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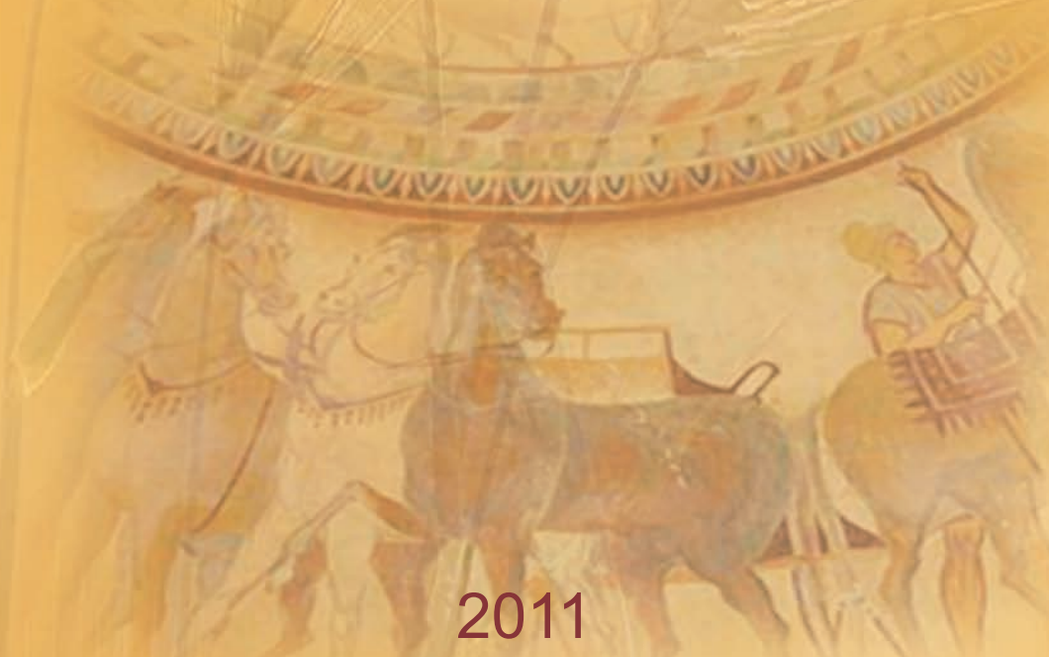

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Superovulation and embryo transfer in goats by using PMSG or FSH

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Abstract. *The objective of this study was to analyze the efficiency of superovulation, embryo collection, transfer and embryo survival in indigenous goat during breeding season. Twenty-four goats-donors were synchronized by placing an intravaginal sponge of medroxyprogesterone acetate (MAP, 60mg) for 17 days. Superovulation was induced in 12 goats by a single injection of PMSG (1200 IU/animal, i.m.), at the time of sponge removal, and in 12 goats by FSH i.m. injections, twice a day, in a total amount of 21mg in reduced consecutive doses, starting at day 15 of the estrus cycle. All donors showing estrus, were bred naturally and 6 days post mating were subjected to a surgical uterine flushing with Dulbecco`s phosphate saline enriched with 3% BSA and fresh embryos were transferred surgically to synchronized recipients. Mean interval to onset of estrus did not differ between the two treatments; however the duration of estrus was shorter in FSH - treated group. There was significant difference ($p<0.05$) between treatments in total ovarian response, in ovulation rate (3.9 ± 0.9 group PMSG, and 12.3 ± 2.0, group FSH) and in embryo recovery (2.3 ± 0.8 for PMSG, and 9.3 ± 1.5 for FSH group). Embryo survival after transfer of embryos was significantly ($p<0.05$) higher for the FSH group (61.1% and 22.2% for FSH-p, and PMSG).*

Keywords: transfer, embryo, superovulation, goat

Abbreviations: FSH – follicle stimulating hormone, MAP – medocsiprogesteronacetatum, PBS – Phosphate-buffered saline, BSA – Bovine Serum Albumin, PMSG – Pregnant Mare's Serum Gonadotropin, DMSO – dimethylsulphoxide, CL– corpora lutea

Introduction

The first successful embryo transfers in livestock species were performed on sheep and goat almost a century ago (Warwick et al., 1934) however, until recently, there has been little commercial embryo transfer activity in the small ruminants. The initial step in a program designed to generate an economically feasible number of embryos is to determine an efficient superovulatory hormonal regimen. Over recent years superovulation regimen for goat have been described using Pregnant Mare Serum Gonadotropin (PMSG) or Porcine pituitary Follicle Stimulating Hormone (FSH) (Moor, 1980; Armstrong et al., 1982). The effectiveness of these hormones in sheep and goat has been compared (Kiessling et al., 1986; Armstrong et al., 1983a). FSH was reported to produce a greater ovulation rate than did a single injection of PMSG with a lower degree of follicular hyper stimulation and lower incidence of large follicles that failed to ovulate (Pendleton et al., 1986; McNatty et al., 1989).

The purpose of the present study was to compare ovulation rate and number and duality of flushed embryos in indigenous dairy goats, during the breeding season, with PMSG or FSH, and the survival percentage of flushed embryos after transferring, as freshet, to recipients.

Material and methods

A total of 42 multiparous indigenous dairy goats were used in the present experiment. Twenty-four goats were used as donors and allocated in two treated groups, 12 goats in each group. Eighteen goats served as controls. All animals, donors and controls, were synchronized by using intravaginal sponges, impregnated with 60 mg medroxyprogesterone acetate (MAP) (Veramix; Upjolin Co.) for a period of 17 days. In twelve donors, at the time of sponge removal, was administered 1200 IU PMSG/goat (intergonan; Intervet Co.) as a single i.m. injection and in the rest 12 donors, 48 hours before sponge removal, began a course of FSH (FSH; Schering Co.) i.m. injections administered twice daily over a 4-day period, a total amount of 21 mg in reduced consecutive doses (4 and 3 mg, 3 and 3 mg, 2 and 2 mg, 2 and 2 mg FSH). Donor and control animals were checked for estrus twice daily, in 12 hours' intervals, beginning 12 hours after sponge removal. All donors showing estrus were hand mated to 4 proven fertile males twice daily from onset to end of estrus.

Eighteen multiparous indigenous dairy goats were used as recipients and synchronizing with the same hormonal treatment with donors, however for a period of 16.5 days. Estrus detection was conducted also twice daily. Six days after the first detection of estrus, embryos were recovered surgically from donors who were off feed and water 24 hours prior to surgery. Reproductive tracts of animals were exteriorized through a mid-ventral incision and each uterine horn was flushed separately from uterotubal junction to uterine corpus with 20 ml Dulbecco's phosphate-buffered saline (PBS), pH 7.2, enriched with 3% Bovine Serum Albumine (BSA) and warmed to 37°C. The flushings were collected in sterile plastic Petri dishes. Each ovary was carefully observed and the total ovarian response (corpora lutea and follicles) was recorded. Collection media from both uterine horns were examined immediately after the recovery under a stereomicroscope, transferred into fresh PBS identical to the flushing medium and recovered embryos were classified.

Transfers were performed to synchronized recipients via mid-ventral incision and exposure of reproductive tract. Two embryos (excellent or good morula or young blastocysts) were transferred to

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each recipient (one embryo/horn) at the top part of the uterine horn even if there was only one ovulation. Number, position and appearances of corpora lutea (CL) and unovulated follicles were recorded. The remaining embryos have been frozen and stored in liquid nitrogen (-196° C) for future purposes. Pregnancy diagnosis was obtained by measuring progesterone level in plasma 21 days after estrus (Terqui and Thimonier, 1974) and by recording the daily estrous behavior. Data were assessed by Student's t-test for the differences between treatment groups and by binomial analysis for the differences between percentage responses.

Results

All goats in three groups showed estrus, however the mean interval of onset of estrus to sponge removal was greater in control goats compared to gonadotrophic treatment groups (42.3 ± 4.7 vs. 25,8 ± 1.9 and 29.0 ± 3.2, p<0.05, Table 1). Duration of estrus was shorter in FSH - treated group compared to PMSG - treated and control groups (24.2 ± 3.4 vs. 37.2 ± 4.3 and 32.5 ± 1.8, p<0.05, Table 1).

Ovarian response was greater in gonadotrophic treated groups compared to controls (6.9 ± 1.1 and 22.5 ± 2.1 vs. 1.3 ± 0.4, p<0.05, Table 2), however the ovarian response in FSH - treated group was also greater than in PMSG - treated group (22.2 ± 2.1 vs. 6.9 ± 1.1, p<0.05, Table 2). The number of corpora lutea (CL ovulation rate) was greater in gonadotrophic treated groups compared to controls (3.9 ± 0.9 and 12.3 ± 2.0 vs. 1.3 ± 0.4, p<0.05, Table 2), however the ovulation rate in FSH - treated group was greater than in PMSG - treated (12.3 ± 2.0 vs. 3.9 ± 0.9 , p<0.05, Table 2).

The number and size of follicles into the ovaries of gonadotrophic treated goats (Table 3) varied significantly. Follicles sized between 2-4 mm and 4-6 mm were greater in FSH - treated group than in PMSG - treated group (53.2% and 40.1% vs. 8.3% and 27.7%, p<0.05), however, in contrast, follicles sized between 6-8 mm and preovulatoires (>8 mm) were smaller in FSH - treated group compared to PMSG - treated group (3.2% and 3.2% vs. 33.3% and 30.5%, p<0.05).

The total number of recovered embryos, the average recovered

Table 1. Mean interval of onset of estrus from sponge removal and duration of estrus in superovulated by PMSG or FSH and control goats

Group	Onset of estrus, hours Mean ± SE	Duration of estrus, hours Mean ± SE
PMSG	25.8±1.9[a]	37.2±4.3[a]
FSH	29.0±3.2[a]	24.2±3.4[b]
Control	42.3±4.7[b]	32.5±1.8[a]

a,b - column value with different superscript differ statistically (p<0.05).

Table 2. Total ovarian response and ovulation rate in superovulated by PMSG or FSH and control goats

Group	Ovarian response		Ovulation rate Mean ± SE
	Number of follicles	Number of CL	
PMSG	36	47(6.9±1.1)1[a]	3.9±0.9[a]
FSH	122	148(22.5±2.1)[b]	12.3±2.0[b]
Control	0	27(1.3±0.2)[c]	1.3±0.2[c]

1 - follicles and CL are included; a,b,c - column value with different superscript differ statistically (p<0.05)

Table 3. Percentage of number and size of follicles into the ovaries of superovulated by PMSG or FSH

Treatment	Percentage (%) of follicles per size (mm)			
	2-4mm	4-6mm	6-8mm	Preovulatoires (>8mm)
PMSG	8.3[a]	27.7[a]	33.3[b]	30.5[a]
FSH	53.2[b]	40.1[b]	3.2[a]	3.2[b]

a,b - column value with different superscript differ statistically (p<0.05)

Table 4. Recovered embryos from goat-donors after superovalution treatment with PMSG or FSH.

Treatment	Embryos recovered			
	Number of donors	Total number of embryos	Embryos per donor Mean ± SE	Recovery rate (%)
PMSG	12	28	2.3±0.8[a]	59.5[a]
FSH	12	112	9.3±1.5[b]	75.6[b]

a,b - column value with different superscript differ statistically (p<0.05)

Table 5. The survival of transferred embryos, recovered from PMSG or FSH treated donor-goats

Treatment of donors	Number of transferred donors	Number of recipients	Ovulation rate of recipients Mean ± SE	Kidding (%)	Kid per transferred embryos
PMSG	18	9	1.4±0.2[a]	22.2[a]	4/18[a]
FSH	18	9	1.3±0.1[a]	77.7[b]	11/18[b]

a,b - column value with different superscript differ statistically ($p<0.05$)

Table 6. Survival of transferred embryos in relation to the position of CL

Position of CL	Number of recipients[1]	Number of CL	Number of pregnant recipient	Kid born per embryo transferred (%)
PMSG	10	10	5	40[a]
FSH	8	17	4	35[a]

[1]Two embryos per recipient, one to each horn; a - column with same superscript were not statistically different ($p<0.05$)

embryos per donor and the recovery rate were greater ($p<0.05$) in FSH - treated group compared to PMSG - treated group (112, 9.3 ± 1.5 and 75.6% vs. 28, 2.3 ± 0.8 and 59.5%, Table 4). The survival of embryos recovered from PMSG - or FSH - treated group in multiparous recipients varied significantly ($p<0.05$). The kidding and the kids per transferred embryos were greater in FSH - treated group compared to PMSG - treated group (77.7% and 11/18 vs. 22.2% and 4/18, Table 5). The survival of transferred embryos in relation to the position of the CL on the ovaries of recipients were found to be similar to those with CL either in one or in both ovaries (Table 6).

Discussion

In the present study an effort has been made to compare the two most widely used gonadotrophic preparations, PMSG and FSH, in provoking superovulation efficiently in indigenous dairy goats and further to determine an appropriate protocol and treatment regimen suitable to this breed. All goats, donors and recipients, exhibiting estrus after synchronization by using intravaginal sponges MAP and the timing data for onset and duration of estrus are similar to those reported in goats by Cameron et al. (1988).

Total ovulation response and ovulation rate was significantly higher in FSH - treated than in PMSG - treated donors, as recorded by Jacques et al. (1989) as well. Premature regression of CL was not observed, when the embryos recovered at day 6. In the ovaries of FSH - treated goats a great number of small premature follicles (2 to 6 mm diameter) were found, on the contrary, in the ovaries of PMSG - treated goats large follicles (>6 mm diameter) were observed and occasionally large cystic anovulatory follicles, evidence of ovarian over stimulation. The latter has also been reported by Armstrong et al. (1983a). This superiority of FSH has been described by others for goats (Armstrong et al., 1983a; Tervit et al., 1986) and cattle (Elsden et al., 1978) and is thought to be due to the considerably more rapid clearance of FSH than of PMSG from the blood circulation (half life approx. 2h compared with 20h for PMSG) (Akbar et al., 1974; Mcintosh et al., 1975).

High rates of fertilization were obtained with both treatments regimen leading to significantly higher embryo recovery from FSH - treated animals. The survival rate of fresh embryos, transferred to multiparous recipient goats, was higher for embryos recovered from FSH - treated group, as judged by the pregnancy rate of the recipients, similar results were also obtained by other investigators (Armstrong et al., 1983b; Nuti et al., 1987). The lower survival rate of the embryos produced by PMSG - treated group must be attributed to several intrinsic factors of the embryos such as chromosome abnormalities, polyspermic fertilization and cleavage abnormalities which were found in sheep by Williams and Long (1980) in superovulate ewes by using PMSG. All this chromosome abnormalities were ascribed to an adverse effect of PMSG. The survival of transferred embryos was not affected either by the number or by the position of the CL in the ovaries of the recipients and these findings are in agreement with the results of Torres and Sevellec (1987) in ewes. In summary, the findings from the present study showed that the FSH superovulation regimen resulted in greater ovulation rate, embryo recovery rare, embryo survival after transferring than in PMSG and, simultaneously, the adverse effect of PMSG was not observed in causing ovarian overstimulation.

Conclusions

Mean interval to onset of estrus did not differ between the two treatments; however the duration of estrus was shorter in FSH - treated group. There was significant difference ($p<0.05$) between treatments in total ovarian response, in ovulation rate (3.9 ± 0.9 group PMSG, and 12.3 ± 2.0, group FSH) and in embryo recovery (2.3 ± 0.8 for PMSG, and 9.3 ± 1.5 for FSH group). Embryo survival after transfer of embryos was significantly ($p<0.05$) higher for the FSH group (61.1% and 22.2% for FSH-p, and PMSG).

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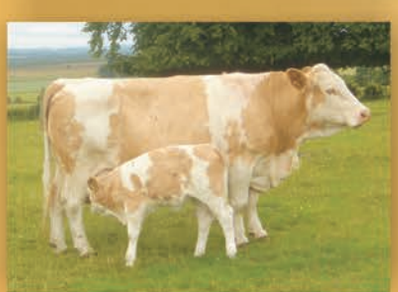

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