Effect of Superovulatory Regimens on Ovarian Response and Embryo Production in Fine Wool Sheep in Tropics

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ABSTRACT : Fine wool sheep (n=18) maintained in a tropical environment were allocated to three treatment groups. Estrus was induced with two injections of $PGF_{2\alpha}$ (10 mg. im) at 10 days interval. Superovulation treatment started 2 days prior to the second injection of $PGF_{2\alpha}$. Each ewe was treated with a total dose of 25 units FSH (Super-OV) i.m. every 12 h over 3 days; Group 2 were also injected i.m. with 200 IU PMSG at the first injection of FSH; Group 3 was treated as in Group 2 and also with GnRH (4 μg Buserelin) at the onset of estrus. The ewes in estrus were mated with a fertile ram. Ovarian examination and recovery of embryo and ova were performed at laparoscopy and laparotomy on day 3 or 4 after mating. Data for onset of estrus, duration of estrus, number of corpora lutea (CL), number of unnovulated large follicle (LF), embryo recovery rate, embryo quality and fertilization recorded for the 3 groups. Ewes in the Group 1 set in estrus later ($p<0.05$; 50.0 ± 7.29 h) than the ewes in Group 2 (24.5 ± 3.58) and 3 (32.5 ± 3.58 h). The duration of estrus, ovarian size and ovarian response (number of CL and LF) did not differ significantly ($p>0.05$) among the 3 groups. The proportion of ewes with a superovulatory response (≥2 CL) was the lowest (50%) in Group 1 treated with FSH alone but ova/embryo recovery (100%) and fertilization (100%) was significantly ($p<0.05$) higher than Group 2 (58.3 and 85.7%, respectively) and Group 3 (48.6 and 50%, respectively). It is concluded that in tropical fine wool sheep, there is no difference in the 3 treatments for yield of good quality embryos but ovarian response and ovulation rate increased on additional use of PMSG and GnRH respectively to FSH alone. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 5 : 595-599*)

Key Words : Sheep, Gonadotrophin, Superovulation, Embryo, Semi-Arid, Tropics

INTRODUCTION

Temperate breed of sheep for fine wool and their higher crosses with native (> 75% exotic inheritance) in tropical condition are maintained for their use in crossbreeding programme to augment production. These sheep encounter different nutritional regimen, managemental practices and harsh environment (high ambient temperature, scarcity of feed and water) as compared to that in temperate regions. The low reproductive efficiency of the fine wool sheep under such environment limits the increase of their population number. One of the methods to multiply sheep at a faster rate is through embryo transfer. There is meagre information available on the superovulatory response of these sheep kept in arid/semiarid tropics (Naqvi and Gulyani, 1996; Naqvi et al., 1998b). There are several intrinsic (age, genetics, ovarian status etc.) and extrinsic (season, nutrition, exogenous gonadotprophin preparations) factors which are known to influence the outcome of a superovulatory treatment (Armstrong and Evans, 1983; Gordon, 1997).

Individual variability in superovulatory response to a treatment is one of the major limitation for economic usage of embryo transfer in animal improvement programmes and less causes for such variability have been explained. Several studies conducted on temperate sheep have placed much emphasis on the factors that contribute to variability in ovarian response to different gonadotrophin preparations either FSH alone or in combination with PMSG and/or GnRH (Gordon, 1997). The objective of this study was to examine the superovulatory response and embryo production after treatment with FSH (Super-OV) alone or in combination with PMSG and/or GnRH in elite fine wool sheep kept in semi-arid tropics.

MATERIALS AND METHODS

Location

The experiment was conducted at the Institute's sheep farm at Avikanagar which is located at longitude of 75°-28'E, latitude of 26°-26'N and altitude of 320 m above mean sea level in the semi-arid region of the country. The climate of this region is essentially tropical. The highest temperatures occur from April to June when mean monthly temperature is about 42℃. The rainfall is erratic and mainly concentrate during July to August. The precipitation ranges from 400 to 700 mm per annum.

Animals

Eighteen adult ewes were randomly selected from a mixed flock of Rambouillet and Bharat Merino sheep. Rambouillet sheep were imported from USA while

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Bharat Merino is a strain developed for fine wool by crossing the native ewes with Rambouillet/Soviet Merino rams and stabilizing the population at 75% exotic inheritance. The ewes were 4-8 years of age and weighing 34.9±0.66 (28.0-38.0 kg). They were grazed 8-10 hours daily on *Cenchrus ciliaresis* pasture intersparsed with seasonal shrub. In addition to grazing a concentrate mixture 200 g/ewe/day was provided to fulfil their nutritional requirement.

Animal treatment

All the ewes were examined for their body condition and observed for estrus occurrence for at least 2 consecutive estrous periods before commencing the treatment for superovulation. The ewes were randomly allocated in equal number (n=6) to three groups. To control the estrus, two injections of prostaglandin $F_{2\alpha}$ (PGF) (Lutalyse, Unichem Ag vet., India), 10.0 mg each, were administered intramuscularly at 10 days interval in all the ewes. Superovulatory treatment commenced two days prior to second PGF injection. Each ewe in the three groups received a total dose of 25 units FSH (Super-OV, Ausa International Inc. USA) twice a day (morning and evening) in constant dose over a period of three days. The ewes in Group 2 and 3 also received a 200 IU pregnant mare serum gonadotrophin (PMSG) (Folligon, Intervet-Netherlands) once as intramuscular injection at the commencement of the superovulation treatment. Treatment to the ewes in Group 3 was the same as in Group 2 but additionally an injection of 4 μg Buserelin (Receptal, Hoechst India Ltd., India), an agonist of gonadotrophin releasing hormone (GnRH), was also administered at the onset of detected estrus.

Estrus detection and mating

Estrus detection was performed by parading aproned rams (ram:ewe=1:10) of high sexual vigour at 6 hourly interval upto 96 hours commencing from the day of second PGF dose. Ewe detected in estrus was mated twice daily (morning and evening) with a ram of Rambouillet breed of proven fertility.

Ovarian examination and embryo recovery

Ovarian examination and embryo recovery on day 3 or 4 after mating were carried out at laparoscopy and laparotomy. The animals were fasted for at least 24 hours prior to laparoscopy and/or laparotomy. Abdominal area anterior to udder was shaved and sprayed with 70% alcohol. Animals were sedated with Xylazine hydrochloride (Xylazine, Indian Immunologicals, India) 0.1 mg/kg body weight and then locally anaesthetized by infiltration of the incision area with 6-8 ml 2% lignocaine hydrochloride (Xylocaine, SGPharm, India). All the ewes treated for superovulation were examined by laparoscopy to determine whether they were worth attempting for surgical embryo collection. The procedure used for laparoscopy has been described elsewhere (Naqvi and Gulyani, 1995). The ewes with ≥ 2 corpora lutea (CL) were marked as having a superovulatory response. Midventral laparotomy was performed in the ewes having superovulatory response and ovarian dimension were measured using a sterile caliper. The procedure for embryo collection was similar to that described elsewhere (Hunter et al., 1955). The fallopian tubes were flushed retrogradely with a flushing media (modified Dulbecco's phosphate buffered saline) introduced near the tubal junction. The tubal washing was collected in sterile petri dish and embryo/ova were searched/examined under stereozoom microscope (Nikon, Japan) at 50× magnification. Fertilization of ova was confirmed by cleavage. Good quality embryos show proper symmetry of cells and no shrinkage, vacuolization or lysis. The numbers of recent ovulations (corpora lutea) and persistent large follicles (LF) were recorded. The ovarian response was estimated by adding number of CL to LF.

Data analyses

The data were expressed as a mean SEM. Data on estrus response, ovarian dimension, number of CL and LF, and total ovarian response were analysed using analyses of variance (Harvey, 1990). The statistical difference among more than two groups were compared using Duncan's multiple range test (DMRT). The proportion data were analysed using chi-square test.

RESULTS

All the ewes in the three treatment groups exhibited behavioural sign of estrus within three days after second PGF injection (table 1). The interval between PGF dose and onset of estrus was longer (50 ±7.24 h; $p<0.05$) in ewes treated with FSH alone (Group 1) as compared to those treated with FSH in conjunction with PMSG (Group 2, 24.5±3.58, and 3, 32.5±3.58 h). The duration of behavioural estrus was the longest in Group 2 (35.0±3.25 h) followed by Group 3 (33.5±5.33 h) and 1 (29.5±3.95 h), but did not approach to statistical significance ($p>0.05$). Two ewes each in Group 1 and 3 (33.3%) had not ovulated at the time of laparoscopy.

Table 2 presents the mean values of ovarian dimension and superovulatory response among the three treatment groups. The size of ovaries was the largest in Group 3 followed by that in Group 2 and 1, respectively, but did not differ significantly ($p>0.05$). Among the ewes responded to superovulatory treatment and subjected to flushing, the total ovarian response (CL+LF) and number of ovulation (total CL)

Table 1. Estrus synchronization in the ewes treated with the combination FSH, PMSG and/or GnRH for inducing the superovulation

Treatment	FSH (Super-OV)	FSH+PMSG	FSH+PMSG+GnRH
No. of ewes	6	6	6
No. (%) of ewes in estrus	6 (100)	6 (100)	6 (100)
No. (%) of ewes ovulated	4 (66.6)	6 (100)	4 (66.6)
Time interval to onset of estrus (h)	50.0±7.29[a]	24.5±3.58[b]	32.5±3.58[b]
Duration of estrus (h)	29.5±3.95	35.0±3.25	33.5±5.33

[a,b] Values without common supersripts differ ($p<0.05$).

Table 2. Ovarian size and superovulatory response in the ewes treated with the combination FSH, PMSG and/or GnRH

Treatment	FSH (Super-OV)	FSH+PMSG	FSH+PMSG+GnRH
1. No. of ewes treated	6	6	6
2. Ovarian dimensions (mm)			
Right ovary			
length	16.6±2.90	20.0±.07	24.7±2.75
Breadth	12.3±1.76	16.2±2.13	17.7±2.13
Left ovary			
length	18.0±2.51	19.5±1.84	22.0±1.77
Breadth	10.3±0.33	15.2±2.09	14.5±1.00
3. Superovulation per ewe treated			
No. of LF	0.6±0.4	2.0±0.8	0.8±0.6
No. of CL	3.0±1.1	4.0±1.2	6.3±2.3
(Range)	(0-7)	(0-8)	(0-16)
Ovarian Response	3.6±0.9	6.0±1.1	7.1±2.0
% of ewes with 2CL	50.0 (3/6)	83.3 (5/6)	66.6(4/6)
4. Superovulation per ewe flushed			
No. of LF	0.0±0.0	1.6±0.9	0.2±0.2
No. of CL	4.0±1.5	4.8±1.2	9.2±2.2
Ovarian Response	4.0±1.5	6.4±1.3	9.5±1.6

Table 3. Fertilization, recovery rate and quality of embryos from superovulated ewes treated with the combination FSH, PMSG and/ or GnRH

	No. of ewes flushed	Total no. of CL	(%) Embryo recovered/ ova ovulated	Fertilization (%)	Good quality embryos (%)
FSH(Super-OV)	3	12	100 (12/12)[a]	100 (12/12)[a]	91.6 (11/12)
FSH+PMSG	5	24	58.3 (14/24)[b]	85.7 (12/14)[ab]	91.6 (11/12)
FSH+PMSG+GnRH	4	37	48.6 (18/37)[b]	50.0 (9/18)[b]	88.8 (8/9)

[a,b] Values without common superscripts differ ($p<0.05$).

were highest ($p<0.05$) in ewes treated with FSH in conjunction with PMSG and GnRH (Group 3). The total number of LF was more ($p<0.05$) in the ewes treated with FSH and PMSG (Group 2) as compared to that in Group 1 and 3. The same trend of results was observed on the basis of number of ewes treated for superovulation but the values did not approach to statistical significance ($p>0.05$).

The results pertaining to embryo recovery and fertilization rates are presented in table 3. The embryo recovery rate was highest ($p<0.05$) in group 1 followed by Group 2 and 3. Fertilization percentage of recovered ova was also higher in Group 1 as compared to Group 2 ($p>0.05$) and Group 3 ($p<0.05$).

DISCUSSION

The use of PGF was effective in synchronizing the superovulatory estrus since majority of ewes exhibited behavioural estrus within 2-3 days after the second

PGF dose as also reported earlier (Naqvi et al., 1998a). The duration of estrus in the ewes was similar to that reported in the natural (Naqvi et al., 1998c) and superovulatory estrus (Naqvi et al., 1998a). The time interval from the second PGF to onset of estrus was longer in ewes treated with FSH alone as compared to ewes which also received PMSG (Group 2 and 3). This is in accordance with the study by Jabbour et al. (1991) who demonstrated that PMSG is highly steroidogenic in sheep as compared to FSH. In the study reported herein, PMSG treated ewes tended to exhibit behavioural estrus earlier than the ewes that received only FSH. Animals stimulated for superovulation set in estrus earlier than the unstimulated ewes on treatment with PGF (Samartzi et al., 1995). The ewes in Group 2 and 3 were stimulated to a greater extent than Group 1 (table 2) as being evident by number of ovulations and large follicles. Ovarian responses (no. of ovulation and no. of LF) were highly variable for all the treatments. The large variation observed in this study reduced the value of statistical analysis and the treatment means for ovarian responses calculated on the basis of per ewe treated could not reach statistical significance ($p>0.05$; table 2). A trend towards an increase in ovarian response due to additional use of PMSG was evident. This comports with other studies which showed higher superovulatory response when PMSG was given in conjunction with FSH-P than FSH-P alone (Ryan et al., 1992; Jabbour et al., 1991). A high proportion of ewes (50%) did not respond to the treatment of FSH alone (Group 1). Eppleston et al (1984) also reported that a proportion of ewes treated with FSH-P alone failed to show superovulatory response regardless of the dose or dose regime. Our results indicated that FSH (Super-OV) was similar to the result of FSH-P inspite of being different commercial preparations. The ovarian response (no. of CL and LF) on treatment with FSH (Super-OV; 25 U) in the present study was comparable to the values reported by Crosby (1993) in Black face Mountain and Cheviot ewes after the treatment with FSH (Super-OV; 36 U).

The adoption and selection of one method of superovulation treatment over another is not only determined by ovulatory response but also by the proportion of ova and embryo recovered, their fertilization rate and yield of good quality embryos. In the present study, the ovulation rate increased to almost double while number of LF decreased when GnRH was administered in ewes treated with FSH and PMSG combination. This is in agreement with the studies on Australian sheep (Ryan et al., 1991; Walker et al., 1986) and Indian sheep (Naqvi and Gulyani, 1998). However the embryo recovery rate in Group 3 ewes (48.6%) was low. This may not be attributed to the faulty application of technique. The collection rate obtained in Group 1 (100%) confirmed that the technique applied was satisfactory. The low embryo recovery rate in Group 3 might be attributed to either loss of ova into peritoneal cavity due to higher ovulation (Mutiga and Baker, 1982) or quick disintegration of unfertilized ova and/or degeneration of embryos during the interval between ovulation and embryo collection because of abnormal maturation of oocytes after PMSG treatment (Moor et al., 1985). Samartzi et al. (1995) also demonstrated that hyperstimulation (total ovarian response) decreased the embryo recovery in Chios sheep. These two factors are additive and tend to explain why lowest recovery rate was obtained in Group 3. In our study PMSG was used in both Group 2 and 3 but higher ovulation rate (9.2 ± 2.25 CL/ewe) was found in Group 3.

The proportion of fertilized ova decreased on administering PMSG in combination with FSH (Group 2 and 3). The low fertilization rate following such treatment may be attributed to either high circulating estrogen (Evans and Robinson, 1980) secreted by unovulated follicles or to abnormal maturation of oocytes (Kumar et al., 1990) which could not be fertilized after ovulation.

It is concluded that PMSG in conjunction with FSH increased the ovarian respose in sheep in tropical environment. However, increase in ovarian response and ovulation rate due to PMSG and GnRH, respectively, negatively influenced the embryo recovery and fertilization rate. Further research is needed to identify the factors associated with low fertilization and embryo recovery in superovulated sheep.

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