Reproductive efficiency of Pelibuey and Romanov × Pelibuey ewes synchronized with synthetic progesterone and low doses of PMSG under a hot environment

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ABSTRACT: Thirty-nine multiparous ewes (19 Pelibuey and 20 Romanov × Pelibuey) treated with fluorogestone acetate impregnated intravaginal sponges were used to evaluate the effects of low pregnant mare serum gonadotropin (PMSG) doses and genotype on their reproductive efficiency under heat stress conditions. The sponge treatment lasted for 12 days, and 24 h before sponge removal, ewes of each genotype were injected with 140 or 280 IU of PMSG. Ewes showing estrus were naturally mated twice. Reproductive performance was not affected ($P > 0.05$) by the dose × genotype interaction. All treated ewes presented estrus signs within a 48-h period after sponge removal. Shorter ($P < 0.05$) estrus interval and higher ($P < 0.05$) fecundity were observed in ewes treated with 280 IU of PMSG compared to those treated with 140 IU. Pelibuey ewes exhibited shorter ($P < 0.01$) estrus interval and greater ($P < 0.01$) fertility as compared with Romanov × Pelibuey ewes. The response to estrus, gestation length, prolificacy, and percentage of single and multiple lambing were not affected ($P > 0.05$) by dose or genotype. In conclusion, under heat stress conditions, low PMSG doses as 140 or 280 IU can be used to successfully induce and/or synchronize the estrus in Pelibuey ewes and their crosses with Romanov, regardless of reduced fertility observed in crossed Pelibuey ewes. If a more predictable and compact estrus is required, administration of 280 IU of PMSG is recommended.

Keywords: hair-breed sheep; estrus synchronization; fertility; eCG; heat stress

Pelibuey is a hair breed of domestic sheep widely distributed in warm regions of the American continent due to its rusticity, adaptability to high environmental temperatures, and presence of reproductive activity throughout the year (Wideus, 1997). However, during the summer season, when climatic conditions are of heat stress, estrus behaviour and pregnancy rate of these sheep is reduced (Arroyo, 2011) as a result of the increase in corticosteroid concentrations and activation of thermoregulation mechanisms that favour homoeothermic conditions of the animal (Marai et al., 2008). In Pelibuey sheep bred in an arid weather, Gastelum-Delgado et al. (in press) reported a 55% decrease of ewes in estrus and a high variability in the duration of estrus signs (8–48 h) when temperature averages exceeded 27°C, the upper limit of the thermoneutral zone for sheep (Fuquay, 1981). However, this negative effect of the heat stress on estrus presence was not constant throughout the summer, only from May to July. Additionally, Avendaño et al. (2004) found out that under the arid conditions of Mexico the percentage of Pelibuey ewes lambed in summer was 33.8% compared to 66.2% in winter. Heat stress alters the ovarian follicular dynamics and the ability of the dominant follicle to exert dominance (Hansen, 2009). This adverse effect on the dominant follicle development reduces plasma concentrations of estradiol, LH, and inhibin. Thus, heat stress decreases the intensity and duration of estrus expressions and increases the incidence of anestrous and silent ovulations (Rodrigues et al., 2007). Some investigations have provided insight into potential means of mitigating the effects of heat stress on reproductive efficiency of sheep. These methods include application of exogenous hormones (Naqvi et al., 2004) and cooling (Finocchiaro et al., 2005). Nevertheless, limited information exists on the use of hormone treatments to induce estrus activity in heat-stressed hair breed ewes or their crosses with prolific wool breeds. Contrarily, in dairy cattle the application of hormones has been widely studied to improve fertility under high environmental temperatures (Hansen et al., 2001).

In hair and wool sheep, vaginal insertion of fluorogestone acetate (FGA) impregnated sponges for 10–12 days in combination with the administration of 400–500 IU of pregnant mare serum gonadotropin (PMSG) prior to or at time of sponge removal have been the synchronization and/or induction protocol more used worldwide, with acceptable results on response to estrus, fertility, and prolificacy (Barret et al., 2004; Avendaño-Reyes et al., 2007; Quintero-Elisea et al., 2011; Kermani et al., 2012). However, the protocol brings about elevated costs due to the high PMSG dose used, therefore economically this management strategy is little profitable for sheep producers. In contrast, the PMSG administration in that protocol is obligated to increase the ovulation rate, fertility, and multiple lambing percentages, since failures in the development of corpus luteum and secretion of progesterone are observed in ewes treated with only synthetic progestagens (Holtz, 2005). These failures are because of inadequate development and maturation of pre-ovulatory follicles that leads to deficiencies in estradiol secretion during the follicular phase (González-Bulnes et al., 2005). Therefore, progesterone treatments with low doses of PMSG may be an alternative to decrease the cost of the protocol, to avoid the formation of a low quality corpus luteum, and to induce the estrus activity in hair ewes during the summer season. In Pelibuey ewes treated with FGA and 250 IU of PMSG during the summer season, Macías-Cruz et al. (2009) found an acceptable reproductive performance (100% of response to estrus, 88% of fertility, and 2.0 lambs per ewe lambed). Therefore, evaluating PMSG doses lower than 250 IU is required to determine the optimal dose. Moreover, low PMSG doses have not been proved in hair ewes crossed with prolific breeds (i.e. Romanov) under high environmental temperatures. Ben Säid et al. (2007) indicated that unlike other non-prolific breeds, prolific breeds require a very small estradiol signal to induce full estrus behaviour. Therefore, we expect to observe a better reproductive response to the application of low PMSG doses in Pelibuey ewes crossed with Romanov than in pure Pelibuey ewes. Thus, the objective of this study was to evaluate the effect of low doses of PMSG on reproductive efficiency of pure Pelibuey and Romanov × Pelibuey ewes under a hot environment.

MATERIAL AND METHODS

The study was conducted during summer season at the Sheep Experimental Unit of the Centro de Bachillerato Tecnológico Agropecuario No. 41, located in the Mexicali Valley, Baja California, northwestern region of Mexico (latitude 114º08'N and longitude 32º06'W). The climate of this region is arid and dry with extreme temperatures in summer (above 40°C) and winter (0°C). In general, the average annual temperature and precipitation are 22°C and 85 mm, respectively (García, 1985). Thirty-nine nonpregnant multiparous ewes, 19 pure Pelibuey and 20 Romanov × Pelibuey, 3–5 years old, and with body condition score of 3.0–3.5 units (on scale 1 to 5) (Russell et al., 1969) were used. Ewes were placed into two pens based on genotype, and fed *ad libitum* with a mixture of chopped forage (50% sudangrass hay and 50% alfalfa hay). Diet was served once a day. The chemical composition of the diet was 895 g of dry matter (DM)/kg of mixture, 120 g of crude protein (CP)/kg of DM, and 2.1 Mcal of metabolizable energy (ME)/kg of DM, which covers the nutritional requirements specified by the National Research Council (2007) for sheep at the time of breeding (ME 1.9 Mcal/kg DM and CP 80 g/kg DM) and gestation (ME 1.9–2.4 kg DM and CP 85–105 g/kg DM). Water was offered without restriction.

All ewes received FGA impregnated intravaginal sponges (40 mg Chronogest) (Intervet Mexico S.A. de C.V., Mexico City, Mexico) for 12 days, and 24 h before sponge removal, ewes of each genotype were divided randomly into two groups (Pelibuey *n*= 9–10 per group, and Romanov × Pelibuey *n*= 10 per group) and intramuscularly injected with

140 or 280 IU of PMSG (Folligon; Intervet Mexico S.A. de C.V.). Between 12 and 48 h after sponge removal, four Dorper rams (2 per pen) with probed fertility were introduced in 3-h intervals to pens for estrus detection and natural mating (30 min). Ewes detected in estrus were recorded and mated twice using a controlled mating system, one at the time of the estrus detection and the other 12 h later. Those ewes detected in estrus and mated for the first time were separated to an adjacent pen until the second mating to facilitate the handling in the remaining females. At parturition, lambing date and number of lambs born were recorded individually for each ewe. Response to estrus (percentage of ewes in estrus after sponge removal), time to estrus (interval of time between sponge removal and onset of estrus), fertility (percentage of ewes lambed from ewes mated), prolificacy (number of lambs born per ewe lambed), fecundity (number of lambs born per ewe mated), single (percentage of ewes lambed with one lamb) or multiple (percentage of ewes lambed with two or more lambs) lambing, and gestation length (interval of time between day of mating and lambing) were determined from collected information. Additionally, weather data were obtained from the Climatic Experimental Station of the Autonomous University of Baja California, localized about 30 km far from the study site. The information regarding maximum, minimum, and average temperature and relative humidity were collected every hour throughout the experimental period, in order to calculate temperature-humidity index (THI) using the following equation (Hahn, 1999):

$$THI = 0.81 \times T + RH\ (T - 14.40) + 46.40$$

where:
T = environmental temperature
RH = relative humidity

All data of estrus behaviour and reproductive performance were analyzed using a model that included the fixed effects of PMSG dose (140 vs. 280 IU) and genotype (pure Pelibuey vs. Romanov × Pelibuey), as well as the interaction dose × genotype. Analysis of Variance was performed for time to estrus, prolificacy, and gestation length using the GLM procedure, while response to estrus, fertility, fecundity, and single and multiple lambing percentages were analyzed with the CATMOD procedure, both using SAS program (Statistical Analysis System, Version 9.0, 2004). Since the interaction dose × genotype was not significant for any response variable, only results for main effects are presented. Mean comparisons were done with Tukey's test for continuous variables, while chi-squared test was performed for categorical variables. Additionally, the time interval from sponge removal until 48 h was divided into three classes (< 24 h, 24–36 h, and > 36 h) to distribute the percentage of ewes in estrus. In each class, the percentage of ewes in estrus was evaluated using the CATMOD procedure.

RESULTS

Climatic conditions observed during the study period are shown in Table 1. During the 5 months of the experiment, averages of environmental tempera-

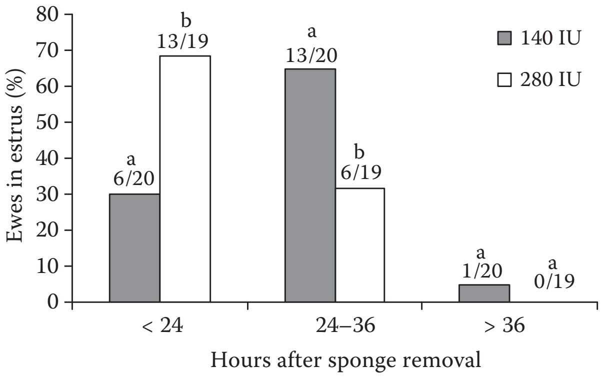

Figure 1. Effect of pregnant mare serum gonadotropin (PMSG) dose on estrus percentage at different times after sponge removal

^{a,b}significant differences within each interval ($P < 0.05$)

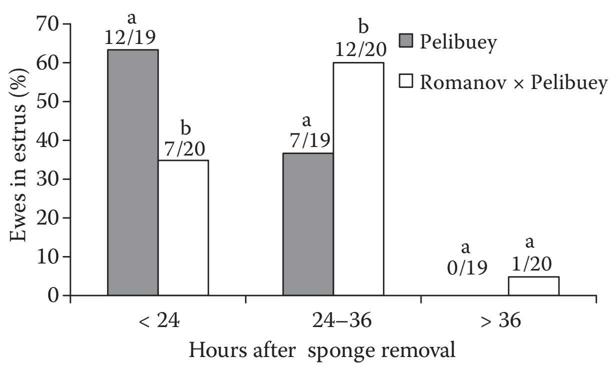

Figure 2. Effect of genotype on estrus percentage at different times after sponge removal

^{a,b}significant differences within each interval ($P < 0.05$)

Table 1. Daily minimum, maximum, and average for the climatic variables environmental temperature (T), relative humidity (RH), and temperature-humidity index (THI) per week during the study

Month/ week	Daily								
	minimum			maximum			average		
	T	RH	THI	T	RH	THI	T	RH	THI
June									
1	17.9	6.0	61.1	41.7	79.0	101.7	29.3	38.9	75.9
2	16.6	6.0	60.0	41.9	66.0	98.5	30.5	24.2	75.0
3	16.6	3.0	59.9	41.9	66.0	98.5	30.8	20.7	74.8
4	20.1	3.0	62.9	44.2	71.0	103.4	33.0	23.7	77.6
July									
1	21.1	7.0	64.0	43.2	72.0	102.1	32.3	34.3	78.7
2	26.6	20.0	70.4	44.0	75.0	104.2	35.2	41.9	83.6
3	26.4	16.0	69.7	45.0	68.0	103.7	35.3	41.3	83.6
4	27.1	14.0	70.1	43.1	75.0	102.8	33.8	43.2	82.1
August									
1	22.7	8.0	65.5	42.9	66.0	100.0	33.7	33.7	80.2
2	20.9	7.0	63.8	42.8	74.0	102.1	32.5	27.5	77.7
3	28.7	16.0	71.9	45.2	79.0	107.3	35.1	46.8	84.5
4	25.7	11.0	68.5	45.2	83.0	108.6	35.9	40.2	84.1
September									
1	16.9	6.0	60.2	45.1	58.0	100.7	31.3	23.9	75.8
2	16.3	14.0	59.9	41.3	79.0	101.1	29.6	37.9	76.2
3	20.2	4.0	62.9	41.4	63.0	96.9	32.0	23.2	76.4
4	16.1	5.0	59.5	42.8	80.0	103.8	30.8	25.8	75.6
October									
1	22.3	10.0	65.3	43.6	89.0	107.7	31.5	43.4	79.4
2	12.3	15.0	56.0	35.0	91.0	93.5	23.3	45.9	69.3
3	17.1	13.0	60.6	37.8	72.0	93.9	28.4	31.7	73.8
4	12.3	24.0	55.9	32.5	89.0	88.8	21.2	57.2	67.5

ture and relative humidity were 31.5°C and 34.9%, respectively, and the combination of both climatic factors produced an average THI of 77.8 units. The highest environmental temperatures and THI were recorded in the 3rd (45.2°C, 107.7 units) and 4th (45.2°C, 108.6 units) week of August, while the lowest in the 2nd (12.3°C, 56.0 units) and 4th (12.3°C, 55.9 units) week of October, the month considered the summer end/autumn start. Maximum relative humidity (91.0%) was detected in the 2nd week of October while minimum (3.0%) in weeks 3 and 4 of June. In general, average temperatures of summer months were above 30°C (30.9–34.3°C), but in October (autumn season) this temperature was below 30°C (26.1°C). Monthly averages of THI were ≥ 72 units (75.8, 82.0, 81.6, 76.0, and 72.1 units for June, July, August, September, and October, respectively).

Reproductive responses of synchronized ewes were not affected by the interaction dose × genotype. All ewes of the study exhibited overt signs of estrus during the 48-h observation period. Within the first 24 h after sponge removal, ewes treated with 280 IU of PMSG had greater ($P < 0.05$) response to estrus than ewes treated with 140 IU of PMSG; opposite results ($P < 0.05$) were observed 24–36 h post the sponge removal (Figure 1). The response to estrus 36–48 h after the sponge removal was similar ($P > 0.05$) between ewes treated with 140 and 280 IU of PMSG. Regarding the effect of ewe genotype on the estrus distribution after sponge removal (Figure 2), it was observed that the response to estrus in Pelibuey ewes was greater ($P < 0.05$) in the first 24 h but lower ($P < 0.05$) between 24–36 h compared with Romanov × Pelibuey ewes. Thirty-six h after the sponge removal, the response to estrus was similar ($P > 0.05$) between genotypes.

With exception of time to estrus and fecundity, reproductive parameters were not affected by PMSG doses (Table 2). Shorter ($P < 0.01$) time to estrus (26.3 vs. 29.5 ± 2.5 h) and higher ($P < 0.05$) fecundity rate (130 vs. 174%) were observed in ewes treated with 280 IU of PMSG than in those treated with 140 IU of PMSG. In addition, ewe genotype affected only time to estrus and fertility (Table 2); earlier ($P < 0.01$) was the onset of estrus (25.5 vs. 30.3 ± 2.5 h) after sponge removal and higher ($P < 0.05$) was the fertility (65 vs. 79%) in Pelibuey ewes than in the crossed ewes.

DISCUSSION

Climatic conditions recorded during the development of the present study were of heat stress, since average temperature (31.5°C) was above the superior limit of the thermoneutral zone indicated for sheep (27°C) (Fuquay, 1981). This heat stress was classified as mild to moderate (72–79 units), given the average THI estimated (77.8 units); environmental conditions in July and August were considered as moderate to severe heat stress (THI 80–89 units) (Burgos Zimbelman and Collier, 2011). In arid regions of the world like that of the

Table 2. Effects of pregnant mare serum gonadotropin (PMSG) dose and genotype on reproductive efficiency of ewes synchronized with synthetic progesterone

	Dose of PMSG (IU)		Ewe genotype	
	140	280	Pb	Rv × Pb
Ewes treated (*n*)	20	19	19	20
Response to estrus (%)	20/20 (100)[a]	19/19 (100)[a]	19/19 (100)[a]	20/20 (100)[a]
Time to estrus (h)	29.5 ± 2.5[a]	26.3 ± 2.5[b]	25.5 ± 2.5[a]	30.3 ± 2.5[b]
Length of gestation (days)	149 ± 2.1[a]	148 ± 2.1[a]	150 ± 2.4[a]	148 ± 2.4[a]
Fertility (%)	14/20 (70.0)[a]	14/19 (73.7)[a]	15/19 (78.9)[a]	13/20 (65.0)[b]
Prolificacy	1.8 ± 0.4[a]	2.2 ± 0.4[a]	2.1 ± 0.4[a]	2.0 ± 0.4[a]
Fecundity (%)	26/20 (130)[a]	33/19 (174)[b]	31/19 (163)[a]	28/20 (140)[a]
Single lambing (%)	3/14 (21.4)[a]	1/14 (7.2)[a]	3/15 (20.0)[a]	1/13 (7.7)[a]
Multiple lambing (%)	11/14 (78.6)[a]	13/14 (92.8)[a]	12/15 (80.0)[a]	12/13 (92.3)[a]

Pb = Pelibuey, Rv = Romanov

[a,b]means with different letters in a row within each factor indicate significant differences at $P < 0.05$

present study, such climatic conditions are common during summer and they negatively affect the estrus behaviour, fertility, and productivity of animals (González-Bulnes et al., 2011). Reserach has provided insight into potential means of mitigating the effects of heat stress on reproductive efficiency of domestic animals (Naqvi et al., 2004; Finocchiaro et al., 2005). However, compared with bovines, in sheep the use of strategies to reduce the effect of the heat stress on reproduction efficiency has been very limited. This study shows that the hormonal treatment with progesterone and low PMSG doses may be used to induce and/or synchronize reproductive activity of hair ewes or their crosses with prolific breeds under high temperatures.

It has been suggested that low PMSG doses are not sufficient to stimulate additional follicular development in ewes, consequently reduced response to estrus and longer estrus interval have been observed (Romano et al., 1996). However, results of the present study show an effectiveness of the protocol used to synchronize or induce the estrus activity in hair ewes maintained in heat stress conditions, since 100% of ewes presented estrus signs within the first 48 h after sponge removal. Other studies performed in similar climatic conditions using hormonal treatments based on FGA and 250 IU of PMSG also reported similar results (Macías-Cruz et al., 2009, 2012). Therefore, the application of 140 or 280 IU of PMSG can be used without affecting estrus behaviour after sponge removal in Pelibuey and Romanov × Pelibuey ewes maintained in conditions of high environmental temperatures.

The estrus distribution was affected by PMSG doses and ewe genotype. Compared with the administration of 140 IU of PMSG, the application of 280 IU of PMSG led to earlier onset of estrus and grouped within the first 24 h after sponge removal. Those results were attributed to a greater follicular development with the PMSG dose of 280 IU, which provoked a greater estradiol production (Kermani et al., 2012). This is in agreement with other studies demonstrating that as PMSG doses increased, the estrus interval was shorter and better predictable, and the onset of estrus and ovulation was more precise (Quintero-Elisea et al., 2010, 2011; Kermani et al., 2012).

Like in the present study, some authors have reported the effect of ewe breed on the estrus behaviour of ewes treated with FGA and PMSG (Emsen and Yaprak, 2006; Ben Säid et al., 2007; Moeini et al., 2007). These authors indicated that prolific breeds compared with other less-prolific improved their estrus behaviour (shorter interval to estrus and more grouped estrus) when they were treated with 300–500 IU doses of PMSG. However, results of this study showed that prolific ewes (Romanov × Pelibuey) presented longer interval to estrus after device removal and a more disperse estrus distribution. There was expected a shorter interval to estrus and the estrus distribution more compacted 24 h post sponge removal for Romanov × Pelibuey ewes, because Romanov breed has naturally higher follicular development, secretion of estrogen, ovulation rate, and prolificacy, and three times more granulosa cells in their pre-ovulatory follicles than other prolific breeds (Ricordeau et al., 1990). Additionally, a very weak estradiol signal to induce full estrus behaviour is sufficient for this breed if compared with other breeds (Ben Säid et al., 2007). Possibly, results of the effect of ewe genotype on estrus behaviour could be related with differences in the adaptation level to heat stress conditions. Pelibuey sheep has the ability to rapidly adapt to extreme climates without affecting its reproductive performance (Tabarez-Rojas et al., 2009); contrarily, Romanov sheep shows low estrus activity under hot environmental conditions (Ricordeau et al., 1990). Genetic adaptation to heat stress is possible both with respect to regulation of the body temperature and cellular resistance to elevated temperatures (Hansen, 2009).

In general, fertility in this study was 71.8%, without the effect of PMS doses, although no control group was considered. Although it was lower than the fertility previously reported by Macías-Cruz et al., 2009, 2012 (89 and 95%, respectively) for Pelibuey ewes treated with FGA and 250 IU of PMSG under arid conditions of northwestern Mexico, the herein ascertained fertility rate exceeded 70%, which is desirable in natural mating systems. The discrepancy among results can be due to variations in climatic conditions among study years. Similarly, PMSG doses did not affect other reproductive parameters such as prolificacy, gestation length, and percentage of single and multiple lambing. Means and percentages reported (Macías-Cruz et al., 2012) for these parameters in hair breed ewes synchronized with FGA and 250 IU of PMSG were very similar to those found in the present study (148.5 ± 2.1 days of gestation, 2.0 ± 0.4 lambs/ewe lambed, 14% of single lambing, and 86% of multiple lambing). Applying 500 IU of PMSG to Pelibuey ewes treated with FGA, Avendaño-Reyes et al. (2007) found slightly greater prolificacy (2.5 ± 0.3 lambs/ewe lambed) than observed in the present study. On the other hand, fecundity was by 44% higher in ewes treated with 280 UI of PMSG, which is due to the numerical difference in single lambing observed in ewes treated with 140 IU, and to the presence of ewes that lambed 3 or 4 offsprings in the group treated with 280 IU. Under thermoneutral conditions, Quintero-Elisea et al. (2011) found also higher fecundity percentage in Pelibuey and Blackbelly ewes treated with 200 IU of PMSG than in those treated with 100 IU. In general, these results suggest that low PMSG doses as 280 IU can be used in combination with FGA impregnated sponges to improve the lamb production in Pelibuey and Pelibuey × Romanov ewes during the season of high environmental temperatures.

Ewe genotype affected the fertility obtained with the synchronization protocol used, being higher in Pelibuey than Romanov × Pelibuey. This result seems to be related with the great adaptation of Pelibuey ewes to heat stress conditions (Tabarez-Rojas et al., 2009). Thus, the possible negative effects of heat stress on results of estrus behaviour of Romanov crossed ewes could be extensive to gestation development and lambing. It is well known that high environmental temperatures produce embryonic losses and fetal deaths during gestation, when ewes cannot adapt to these climatic conditions (Marai et al., 2008). Additionally, reproductive parameters associated with lamb production were not affected by ewe genotype. In contrast, other studies carried out during spring under arid climate reported similar fertility but different prolificacy, fecundity, and percentage of single and multiple lambing between Awassi and Red Karaman ewes (Emsen et al., 2006), and between Brown and Black Awassi ewes (Kridli et al., 2009). This discrepancy among results was related to variations in environmental conditions recorded in each study (Kermani et al., 2012).

CONCLUSION

In conclusion, the combination of FGA impregnated intravaginal sponges with low PMSG doses (140 or 280 IU) can be used effectively to induce and/or synchronize the estrus presence in Pelibuey and Romanov × Pelibuey ewes under heat stress conditions. Additionally, administration of 140 or 280 IU of PMSG 24 h before sponge removal is equally effective to improve fertility and lamb production. In contrast, greater fertility is observed in Pelibuey ewes than in their crosses with Romanov when they are treated with this protocol. Finally, administration of 280 IU of PMSG is recommended to obtain a more predictable and compact estrus, and to assure an acceptable reproductive performance of ewes treated.

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