibodies did not negatively affect the prolificacy rates. There was no clear tendency comparing prolificacy rates among treatments within similar antibody classes. While comparison among antibody classes within treatments showed that the <3% class was the most favourable for high ovulation rates for T2 and T4 ($p<0.01$).

DISCUSSION

Anti-PMSG antibody liaison levels: Anti-PMSG antibodies were reported for various species, cattle (Jainudeen *et al*., 1966); goats (Baril *et al*., 1992a; 1992b); rabbits (Canali *et al*., 1991) and sheep. For the latter, they were found in the Finssheep (Rainio, 1992) and Lacaune (Brice *et al*., 1995; Bodin *et al*., 1997) breeds. Antibodies were studied in the various species following different PMSG injection protocols.

Antibody base level corresponds to that recorded before the first PMSG injection while the liaison level corresponds to that of antibody following the preceding PMSG administration. In this study, the liaison antibody level observed for control ewes (mean level was 0.85±0.23% with 77% of ewes having <-1.5% levels) was slightly higher than levels reported for the Lacaune (mean level was 0.69% with 95% of ewes having <1.5%) breed (Brice *et al*., 1995; Bodin *et al*., 1997). Furthermore, there were no significant differences among treatments for low antibody levels (Fig. 1). Liaison levels rarely reached 9-12% in contrast to reports on goats where the effects of PMSG treatments on antibody production where highly significant (Drion *et al*., 2001). Likewise, the residual antibody levels produced after one, two and three successive hormonal treatments were comparable and were higher than that of control ewes (T1), witch have not been treated before ($p<0.01$). Differences between antibody levels from repeated (T2, T3 and T4) and first PMSG usage disappeared in day 40 (mean levels ranged from 3.64±0.61% to 3.98±1.28%) (Fig. 1 and Table 1). Significant differences between the peak and day 40 antibody levels ($p<0.05$) for all treatments and comparable residual antibody levels for ewes having 1, 2 or 3 preceding PMSG treatments suggest that Ouled Djellal ewes were able to eliminate antibodies incurred from previous PMSG treatments. These results are contradictory to other reports on sheep (Rainio, 1992; Brice *et al*., 1995; Bodin *et al*., 1997) and goats (Baril *et al*., 1992a; 1992b; 1996; 1998). These authors found different residual antibody levels before any new PMSG treatment. They suggested that the elimination process of residual antibodies, by ewes and goats between two consecutive PMSG treatments, is slow, which does not seem the case for the Ouled Djellal breed. The Ouled Djellal ewes manifested low accumulation peak (Fig. 1) compared to the Lacaune breed for example (Brice *et al*., 1995; Bodin *et al*., 1997). In fact, regardless of the timing of peak, 7th to the 18th day following PMSG injection, at day 40 (peak-j40), as before the current PMSG treatment, ewes of this study efficiently eliminated residual antibodies (peak-residual) by 41-50%, which may explain similarities between binding rates observed during these phases ($p>0.05$) (Fig. 1 and Table 1).

Trends of temporary evolution of antibody anti PMSG binding levels were similar for all treatments. It showed three distinct phases, an increasing phase (between residual antibody levels and 7 day following PMSG injection, a plateaus (day 7 till day 18) and a decreasing phase (day 18 till day 40). The kinetic of these antibody levels correspond to the secondary immuno-humeral response scheme, a short latent phase, a second phase where response is steady and higher than the primary one and a third decreasing lengthy phase (Pastoret *et al*., 1990). Peak antibody binding levels were late for ewes receiving a single PMSG treatment (day 18 for control ewes), early for ewes with frequent PMSG treatments (day 7 for T4 ewes) and intermediate (day 14) for T2 and T3 ewes. This may explain the immunisation and accumulation of anti-PMSG antibody by treated animals. There was however a great variation among ewes (Fig. 1) with respect to residual antibody levels and their evolutions within the same treatment (for T2 or T3) in the same period (peak-day 40). This is in agreement with reports on goats (Roy *et al*., 1999; Baril *et al*., 1998) suggesting great variability in the immune response to PMSG.

Repeated use PMSG may result in a reduced efficiency in oestrus synchronisation in goats (Baril *et al*., 1992a; 1992b; 1998) and in sheep (Brice *et al*., 1995). Results from experiments on oestrus induction and synchronisation were variable (Folch *et al*., 1982; 1985; Khaldi and Lassoued, 1988). A first PMSG use seems to be the most effective in synchronizing. In this study, all ewes manifested oestrus in a 1 hour period 48 h following PMSG injection in agreement with many works (Dutt, 1953; Cullen *et al*., 1968; Chupin and Mauleon, 1976; Lahlou Kassi, 1987; Gouro and Yénikoye, 1991; Boly *et al*., 2000). PMSG has a like FSH (Follicle Stimulating Hormone) action on follicle growth, on oestradiol level and oestrus. Furthermore, PMSG has a tendency to lengthen oestrus duration following the increase in the number of maturing follicles (Land *et al*., 1973; Hay and Moor, 1975; Boly *et al*., 2000). Results obtained for the <3-7% residual antibody class for all treatments showed no negative effects of antibody levels on oestrous compared to control levels. These results are not in agreement with those obtained on goats in some studies (Baril *et al*., 1992a, b, 1998) where oestrus manifestation weakened following a second PMSG treatment when antibody binding levels were high. However, results by classes of residual antibodies revealed a tendency for a delaying effect of residual antibodies in the 3-7% class on oestrus occurrence from repeated use of PMSG in the case of Ouled Djellal ewes This effect became important ($p<0.05$) following two or three PMSG treatments. Residual antibodies lower than the 3% level, were not associated with any depressing effects on oestrus in agreement with Baril *et al*. (1992a, b) who reported high oestrus levels when antibody levels before treatment are low (<5%) and vice versa. Similarly for ewes (Brice *et al*., 1995) the highest and lowest LH peaks were obtained when residual antibody levels were <3 and > 12%, respectively.

The occurrence and distribution of oestrus 60 mn following the start of heat monitoring, 48 h after PMSG injection, were comparable among treatments except for the results obtained at the end of the first and second repetition of T2 and T3), where 30% and 17% of ewes were detected in oestrus after 2-3 and 2-5 h, respectively. Consequently, residual antibodies would unfavourably affect oestrus only after 2 or 3 PMSG injections. Likewise, 27% and 12.5% of ewes showed delays in oestrus manifestation following 2 or 3 PMSG injections in the 3-7% residual antibody class level (Table 3). These results are in agreement with most studies (Baril *et al*., 1992a; 1992b; Brice *et al*., 1995; Drion *et al*., 2001). The frequency of late oestrus for goats with residual antibody levels > 5% were higher than those observed for goats with <5% residual antibody levels (Baril *et al*., 1996, 1998). In ewes, highest LH peaks were recorded on early animals with <3% antibody residual levels, while unsatisfactory results were obtained on late animals with > 12% anti-PMSG antibody liaison levels (Brice *et al*., 1995). The latter authors found that antibody liaison levels greatly affect the peak of LH. They reported that 75% of ewes with binding level<3% had highest LH levels between 32 and 48 h following PMSG injection and only 35% of ewes with levels> 35% had LH peaks in the same period. In our study, the efficiency of PMSG usage to improve sexual characteristics and reproductive performances was not compromised by the presence of anti-PMSG antibody in the blood of Ouled Djellal ewes. Those observations differ from reports on other breeds (Rainio, 1992; Baril *et al*., 1992a; Brice *et al*., 1995; Baril *et al*., 1996; 1998; Drion *et al*., 2001). Actually, repeated use of PMSG on Ouled Djellal ewes did not negatively affect reproductive performances even though it had resulted in late oestrus in the 3-7% residual antibody class. Late oestrus manifestation was associated with reduced fertility in AI schemes in goats (Rainio, 1992; Baril *et al*., 1992a; Baril *et al*., 1996; 1998; Drion *et al*., 2001) and reduced fertility and with lesser degree ovulation rate in ewes (Brice *et al*., 1995) which was not the case for Ouled Djellal ewes in natural breeding (PMSG treatments+natural breeding). However, results on prolificacy rates were comparable to these reported on goats (Drion *et al*., 2001). Discrepancies in results on the

efficiency of repeated use of PMSG might be explained by the breeding mode, natural breeding vs. artificial insemination and probably by breed differences in dealing with residuals antibodies. Some authors (Baril *et al*., 1992a; Brice *et al*., 1995) suggest that residual anti-PMSG antibodies when present limit reproduction performances in ewes and goats. High residual antibody levels generate a bad oestrus and ovulation synchronisations in ewes (Baril *et al*., 1992a) and goats (Brice *et al*., 1995). Relations between fertility and delays between oestrus and insemination are well known in cattle (Trimberger, 1948), goats (Baril *et al*., 1993) and ewes (Brice *et al*., 1995). Furthermore, lengthening the waiting time of the ovocyte will increase the embryonic mortality (Killeen and Moore, 1970a) and the efficiency of spermatozoid transportation depend greatly on the moment of the artificial insemination (Killeen and Moore, 1970b) consequently, reducing ovulation and parturition rates (Brice *et al*., 1995). Negative effects of residual antibodies on reproduction are important when the second PMSG treatment is applied in the same sexual season as the first one in goats, especially for those with antibody levels >10% (Baril *et al*., 1992a; 1996; 1998) and in ewes when the treatment is applied one month following the previous treatment (Rainio, 1992). In ewes, reductions in fertility rates were observed following elevated residual antibody levels even if consecutive PMSG treatments were one year apart (Brice *et al*., 1995; Bodin *et al*., 1997). These authors found up to 16 point differences between fertility measures in the extreme binding antibody levels (<6 and 6-12%). Ouled Djellal ewes seemed less sensitive to elevated residual antibodies compared to other breeds. In addition to comparable antibody binding levels for 2, 3, or 4 PMSG treatments, ewes were able to eliminate antibodies in the peak-residual phase following their moderate accumulation, compared to other breeds (Brice *et al*., 1995; Bodin *et al*., 1997) in the base or residual-peak phase.

Natural breeding seems to be the method of choice in the case of repeated use of PMSG to induce and synchronise oestrus cycles in Ouled Djellal ewes, as was the case for the Lacaune breed (Brice *et al*., 1995). Prolificacy rates and fertility of Ouled Djellal ewes were not affected by elevated residual antibody levels and there was no clear tendency of these reproductive traits variations among treatments. That proves otherwise, that the parameters of reproduction are not solely influenced by residual anti-PMSG antibodies. Similar remarks have been observed for fertility after artificial insemination in goats (Baril *et al*., 1998).

CONCLUSION

Intramuscular injection of an intermediate dose (500 UI) of PMSG has lead to the production of anti-PMSG antibodies. Residuals antibody levels, present before the next PMSG treatment, were similar among two, three and four PMSG consecutive treatments but were different from the control treatment base level, where Ouled Djellal ewes received a first PMSG dose. There was an important variability in ewes' individual responses to various treatments. Ouled Djellal ewes showed a great ability of antibody elimination following their accumulation from the repeated use of PMSG. There were no significant differences in the temporal evolution of accumulated anti-PMSG antibodies among various treatments and during peak levels reached between the 7th and 18th day following the injection. There were no depressing effects of antibodies on oestrus for ewes in the <3-7% residual antibody levels. However, both of oestrus occurrence and frequency distribution were unfavourably affected in the 3-7% residual antibody class. The efficiency of the gonadotrophin hormone was not compromised by the presence of anti-PMSG antibody of Ouled Djellal ewes. Repeated use of PMSG seems not to affect reproductive performances of these ewes in natural breeding schemes. Natural breeding might be effectively used on ewes repeatedly treated by FGA and PMSG on 8 month intervals to successfully have 3 lambing in 2 years for these breed.

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