OVULATORY RESPONSE AND EMBRYO RECOVERY IN RAMBOUILLET SHEEP USING FSH

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ABSTRACT

Rambouillet sheep (n=15) were subjected to superovulation treatment using FSH (Folltropin-V, Bioniche Animal Health, Canada) during the period between August and November, 2013. The sheep were randomly divided into two groups. Group 1 (n=7) animals were administered 80 mg of NIH-FSH-P1 in six divided doses for 3 days at 12 hour interval in a tapering doses schedule (20 mg, 20 mg, 15 mg, 15 mg, 5 mg and 5 mg) where as ewes of Group 2 (n=8) were given 100 mg NIH-FSH-P1 in the tapering dose schedule (25 mg, 25 mg, 15 mg, 15 mg, 10 mg and 10 mg) as that of group 1. All the ewes received progesterone (P_4) impregnated vaginal sponges for 12 days with FSH treatment initiated either on day 9 or 11 and 200 IU eCG along with first/ fifth FSH injection. P_4 sponges were withdrawn at the time of penultimate FSH injection. Oestrus detection was done using apronized rams from 24-48 hr after sponge withdrawal and those in oestrus were naturally served for 2-3 times at 12 hr interval. Embryos were collected by retrograde surgical technique between day 3 and 5 after oestrus. Animal with two or more corpora lutea (CL) were considered to have responded to superovulation (SOV) treatment. A 57.14 % (4/7) and 75% (6/8) ewes of group 1 and 2, respectively responded to SOV treatment. The ovarian response was highly variable and ranged between two and eight CL irrespective of groups. The mean ovarian response was 2.0±1.06 (14/7) and 2.37 ± 0.8 (19/8) in groups 1 and 2, respectively. The mean number of transferable embryos in groups 1 and 2, respectively was 1.0± 0.57 (4/4) and 1.83 ±0.75 (11/6). The mean ovarian response as well as embryo recovery did not differ significantly (P >0.05) between groups. From the results it may concluded that the reasons for poor ovulation rate (over all 2.2 ± 0.63; 33/15), high anovulation rate (over all 3.4 ± 0.93; 51/15), poor embryo recovery rate (45.45%; 15 embryos/33 CL) as well as less no. embryos recovered per animal (over all 1.50 ± 0.5) need to be investigated and warrant further studies.

Key words: Sheep, superovulation, FSH, embryo

Introduction

Long generation interval coupled with limited prolificacy is the major limiting factor for faster multiplication in sheep. The technique of multiple ovulation and embryo transfer (MOET) has been developed in this species to accelerate genetic improvement by increasing the number of offspring produced. However, successful integration of Embryo Transfer Technology (ETT) into breeding programme largely depends on the number of viable embryos recovered as well as the conception rate following ET. The uncertainty as well as variability in ovulatory response is a frustrating task for extensive exploitation of superior ewes to realize more number of embryos. The response to superovulation as well as the yield of viable embryos depend on various factors such as photo period (Mutiga and Baker, 1984), season (Mufti *et al.*, 1997, Chagas *et al.*, 2003), ovarian follicular status (Gonzalez-Bulnes *et al.*, 2000), hormone used (Driancourt and Fry, 1992), dose schedule (D'Alassandro *et al.*, 2005), repeated superovulation (Magarey *et al*., 2003) etc. Information on the superovulation of Rambouillet sheep in tropical climate is very scanty. The present experiment was an attempt to assess the ovulatory response and embryo recovery in Rambouillet sheep following administration of purified FSH in two different dose schedules.

Materials and Methods

The experiment was conducted during the peak breeding period between August and November at Govt. sheep breeding farm, Panthal, located near Katra, District Reasi of Jammu (J & K) at latitude of 32°58'29"N and 74°58'35"E and at an altitude of 1671 meters. The annual rainfall in the area is about 130 cm and the maximum and minimum temperature during this period was 29.6°C and 11.2°C, respectively. Parous, cycling, non-pregnant Rambouillet sheep (n=15) of 6-8 years age and weighing 40-50 kg were randomly divided into two groups. They were grazed daily from 07:00 AM to 06:00 PM on green pastures interspersed with seasonal shrubs. Additionally each ewe was given daily 6 kg green/dry fodder (Oat/maiz/barseem) and 500gm concentrate mixture. Animals of group 1 (n=7) animals were administered i/m 80 mg of NIH-FSH-P1 (Folltrpin V, Bioniche Animal Health, Canada) in six divided doses for 3 days at 12 hr interval (6 AM and 6 PM) in a tapering dose schedule (20 mg, 20 mg, 15 mg, 15 mg, 5 mg and 5 mg) where as ewes of group 2 (n=8) were given 100 mg NIH-FSH-P1 in the same tapering dose schedule (25 mg, 25 mg, 15 mg, 15 mg, 10 mg and 10 mg) as that of group 1. All the ewes received progesterone (0.35 gm) impregnated vaginal sponges for 12 days with FSH treatment initiated on day 10 and 200 IU eCG (Folligon, Intervet, Holland) along with first/ fifth FSH injection. The vaginal sponges were withdrawn at the time of penultimate FSH injection. Oestrus in gonadotropin treated ewes was detected by using apronised ram of high sexual vigor every 8 hrs for 30 minutes, starting at 24-48 hrs after sponge withdrawal and those in oestrus were naturally mated for 2-3 times at 12 hr interval using Rambouillet ram of proven fertility.

Between day 3 and 5 after super oestrus, the ewes were

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withheld feed and water from 24 and 12 hours, respectively prior to surgery. The abdominal area anterior to the udder was shaved and disinfected with 70% alcohol and the ewes were sedated by administering xylaxine @ 0.05 mg/kg body weight followed by ketamine @ 2-5 mg/kg body weight 10 minutes later. The surgical area was locally infiltrated with 4-5 ml of 2% lignocaine hydrochloride (Lox, Neon Laboratories Limited, Mumbai, India). Each ewe was subjected to laparoscopic ovarian examination to assess the ovarian response and those having ≥2 functional corpora lutea (CL) with good morphological appearance consistent with their age, were subjected to mid ventral laparotomy for embryo collection. Following laparotomy, the ovarian response on each ovary was quantified by counting the number of CL and anovulatory follicles of ≥ 5mm (Naqui and Gulyani, 1999). A PVC tube of 2 mm inner diameter was inserted into the fimbria and each fallopian tube was flushed retrogradely (Hunter *et al.,* 1955) by injecting 20 ml Dulbecco's phosphate buffered saline containing 0.1% BSA from utero-tubal junction towards fimbriated end. The collected tubal washings were examined under stereozoom microscope (Olympus, Japan) at 20-63x magnification. The embryos were evaluated morphologically for their stage and grade as per IETS recommendations. The data was analysed as per Snedecor and Cochran (1994).

Results and Discussion

A total of 33 ovulations (2.2 ± 0.63) were observed and 15 viable embryos (mean 1.5 ± 0.5) were recovered from 10/15 (66.67%) ewes that responded (≥2CL) to superovulatory treatment (Table 1). The ovulatory response varied considerably among ewes (range 2-8 CL) which may be attributed to ovarian status of the donor at the time of gonadotropin treatment (Gonzalez-Bulnes *et al.*, 2000). Wide variation in ovarian response was also reported elsewhere in Rambouillet (Naqui and Gulyani, 1999) and Corridale (Mufti *et al*., 1997) breeds of sheep superovulated with FSH-P. The incidence of superovulatory response was 57.14 and 75.0%, respectively in groups 1 and 2 and the difference was not significant (P>0.05). In the present study, the mean ovarian response was 2.0±1.06 (14/7) and 2.37 ± 0.8 (19/8) in groups 1 and 2 respectively with no significant difference (P>0.05) between groups. It is quiet unusual that the mean ovarian response was exceedingly low irrespective of the groups, in contrast to previous reports (Ishwar and Memon, 1996; Naqui *et al*., 2001; Forcada *et al*., 2011). This could be attributed to variation in breed, hormone used and dosage schedules (Torres and Cognie, 1984). It has been observed that the reproductive performance of Rambouillet sheep was low (0.34 - 0.67 lambs/ ewe/year) in sub temperate and semi arid environments (Tomer and Mahajan, 1980; Nivasasrkar *et al*., 1983). In general the animals with high fecundity are known to be more responsive to gonadotropin stimulation than those with low fecundity (Cahill and Dufour, 1979). The mean number of transferable embryos also did not differ significantly (P>0.05) between groups (1.0± 0.57 vs. 1.83 ±0.75). In the present experiment, the low mean viable embryo recovery might be the direct reflection of poor ovarian response. However, all the recovered embryos were found to be viable indicating the fertility of ram as well as lack of deleterious effect of hormonal treatment on sperm transport. In general, fertilization rates are compromised in high responding donors due to either impaired sperm transport (Evans and Armstrong, 1984) or sub optimal ovum quality (Moor *et al*., 1985). The embryo recovery rate was observed to be low in this study (45.45%; 15 embryos/33 CL). Zhongle Chang *et al.* (2006) reported a recovery rate of 45-92% in different age groups of goats. From the results, it may concluded that the reasons for poor ovulation rate (over all 2.2 ± 0.63; 33/15), high anovulation rate (over all 3.4 ± 0.93; 51/15), poor embryo recovery rate (45.45%; 15 embryos/33 CL) as well as less no. embryos recovered per animal (over all 1.50 ± 0.5) need to be investigated and warrant further studies.

Table 1: Ovulatory response and embryo recovery in super ovulated Rambouillet sheep

Attributes	Treatment		Total
	FSH 80 mg (Group 1)	FSH 100 mg (Group 2)	
No. superovulated	07	08	15
No. responded (≥ 2 CL), (%)	04 (57.14)	06 (75.0)	10 (66.67)
Total ovulations (CL)	14	19	33
Mean No. of ovulations	2.0 ±1.06[a] (14/7)	2.37± 0.8[a] (19/8)	2.2±0.63 (33/15)
Mean No. of anovulatory follicles	2.0 ±0.85[a] (14/7)	4.63±1.5[a] (37/8)	3.4±0.93 (51/15)
Mean No. of viable embryos	1.0±0.57[a] (4/4)	1.83±0.75[a] (11/6)	1.5±0.5 (15/10)
Mean No. of viable embryos	1.0±0.57[a] (4/4)	1.83±0.75[a] (11/6)	1.5±0.5 (15/10)

Means with similar superscripts with in an attribute do not differ significantly (P<0.05)

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