



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2020; 9(6): 375-380

© 2020 TPI

www.thepharmajournal.com

Received: 14-04-2020

Accepted: 17-05-2020

Anil Kumar Pandey

Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu, Jammu Kashmir, India

Utsav Sharma

Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu, Jammu Kashmir, India

Anil Kumar Kaul

Department of Sheep Husbandry Jammu, Govt. of Jammu & Kashmir, Jammu, Jammu Kashmir, India

Sudhir Kumar

Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu, Jammu Kashmir, India

Nishi Pande

Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu, Jammu Kashmir, India

SEH Andrabi

PhD Scholar, Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu, Jammu Kashmir, India

Corresponding Author:

Anil Kumar Pandey

Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, Jammu, Jammu Kashmir, India

Response to FSH (Folltropin-V) and hCG treatment on superovulation and embryo production in sheep under sub-tropical climate

Anil Kumar Pandey, Utsav Sharma, Anil Kumar Kaul, Sudhir Kumar, Nishi Pande and SEH Andrabi

DOI: <https://doi.org/10.22271/tpi.2020.v9.i6f.4796>

Abstract

The effectiveness of FSH (Folltropin-V) in two different doses (80 mg and 100 mg) with or without use of hCG was assessed on achieving superovulation and embryo production in sheep under sub-tropical climate of India. During the breeding season, 39 crossbred ewes were synchronized using progesterone impregnated intravaginal sponges kept in situ for 12 days. Superovulation treatment was given in two groups (80 mg FSH and 100 mg FSH) in six tapering dose schedule. Each group was subdivided in two groups, without hCG treatment or with hCG treatment 24hrs after removal of progesterone implant, thus making four treatment groups. The ewes were observed for estrus every 4h, on the 5th day following estrus ovarian response was evaluated using rigid laparoscope and ewes showing superovulatory response were subjected to embryo collection using retrograde flushing of uterine horns after catheterization of the fimbriated end of the fallopian tube. No differences were found among the treatment groups in incidence of estrus and percentage of ewes showing superovulatory response. Treatment with hCG improved percent ovulation and fertilization percentage in superovulating ewes at higher dose of FSH (100 mg). Although additional treatment with hCG improved total ovarian response and percent ovulations, the mean number of viable embryos and number of unfertilized ova remained statistically unaffected. It is concluded that the FSH (folltropin-V) at 80 mg in six reducing dose schedule is suitable and economical for superovulation in ewes.

Keywords: FSH, Superovulation, hCG, embryo transfer, Folltropin-V

1. Introduction

Reproductive biotechnologies have been a boon for increasing productivity of livestock. Multiple ovulation and Embryo transfer (MOET) in sheep, is useful in reducing generation interval and maximizing production of lambs from genetically superior breeding stocks (Wuliji *et al.*, 1995) [58]. The success and economy of MOET programmes largely depends on the donor's ability to produce embryos and the recipient's pregnancy rate. However, this technology is not so popular on the sheep breeding farms due to highly variable response to superovulation treatments and inconsistency in production of quality embryos among various treatments. Superovulation and embryo production in sheep are variable between the ovarian follicular status (Gonzalez-Bulnes *et al.*, 2000) [21], hormone used (Driancourt and Fry, 1992) [16], dose schedule (D'Alessandro *et al.*, 2005) [13], season (Chagas *et al.*, 2003) [9], re-use of animals (Magarey *et al.*, 2003) [37] etc. Folltropin-V is highly a purified porcine FSH hormone and due to its short half life multiple injections are required to achieve superovulation. The hCG is commonly used as a substitute for LH to initiate luteinisation in the absence of an endogenous LH surge in treated cows and ewes (Kamomae *et al.*, 1989) [29]. It maintains the life span of CL in the treated cows and stimulates the synthesis of progesterone (Litch and Condon, 1988) [32]. It has been tested beneficial in cyclic goats for superovulation using eCG (Urnaru *et al.*, 2013) [55]. Hormonal protocols use for superovulation needs to be standardized at every place for the optimum response and the information on the superovulation of sheep using Folltropin-V in sub-tropical climate is very scanty. The present experiment was aimed to evaluate two doses of FSH (Folltropin-V) with or without additional treatment of hCG in achieving superovulation and embryo production in sheep.

Material and Methods

The present study was carried on crossbred ewes housed at Sheep Breeding Farm, Panthal,

Reasi District of Jammu, India (latitude of 32°58'29"N), during starting of the breeding season (August to November), with environmental temperature fluctuating between a minimum of 8°C and a maximum of 30°C. All the ewes were kept under loose housing system in a clean and hygienic condition and fed daily 6 kg green/dry fodder (Oat/maiz/barseem) and 500 gm concentrate mixture besides routine grazing of 8-10hrs on green pastures.

2.1 Estrus synchronization and superovulation treatment

Thirty nine mature non lactating crossbred ewes were synchronized using intravaginal progesterone impregnated sponge (procured from CSWRI, Avikanagar) kept in-situ for 12 days. Superovulation treatment was initiated with administration of serum gonadotropin (eCG, Folligon, Intervet) @ 200 IU at the time of first FSH injection on day 10 using FSH (Folltropin-V) Bioniche Animal Health, Canada) in six divided doses at 12 hours interval. Progesterone sponges were removed on the 5th FSH injection i.e. 12hrs before last dose of FSH.

2.2 Grouping of animals

Animals were randomly grouped in four different groups i.e. FSH @ 80 or 100 mg each with or without administration of hCG @ 500 IU given at 24hrs after removal of progesterone sponge.

Group-I(n=11) : Ewes were treated with 80 mg of NIH-FSH-P1 in six divided reducing dose regimen of 20 mg, 20 mg; 15 mg, 15 mg; 5 mg and 5 mg.

Group-II(n=9): Ewes were treated with 80 mg of NIH-FSH-P1 in six divided reducing dose regimen of 20 mg, 20 mg; 15 mg, 15 mg; 5 mg and 5 mg along with Inj. hCG (Chorulon, Intervet) @ 500 I.U. 24 hr after P4 implant withdrawal.

Group-III(n=10): Ewes were treated with 100 mg of NIH-FSH-P1 in six divided reducing dose regimen of 30 mg, 30 mg; 15 mg, 15 mg; 5 mg and 5 mg.

Group-IV(n=9): Ewes were treated with 100 mg of NIH-FSH-P1 in six divided reducing dose regimen of 30 mg, 30 mg; 15 mg, 15 mg; 5 mg and 5 mg along with Inj. hCG (Chorulon, Intervet) @ 500 I.U. 24 hr after P4 implant withdrawal.

2.3 Estrus detection of donor and recipient ewes

Estrus detection in donor and recipient ewes was carried out using an aproned ram with 30 minutes duration at an interval of 4 hrs after removal of implant.

2.4 Superovulatory response assessment

Superovulatory response was assessed on 5th day after breeding using a laparoscope (2.5mm diameter and 30cm length) after restraining ewes in on a specially designed lap cradle. Prior to examination food and water was withdrawn for 12hrs and abdominal area anterior to udder was shaved, scrubbed with diluted savlon antiseptic solution, dried with sterile gauze and sprayed with 70% alcohol. Sedation was achieved using Inj. xylazine @ 0.29 mg per kg body weight and local anesthesia by subcutaneous infiltration of Inj. lignocaine 2% (~ 5ml) at the line of incision, 5-7 cm anterior to udder and 3-4 cm each side of the mid ventral line. Pneumoperitonium using 4-5 L CO₂ gas was achieved using endoflator device during the examination. Endoscope was sterilized by dipping in 70% alcohol solution followed by rinsing with double distilled water in between each examination. Each ewe was subjected to endoscopic viewing of ovaries to assess the response to superovulation treatment which was again confirmed after laparotomy and exteriorization of ovaries. The number of Corpus Luteum

(CL) and Anovulatory Follicles (AOF, ≥ 5 mm in size) on each ovary were counted and ewes having >2 CL were considered responded to superovulation treatment (Naqui and Gulyani, 1999).

2.4 Embryo collection and evaluation

Retrograde flushing of the uterine horns was performed by catheterization of fimbriated end of the fallopian tube using 5G infant feeding tube and introduction of flushing media (Euroflush, IMV Technologies) using IV cannula inserted near the bifurcation of uterine horns (D'Alessandro *et al.*, 2001). The washings collected into sterile petri dishes were used for searching, evaluation and gradation of embryos under stereo zoom microscope (Olympus, Japan) at 200X using IETS recommendations. The data was analysed as per Snedecor and Cochran (1994)^[31].

3. Results and Discussion

3.1 Incidence and onset of estrus

The incidence (%) of estrus after superovulation treatment using Folltropin-V in two varying doses with and without hCG groups are presented in table-1. The incidence of estrus was 100% in Group I (FSH 80 mg + eCG 200 IU, n=11), Group III (FSH 100 mg + eCG 200 IU, n=10) and Group IV (FSH 100 mg + eCG 200 IU +hCG, n=9) animals, whereas it was 88.89% in Group II (FSH 80 mg + eCG 200 IU+hCG, n=9) animals. Incidence of estrus in cumulative Group I and II (80 mg FSH) was 95 percent and in cumulative Group III and IV (100 mg FSH) it was 100 percent. The incidence of estrus in the treated ewes did not differ among the four groups. The mean time required for the onset of estrus after withdrawal of progesterone sponge in the donor ewes ranged from 3.00hrs to 27.00 hrs (table 2) which varied non significantly between the groups. High estrus induction response are comparable with the Torres and Cognie (1984) who used FSH-P and other studies where single injection of eCG was used on the onset of start of FSH treatment (Forcada *et al.*, 2011; Naqvi and Gulyani, 1998; Naqvi and Gulyani, 1999)^[19, 42, 44]. The exogenous administration of FSH improves the number of dominant follicles development (Mehmood *et al.*, 2012)^[41]. The change from negative to positive feedback between oestradiol and LH when secretion of oestradiol by healthy large antral follicles is enhanced during the follicular phase (Baird, 1983)^[2], induces oestrus behavior and the occurrence of the preovulatory LH rise (Baird and McNeilly, 1981)^[3]. Some of the non responding ewes might have initial follicular development but no preovulatory LH rise or ovulation (Gonzalez-Bulnes *et al.*, 2002)^[20, 22] and may be considered low ovulators (Dattena, 1989)^[15].

The distribution of occurrences of estrus in ewes after superovulation treatment showed that most of the ewes had shown estrus over shorter period of time and synchronization treatment was efficient which is in agreement with the results of other studies that eCG and FSH combination hastens the onset of estrus in ewes. (Naqvi and Gulyani, 1999; Boscos *et al.*, 2002; Blanco *et al.*, 2003 and Junior *et al.*, 2006)^[42, 6, 5, 33]. However longer interval to onset of estrus (36.0 \pm 2.7h) was reported by Cordeiro *et al.* (2003)^[12] in Santa Ines ewes than that observed in our experiment, the differences may be due to breed differences and higher frequency of oestrus detection practiced in the present study. The onset of estrus after progesterone withdrawal was little earlier in animals receiving lower FSH dose though the mean differences between the groups were statistically non significant ($p>0.05$). Okada *et al.*, 2000^[45] observed slightly earlier onset of estrus in ewes having normal CL compared to abnormal CL development after superovulatory treatment. The differences in onset of estrus after treatment may vary due to changing environment during the course of study, individual animal response or stress related to the handling of animals.

Others studies (Torres *et al.*, 1987; Martemucci *et al.*, 1988) [53, 38] had shown a significant relationship between onset of estrus and

ovulation rates.

Table 1: Effect of superovulation treatment on incidence of estrus (%) after removal of progesterone sponge.

Treatment Groups (n=Number of animals treated)	Number of animals observed in estrus (%)	
FSH 80 mg (n=20)	Group I (n=11)	11(100%)
	Group II (n=9)	8(88.89%)
FSH 100mg (n=19)	Group III (n=10)	10(100%)
	Group IV (n=9)	9(100%)

Table 2: Time required for onset of estrus after removal of progesterone sponge.

Treatment Groups	Onset of estrus (hrs) Mean±SE (Range)	
Group I (n=11)	11.50±2.17 (3.00-21.00)	11.37±1.37(3.00-21.00)
Group II (n=8)*	11.25±1.47 (9.00-21.00)	
Group III (n=10)	12.60±1.25 (9.00-18.00)	14.21±1.19(9.00-27.00)
Group IV (n=9)	16.00±2.00 (9.00-27.00)	
Total (n=38)	12.79±0.92(3.00-27.00)	

*one ewe was not observed in estrus.

3.2 Ovarian Response

In this experiment the overall mean number of ovulations observed on the basis of CL number counted on the ovarian surface varied from 0- 14 with a mean of 5.68±0.64 ovulation per ewe (table 3). The values were similar to the results obtained by Naqvi and Gulyani, (1999) [42] using FSH-P @18mg over three days (5.6 ±1.99 Cl/ewe) and PMSG+ FSH(Ovagen) 7.2mg over four days(6.6±1.99 Cl/ewe) in Rambouillet ewes and in Bharat Merino ewes (5.6±2.33 Cl/ewe) by Naqvi and Gulyani, (1996) [43] maintained at semiarid tropics of India but the values are slightly lower than that reported in Suffolk ewes (7.1±0.84) by Mc Kelvey (1994) [40], Morada Nova (white variety) ewes (10.2±1.2) by Lopes Junior *et al.* (2006) [33] and in Merino ewes (13.8± 2.2) by Bruno-Galarraga *et al.* (2014) [8]. The large anoulated follicles present on some ovaries reflected high degree of hyperstimulation of ovaries (Naqvi and Gulyani, 1999) [42].

Group wise analysis of results (table 3) shows significantly higher ($p<0.05$) mean ovulation per ewe in the Group II ewes compared to the Group III. The total ovarian response (Cl+ anovulated large follicles) was also high in the Group II and lowest in the Group III with non significant differences between the other groups. One ewe in each of the Group I, II and III failed to ovulate. The results corroborate with the others findings that best response was obtained only from about one third of the donors (Seidel and Seidel, 1991; Greve *et al.*, 1995) [50, 24]. The multiple ovulation responses were related to the population of gonadotrophin-responsive follicles (2-3 mm) available, at the time of gonadotrophin treatment (Veiga-Lopez *et al.*, 2005) [55]. Higher ovulation in Group-IV indicated availability of gonadotrophin responsive small antral follicles at the onset of superstimulation (Scaramuzzi *et al.*, 2011) [49].

Table 3: Effect of superovulation treatment on total ovarian response.

Treatment groups (n)	Mean±SE (Range) Number of ovulations (CL)	Mean±SE (Range) Anovulated large follicles	Mean±SE (Range) Total ovarian response (CL+ anovulated large Follicle)
Group I (11)	4.91±0.87 ^{ab} (0-8)	3.55±1.27 (0-12)	8.46±0.95 (4-12)
Group II (7)	8.43±1.93 ^a (0-13)	3.00±1.02 (0-6)	11.43±2.81 (1-19)
Group III (10)	4.30±1.44 ^b (0-14)	2.30±0.60 (0-6)	6.60±1.40 (1-16)
Group IV (9)	6.00±0.76 ^{ab} (2-9)	4.44±1.20 (0-9)	10.44±1.60 (3-16)
Total (37)	5.68±0.64 (0-14)	3.32±0.53 (0-12)	9.00±0.83 (1-19)

Means bearing different superscript differ significantly ($p<0.05$) within the columns

3.3 Superovulation percentage

The superovulation percentage (table 4 and table 5) was highest in the treatment Group IV (89%) ewes compared to Group III (50%). It shows that hCG was found to be effective in increasing ovulation rate in the two doses of FSH (80 mg and 100 mg) while highest percent increase (38.89%) was observed higher dose of FSH (Group IV), though anoulated large follicle numbers were not reduced and were highest in the Group IV ewes. Lamraoui *et al.* (2014) [30] used repeated injections (day 0 and 2 after sponge removal) of hCG @ 500 IU in PMSG superovulated ewes and observed higher number of CL per ewe (10.50±5.54) compared to control (6.33±1.15). Braden and Moule (1962) [7] and Hunt *et al.* (1971) [27] observed considerable variations in the ovulatory response and low fertility among eCG-hCG induced ewes in anestrus season.

The hCG is commonly used as a substitute for LH to initiate luteinisation in the absence of an endogenous LH surge in treated cows and ewes (Kamomae *et al.*, 1989) [29]. It maintains the life

span of CL and stimulates the synthesis of progesterone (Litch and Condon, 1988) [32]. Embryo production in superovulated ewes are compromised in approximately 20-30% of the ewes (Gonzalez-Bulnes *et al.*, 2000) [21] possibly by a deficient or inexistent preovulatory LH surge (Gonzalez-Bulnes *et al.*, 2003) [23] or when the follicles are in non-responsive state due to a downregulation of the granulosa and theca LH receptors (Lopez-Diaz & Bosu, 1992). Use of LH at the end of exogenous FSH treatment may increase the ovulation rate and the number of recovered embryos Cognié *et al.* (1986) [10], though the procedures are still unclear and may differ between different genotypes (Picazo *et al.*, 1996) [46]. Excessive ovarian stimulation can yield low ovulations because of its reduced sensitivity to LH (Saumande and Chupin, 1996) [48]. Results presented show beneficial effect of adding hCG in the protocol by increasing number of ovulations, total ovarian response and proportion of ewes showing superovulation.

Table 4: Ovarian response in superovulated (>2 CL) ewes in different groups

Animal observed in estrus (n)	Proportion of ewes showing >2 CL(%)	Mean±SE (Range) Number of ovulations (CL)	Mean±SE (Range) Anovulated large follicles	Mean±SE (Range) Total ovarian response (CL+ anovulated large Follicle)
Group I (11)	8/11(73%)	6.29±0.64 ^a (4-8)	1.29±0.57 ^b (0-4)	7.57±1.09 ^b (4-11)
Group II (7)	6/7(86%)	10.00±1.90 ^a (3-13)	3.40±1.40 ^{ab} (0-6)	13.40±3.19 ^a (3-19)
Group III (10)	5/10(50%)	7.60±1.94 ^a (4-14)	1.60±0.40 ^{ab} (0-2)	9.20±2.06 ^{ab} (6-16)
Group IV (9)	8/9(89%)	6.43±0.75 ^a (3-9)	4.71±1.46 ^a (0-9)	11.14±1.68 ^{ab} (5-16)
Total (37)	27/37(73%)	7.38±0.66 (3-14)	2.79±0.60 (0-9)	10.17±1.0 (3-19)

Table 5: Effect of treatment on superovulation percentage in treated ewes.

Treatment group	Animals in heat	Number of animals exhibiting >2 CL	Superovulation%	Percent Increase in ovulation
Group I	11	8	72.73	12.98%
Group II	7	6	85.71	
Group III	10	5	50.00	38.89%
Group IV	9	8	88.89	
Total	37	27	72.97	

Table 6: Effect of treatment on embryo recovery percentage.

Number of animals flushed	Total CL observed	Total Embryo	Total Unfertilized ovum	Total structures recovered	Embryo Recovery %	Total structures Recovery %	Fertilization %
I (7)	44	12	3	15	27.27	34.09	80.00
II(5)	50	5	9	14	10.00	28.00	35.71
III(6*)	40	12	6	18	30.00	45.00	66.67
IV(7)	45	10	1	11	22.22	24.45	90.91
Total (24)	179	39	19	58	21.79	32.40	67.24

*One ewe with only 2 CL was also used and one embryo was recovered.

Table 7: Mean±SE (Range) Embryo, UFO and Total recovered structures in different groups.

Number of animals flushed	Mean±SE (Range) Embryo	Mean±SE (Range) UFO	Mean±SE (Range) Total recovered structures
I (7)	1.71±0.68 ^a (0-5)	0.43±0.43 ^a (0-3)	2.14±0.74 ^a (0-5)
II(5)	1.00±0.63 ^a (0-3)	1.60±1.60 ^a (0-8)	2.60±1.47 ^a (0-8)
III(6*)	2.00±0.63 ^a (0-4)	1.00±0.63 ^a (0-3)	3.00±0.78 ^a (0-6)
IV(7)	1.42±0.65 ^a (0-5)	0.14±0.14 ^a (0-1)	1.57±0.65 ^a (0-5)
Total (24)	1.56±0.32(0-5)	0.72±0.36(0-8)	2.28±0.43(0-8)

Means bearing different superscript differ significantly ($p \leq 0.05$) within the columns

*One ewe with only 2 CL was also used and one embryo was recovered.

3.4 Recovery of Embryo

The embryo recovery percentage throughout this experiment ranged from 10-30 percent with an average of 21.79 percent, which is quite lower than the usual rates obtained under the traditional decreasing dose FSH protocols (D'Alessandro et al., 1996) [14]. Forcada et al. (2012) [18] observed 50-60% recovery rate while Ramon-Ugalde et al. (2008) [47] observed 70% recovery rate after uterus flushing in superovulated ewes. Very low recovery rate was observed by López-Saucedo et al. (2013) [35] in wild Barbary sheep of Mexico. Since pedigree records of born lambs were maintained in the breeding farm where this experiment was performed, single male was used for breeding which may be a cause of low fertilization and low embryo recovery rate in our experiments, this may be improved in future by use of two males or intra uterine insemination (López-Saucedo et al., 2013) [35], which require further standardization. According to Leoni et al. (2001) [31], the recovery rates could be reduced by the ovarian hypertrophy which might have obstructed the fimbriae to capture the oocytes after ovulation. Ovarian hypertrophy was also observed by Forcada et al. (2012) [18] in their FSH+eCG simplified treatment in ewes. Low recovery of flushing fluid from some of the fallopian tubes observed in some of the ewes under these experiments may also be contributing to lower results. Differences in the recovery rate are usually related

to the operator and flushing conditions (Mayorga et al., 2011) [39]. The total structures recovered ranged from 24.45% to 45% which were also lower than other workers using FSH for superovulation. After ovulation all ova are expected to be in the oviduct (Hafez, 1987) [25] but in superovulated animals due to the altered hormonal profile (Wilmut et al., 1985) [57] and in some cases due to ovarian hypertrophy (Forcada et al., 2012) [18] only a number of ova gain entry in the fallopian tube and exhibit an accelerated transport in the genital tract (Hawk et al., 1988) [26]. It is also known that degenerated embryos do not follow the normal pattern of transport in the uterus and often are quickly disintegrated (Foote and Ellington, 1988) [17] only a proportion of the fertilized ova develop to morulae or blastocysts (Jabbour and Evans 1991) [28]. Identification and isolation of degenerated embryos is difficult since very often they are blocked by masses of epithelial cells or they float in the flushing medium (Lymberopoulos et al., 2001) [36].

The highest fertilization rate observed in group IV (eCG + 100 mg FSH + hCG) indicated beneficial effect of hCG while in Group-II low fertility may be attributed to low embryo recovery percentage. Lower recoveries of viable embryos may account of the possible fertilization failure and/or degeneration of embryos prior to flushing, when there is a higher ovulation rate (Armstrong & Evans, 1983) [1]. Since natural mating was used in

this experiment, it might have defective intrauterine sperm migration (Betteridge 2006). Besides this, abnormal profile of ovarian hormones might have modified the oviductal and uterine transport of ova and embryos within the genital tract (Leoni *et al.*, 2001) [31]. Increase in the ovulation rate under Group II, indirectly has negatively affected embryo recovery percentage total structures recovered and fertility percentage of recovered structures while this effect was compensated by the little higher dose of FSH seen in the Group IV as high embryos fertility (90.91%) and low number unfertilized ovum in the Group III. The mean embryo, UFO and total recovered structure per ewes observed in the ewes showing superovulatory response under different treatments groups has not shown significant statistical differences, this is due to the wide variation observed in the response of individual ewes in each group, lower number of animals in each group and due to small differences in the dose of FSH used between the groups. The results from the current study affirms the finding of others that superovulation treated ovary have follicles with disturbed functionality, and compromised competence of their oocytes to develop into viable embryos (Veiga-Lopez *et al.*, 2008) [56].

Conclusion

In our study we have achieved satisfactory superovulation response by use of FSH(Follitropin-V) in 80 mg and 100 mg reducing dose schedule. Additional hCG given 24h after last FSH dose may increase the ovulation rate, and total recovered structures, however lower dose of FSH has resulted equally good superovulation response and good quality embryos. In the view of economy of the protocol use of 80mg FSH without addition of hCG will be useful for achieving superovulation and embryo recovery embryos in the crossbred ewes, though more research is recommended for achieving higher embryo recovery rate in the superovulated ewes.

Acknowledgments

We are thankful for the help and support provided by the Director, Sheep Husbandry Department of Jammu and the Hon'ble V. C., SKUAST-J for conducting this research work.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Govt. of India

References

1. Armstrong DT, Evans G. Factors influencing success of embryo transfer in sheep and goats. *Theriogenology*. 1983; 19:31-42.
2. Baird DT. Factors regulating the growth of the preovulatory follicle in sheep and human. *J. Reprod. Fertil*. 1983; 46:343-352.
3. Baird DT, McNeilly AS. Gonadotrophic control of follicular development and function during the oestrous cycle in the ewe. *J Reprod Fertil Suppl*. 1981; 30:129-133.
4. Betteridge KJ. A history of farm animal embryo transfer and some associated techniques. *Animal Reproduction Science*. 2003; 79(3-4):203-244.
5. Blanco M, Simonetti L, Rivera O. Embryo production and progesterone profiles in ewes superovulated with different hormonal treatments. *Small Ruminant Research*. 2003; 47(3):183-191.
6. Boscós CM, Samartzi FC, Dellis S. Use of progestagen gonadotropin treatments in estrus synchronization of sheep.

7. Braden AWH, Moule GR. Induction of ovulation in anoestrus ewes. *Australian Journal of Experimental Agriculture*. 1962; 2:75-77.
8. Bruno-Galarraga MM, Cueto M, Gibbons AE, Pereyra-Bonnet F, Catalano R, Gonzales-Bulnes A *et al*. Repeatability of superovulatory response to successive FSH treatment in Merino sheep. *Small Ruminant Research*. 2014; 120:84-9.
9. Chagas E Silva J, Lopes da Costa L, Cidadoo R, Robalo Silva J. Plasma progesterone profiles, ovulation rate, donor embryo yield and recipient embryo survival in native Saloia sheep in the fall and spring breeding seasons. *Theriogenology*. 2003; 60:521-532.
10. Cognie Y, Chupin D, Saumande J. The effect of modifying the FSH/LH ratio during the superovulatory treatment in ewes. *Theriogenology*. 1986; 25:148. (Abstract).
11. Cognie Y. State of the art in sheep-goat embryo transfer. *Theriogenology*. 1999; 51(1):105-116.
12. Cordeiro MF, Lima-Verde JB, Lopes-Júnior ES, Teixeira DIA, Farias LN, Salles HO *et al*. Embryo recovery rate in Santa Inês ewes subjected to successive superovulatory treatments with pFSH. *Small Ruminant Research*. 2003; 49(1):19-23.
13. D Alessandro AG, Martemucci G, Taibi L. How the FSH/LH ratio and dose numbers in p-FSH administration treatment regimen and insemination schedule affect superovulatory response in ewes. *Theriogenology*. 2005; 63:1764-1774.
14. D'Alessandro A, Martemucci G, Totada F, Gambacorta M, Manchisi A. Superovulation and embryo production in ewes using a commercial p-FSH. *Small Ruminant Research*. 1996; 19(3), 255–261.
15. Dattena M. Multiplication of five breeds of "Exotic" sheep in New Zealand using the technique of embryo transplantation. Master of Philosophy Thesis. 1989. Massey University, New Zealand.
16. Driancourt MA, Fry RC. Effect of superovulation with pFSH or PMSG on growth and maturation of the ovulatory follicles in sheep. *Animal Reproduction Science*. 1992; 27(4):279-292.
17. Foote RH, Ellington JE. Is a superovulated oocyte normal? *Theriogenology*. 1988; 29(1):111-123.
18. Forcada F, Sánchez-Prieto L, Casao A, Palacín I, Cebrián-Pérez JA, Muñio-Blanco MT *et al*. Use of laparoscopic intrauterine insemination associated with a simplified superovulation treatment for *in vivo* embryo production in sheep: a preliminary report. *Anim Prod Sci*. 2012; 52:111-1116.
19. Forcada F, Ait Amer-Meziane M, Abecia JA, Maurel MC, Cebrian-Perez JA, Muñio-Blanco T *et al*. Repeated superovulation using a simplified FSH/eCG treatment for *in vivo* embryo production in sheep. *Theriogenology*. 2011; 75:769-776.
20. González-Bulnes A, Santiago-Moreno J, Cocero MJ, Souza CJH, Groome NP, García-García R *et al*. Measurement of inhibin A and follicular status predict the response of ewes to superovulatory FSH treatments. *Theriogenology*. 2002; 57:1263-1272.
21. Gonzalez-Bulnes A, Santiago-Moreno J, Cocero MJ, Lopez-Sebastian A. Effects of FSH commercial preparation and follicular status on follicular growth and superovulatory response in Spanish Merino ewes. *Theriogenology*. 2000; 54(7):1055-1064.
22. Gonzalez-Bulnes A, Garcia-Garcia R, Souza C, Santiago-Moreno J, Lopez-Sebastian A, Cocero M *et al*. Patterns of Follicular Growth in Superovulated Sheep and Influence on Endocrine and Ovarian Response. *Reproduction in Domestic Animals*. 2002; 37(6):357-361.
23. González-Bulnes A, García-García RM, Santiago-Moreno J,

- Domínguez V, López Sebastián A, Cocero MJ *et al.* Reproductive season affects inhibitory effects from large follicles on superovulatory response in ewes. *Theriogenology*. 2003; 60:281-288.
24. Greve T, Callesen H, Hyttel P, Hoier R, Assey R. Effects of exogenous gonadotrophins in oocyte and embryo quality in cattle. *Theriogenology*. 1995; 43(1):41-50.
 25. Hafez ESE. *Reproduction in Farm Animals*. 5th Edition, Lea and Febiger, Philadelphia, 1987, 315-481.
 26. Hawk HW, Conley HH, Wall RJ, Whitaker RO. Fertilization rates in superovulating cows after deposition of semen on the infundibulum, near the uterotubal junction or after insemination with high numbers of sperm. *Theriogenology*. 1988; 29:1131-42.
 27. Hunt WL, Addleman D, Bogart R. Induction of multiovation in the ewe following synchronization of estrus. *Journal of Animal Science*. 1971; 32(3):491-495.
 28. Jabbour HN, Ryan JP, Evans G, Maxwell WM. Effects of season, GnRH administration and lupin supplementation on the ovarian and endocrine responses of merino ewes treated with PMSG and FSH-P to induce superovulation. *Reproduction, fertility, and development*. 1991; 3(6):699-707.
 29. Kamomae H, Kaneda Y, Domeki I, Nakahara T. Blood LH level and induced ovulation after hCG and PMSG treatment in ovarian quiescent cattle. *Nihon Juigaku Zasshi*. 1989; 51(3):467-473.
 30. Lamraoui R, Afri-Bouzebdia F, Bouzebdia Z, Franck M, Gherissi DE. Effect of Repeated Administration of hCG on Ovarian Response in PMSG-superovulated Ouled Djellal Ewes (Algeria). *Tropicicultura*. 2014; 32:10-15.
 31. Leoni G, Bogliolo L, Pintus P, Ledda S, Naitana S. Sheep embryos derived from FSH/eCG treatment have a lower *in vitro* viability after vitrification than those derived from FSH treatment. *Reproduction Nutrition Development*. 2001; 41:239-246.
 32. Litch SJ, Condon WA. Interaction of hCG and Lutalyse on steroidogenesis of bovine luteal cells. *Molecular and Cellular Endocrinology*. 1988; 57(102):81-85.
 33. Júnior ESL, Maia ELMM, Paula NRO, Teixeira DIA, Villarroel ABS, Rondina D *et al.* Freitas VJF. Effect of age of donor on embryo production in Morada Nova (white variety) ewes participating in a conservation programme in Brazil. *Tropical Animal Health and Production*. 2006; 38(7-8):555-561.
 34. Lopez-Diaz MC, Bosu TWK. A review and an update of cystic ovarian degeneration in ruminants. *Theriogenology*. 1992; 37(6):1163-1183.
 35. López-Saucedo J, Ramón-Ugalde JP, de Barroso-Padilla JJ, Gutiérrez-Gutiérrez AM, Fierro R, Piña-Aguilar RE. Superovulation, *in vivo* Embryo Recovery and Cryopreservation for Aoudad (*Ammotragus Lervia*) Females Using Osmotic Pumps and Vitrification: A Preliminary Experience and its Implications for Conservation. *Tropical Conservation Science*. 2013; 6(1):149-157.
 36. Lymberopoulos AG, Amiridis GS, Kühholzer B, Besenfelder U, Christodoulou V, Vainas E *et al.* Fertilization and embryo recovery rates in superovulated Chios ewes after laparoscopic intrauterine insemination. *Theriogenology*. 2001; 55(9):1855-1862.
 37. Magarey GM, Rodger JC, Buist JM, Mate KE. Effects of repeated superovulation and surgical oocyte collection on ovarian response and natural breeding ability of the tamar wallaby (*Macropus eugenii*). *Reprod*. 2003; 125:701-707.
 38. Martemucci G, Gambacorta M, Totada F, Manchisi A, Bellitti E. Induzione della superovulazione nella pecora con PMSG, FSH-p, hMG, per il trapianto di embrioni (Superovulatory response to treatment with PMSG, FSH-p or hMG in ewe for embryo transfer). *Zoot. Nutr. Anim*. 1988; 15:379-386.
 39. Mayorga I, Mara L, Sanna D, Stelletta C, Morgante M, Casu S *et al.* Good quality sheep embryos produced by superovulation treatment without the use of progesterone devices. *Theriogenology*. 2011; 75(9):1661-1668.
 40. Mc Kelvey WAC. Recent research on AI and MOET in sheep and their commercial application in UK. In: *Proceedings of the Embryo Transfer Workshop*, Hamilton, New Zealand, 1994, 96-107.
 41. Mehmood MU, Mehmood S, Riaz A, Ahmad N, Sattar A. Superovulatory response in summer anestrus buffaloes and cattle treated with estrus synchronization protocol. *The Journal of Animal & Plant Sciences*. 2012; 22(4):888-893.
 42. Naqvi SMK, Gulyani R. Ovarian response and embryo recovery to different superovulatory regimes in Rambouillet ewes under semi-arid conditions. *Small Ruminant Research*, 1999; 34(2):127-131.
 43. Naqvi SMK, Gulyani R. Superovulatory response of Bharat Merino ewes to FSH-p during different seasons. In: *Proceedings of the 13th International Congress on Animal Reproduction*, Velhoven, 1996, 4-5
 44. Naqvi SMK, Gulyani R. The effect of gonadotropin releasing hormone and follicle stimulating hormone in conjunction with pregnant mare semen gonadotropin on the superovulatory response in crossbred sheep in India. *Tropical Animal Health and Production*. 1998; 30:369-376.
 45. Okada A, Kamada S, Jeon CW, Miyamoto A, Fukui Y. Incidence of abnormal corpus luteum in superovulated ewes. *J Reprod Dev*. 2000; 46:397-402.
 46. Picazo RA, Cocero MJ, Barragán ML, López Sebastián A. Effects of LH administration at the end of an FSH superovulatory regimen on ovulation rate and embryo production in three breeds of sheep. *Theriogenology*. 1996; 45(5):1065-1073.
 47. Ramon-Ugalde JP, Folch J, Cocero MJ, Piña-Aguilar RE, Alabart J. Embryo recovery from the oviduct in superovulated ewes: a method to improve MOET systems. *Czech Journal of Animal Science*. 2008; 53:45-51.
 48. Saumande J, Chupin D. Induction of superovulation in cyclic heifers: The inhibitory effect of large doses of PMSG. *Theriogenology*. 1986; 25:233-247.
 49. Scaramuzzi RJ, Baird DT, Campbell BK, Driancourt MA, Dupont J, Fortune JE *et al.* Regulation of folliculogenesis and the determination of ovulation rate in ruminants. *Reproduction, Fertility, and Development*, 2011; 23:444-467.
 50. Seidel Jr EG, Seidel MS. *Training manual for embryo transfer in cattle*. Food and Agriculture Organization, 1991. *Animal Production and Health Paper*. 77: U. N.
 51. Snedecor GW, Cochran WG. *Statistical methods*. 6th edn. Iowa state University press, Ames, 1976.
 52. Torris S, Cognie Y. Superovulation and egg transfer in the ewe. *Reprod.Nutr*. 1984; 24:623-631.
 53. Torres S, Cognie Y, Colas G. Transfer of Superovulated sheep embryos obtained with different FSH-P. *Theriogenology*. 1987; 27:407-419.
 54. Umaru MA, Garba HS, Adeyanju JB, Bello A, Oyedipe EO, Buhari S *et al.* Oestrus synchronization and superovulation in the red Sokoto doe (RSD) in Sokoto, Nigeria. *Scientific Journal of Zoology*. 2013; 2(1):6-11.
 55. Veiga-Lopez A, Gonzalez-Bulnes A, Garcia-Garcia RM, Dominguez V, Cocero MJ. The effects of previous ovarian status on ovulation rate and early embryo development in response to superovulatory FSH treatments in sheep. *Theriogenology*. 2005; 63(7):1973-1983.
 56. Veiga-Lopez A, Dominguez V, Souza CJH, Garcia-Garcia RM, Ariznavarreta C, Tresguerres JAF *et al.* Features of follicle-stimulating hormone-stimulated follicles in a sheep model: keys to elucidate embryo failure in assisted reproductive technique cycles. *Fertility and Sterility*. 2008; 89(5):1328-1337.
 57. Wilmut I, Sales DI, Ashworth C. The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. *Theriogenology*. 1985; 23(1):107-19.
 58. Wuliji T, Montgomery GM, Dodds KG, Andrews RN, Beattie AE, Turner PR *et al.* Establishing a flock for gene mapping in wool traits. In: *Proceedings of the New Zealand Society of Animal Production*, 1995; 55:285-288.