Research Article

DOI: 10.48165/ijar.2021.42.2.10

The Indian Journal of Animal Reproduction

The official journal of the Indian Society for Study of Animal Reproduction

ACS Publisher www.acspublisher.com

Year 2021, Volume-42, Issue-2 (Dec)

ISSN 0970-2997 *(Print)*

Effect of Two Different FSH Doses on Superovulatory Response and Embryo Production in Sahiwal Cows

Nidha Imtiyaz[1], Parkash Singh Brar[1], Narinder Singh[2*] and Vijay Singh Malik[3]

[1]Department of Veterinary Gynaecology and Obstetrics
[2]Directorate of Livestock Farms
[3]Department of Veterinary Physiology and Biochemistry Guru Angad Dev Veterinary and Animal Sciences University, Ludhian-141004, Punjab, India.

ABSTRACT

The study was conducted to evaluate the effect of two different doses of FSH on superovulatory responses in Sahiwal cows. Twelve normally cyclic pluriparous Sahiwal cows weighing between 350 and 450 kg were superstimulated using two different doses of Folltropin-V (100 mg vs 200 mg of NIH FSH PI). Prior to superstimulation, the cows were administered estradiol-17β (2 mg, IM) with placement of intravaginal progesterone releasing device (TRIU-B) at the random stage of the estrous cycle (Day 0). From day 4 onward, cows were administered Folltropin-V @100 mg in the group 1 and 200 mg of NIH FSH PI in the group 2; the total dose was divided into 8 tapering doses given at 12 hourly intervals in both the groups. Prostaglandin F2α analogue (PGF2α) was administered at day 6 and 6.5; TRIU-B was removed with the last FSH injection. All cows in both the groups were inseminated twice at 12 and 24h of LH treatment. Daily ultrasonography was performed to record the size and number of follicles and superovulatory response. Results showed that the number of small follicles (≤7 mm) present at the time of initiation of superstimulatory treatment did not differ between both the groups (23.3±3.93 vs. 22.3±1.17, p>0.05). Following superstimulatory treatment, the average number of small follicles reaching ovulatory size (>10 mm) were 18.0±4.04 and 19.2±1.11 in the group-1 and the group-2, respectively; the difference was non-significant (p>0.05). Similarly, the total embryos and transferable embryos recovered also did not differ between the group-1 and the group-2 (9.0±4.02 vs 10.7±2.78; p>0.05, and 3.5±1.26 vs 3.3±1.84; p>0.05, respectively). The study showed that low dose of follicle stimulating hormone i.e. 100 mg is equally effective as 200 mg of NIH FSH PI in inducing superstimulation and embryo production in Sahiwal cows.

Key words: Dosage, Embryo, FSH, Sahiwal, Superovulation.

How to cite: Imtiyaz, N., Brar, P.S., Singh, N., & Malik, V.S. (2021) Effect of Two Different FSH Doses on Superovulatory Response and Embryo Production in Sahiwal Cows.
The Indian Journal of Animal Reproduction, 42(2), 56–64. https://doi.org/10.48165/ijar.2021.42.2.10

INTRODUCTION

Bos *indicus* breeds of cattle have been playing an important role in providing food and livelihood to rural masses in India and many other Asian countries. These breeds are more resilient to higher temperature, more resistant to tick born and other tropical diseases compared to Bos *taurus* and their crosses (Khan *et al.,* 2008). Sahiwal is considered

*Corresponding author.
E-mail address: narinder2002us@gmail.com (Narinder Singh)
Received 26-12-2022; Accepted 26-01-2023

the best milch breed of Bos *indicus* cattle. The availability of Sahiwal cows having good milk production ability is very limited and there is need to improve and propagate this breed for sustainable milk production in the country. The reproductive techniques like multiple ovulation & embryo transfer (MOET) involving superovulation, in vivo embryo collection & transfer has the potential for the faster dissemination of Sahiwal germplasm through production of future breeding sires and bull mothers.

The ovarian response to the superstimulatory treatment is one of the most important determinants of success of embryo transfer program. The conventional superovulation protocols developed & standardized had used 400 mg NIH-FSH-P1 of a partially-purified pituitary extract or 28 to 50 mg (Armour) of a crude pituitary extract, administered intramuscularly twice daily, divided in tapering or constant doses over 4 or 5 days in Bos *taurus*. Similar protocols with dosage regimen varying from 200 to 400 mg NIH-FSH-P1 have been used for superovulation in Bos *indicus* including Sahiwal. However, Bos *indicus* breeds of cattle are considered more sensitive to exogenous gonadotrophins than Bos *taurus* (Randel, 1984) and required 20-30% less dose of gonadotrophins compared to Bos *taurus* (Lewis, 1992). Due to this it was hypothesized that the Sahiwal cows may give satisfactory superovulatory response at lower i.e. 100 mg NIH-FSH-P1 dose of FSH. A few attempts with reduced dose of FSH had been made in Bos *indicus* cows with variable success (Dudukuri, 2015; Singhal *et al.,* 2017; Singh *et al*., 2018). Therefore, the present study was planned with the objective to evaluate the effect of lowering FSH doses to 100 mg NIH-FSH-P1 on superovulatory response and embryo production in Sahiwal cows.

MATERIALS AND METHODS

Experimental animals

The present study was conducted on twelve pluriparous Sahiwal cows (n=12), normally cyclic, weighing between 370 to 450 kg with body condition score (BCS) of more than 3 on scale of 5, maintained at Dairy farm, Directorate of Livestock Farm, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The selected cows were non pregnant, free from uterine abnormalities, maintained under loose housing system; fed with chaffed green fodder, wheat straw, concentrates, common salt, mineral mixture and *ad libitum* drinking water.

Preliminary trial

A preliminary train was conducted before the actual start of the study to select dosage regimen of FSH whether to be kept constant or at the tapering schedule. To select the appropriate manner (constant vs. tapering) of FSH administration, a preliminary trial was conducted to evaluate the efficacy of 100 mg-NIH-FSH-P1 administered in constant vs. tapering doses. Two Sahiwal cows were superstimulated using 100 mg NIH-FSH-P1, divided in eight constant or tapering doses in cross over design. The results of preliminary trial are presented in Table - 1. The average number of CLs was more in the cows administered tapering doses compared to the constant dose schedule (11.0±2.00 vs 1.5±0.5, respectively). The average recovery of total and transferable embryos in the tapering dose schedule in two cows was 6.5±0.50 and 3.5±0.50 respectively, whereas, flushing was not attempted due to poor superovulatory

Table 1: Preliminary trial to evaluate effect of FSH (100 mg NIH-FSH P1) administered in constant vs. tapering doses on superovulatory response in Sahiwal cows.

Parameter			100 mg NIH-FSH-P1	
			Constant dose schedule (n=2)	**Tapering dose schedule (n=2)**
Day	Follicle size		Average no. of Follicles	Average no. of Follicles
Day- 4 (Start of FSH treatment)	a.	≤7 mm	13.0±3.00	14.5±3.5
	b.	>7-10 mm	0.0±0.00	0.0±0.00
	c.	>10 mm	0.5±0.50	0.5±0.50
Day- 8 (Day of Estrus)	a.	<4 mm	9.0±3.00	3.0±2.00
	b.	>7-10 mm	3.0±1.00	6.0±0.00
	c.	>10 mm	2.5±0.50	8.5±2.50
Av. number of CL's On Day 15			1.5±0.5	11.0±2.00

response in the two cows administered with constant dose schedule. The each dose of FSH in Constant doses regimen was 12.5 mg NIH-FSH P1, which appeared insufficient to support the growth multiple follicles, whereas, the initial dose used in tapering schedule was 15 & 20 mg NIH-FSH-P1 for first two days, which supported the growth of small follicles and was able to superstimulate the treated cows. On the basis of results of the preliminary trial; tapering dose regimen was selected for use in the present study.

Treatment groups

The cows were scanned & equally allocated according to the average number of antral ovarian follicles, age and live body weight into two groups i.e. Group- I: 100 mg group and Group- II: 200 mg group. Cows of the both groups were administered 2 mg of Estradiol-17β + 100 mg of progesterone (Sigma Aldrich, India) intra muscularly along with insertion of Progesterone releasing device; TRIU-B impregnated with 958 mg of progesterone (Virbac Animal Health India Pvt. Ltd., India) on the random day of estrous cycle (referred as day 0). On day 4 i.e. expected day of follicular wave emergence, superstimulation treatment was initiated by using Follicle stimulating hormone; Folltropin-V (Bioniche Animal Health, Inc., Belleville, On, Canada) @100 mg NIH-FSH-P1 divided in eight tapering doses i.e. 20, 20, 15, 15, 10, 10, 5 & 5 mg NIH-FSH P1 in the group 1 and @ 200 mg of NIH-FSH-P1 divided in eight tapering doses i.e. 40, 40, 30, 30, 20, 20, 10 & 10 mg NIH-FSH P1 in the group 2, administered at 12 hourly intervals over a period of 4 days.

All the cows were administered two doses of 500 µg PGF2α analogue (Cloprostenol, Inj. Estrumate – Intervet India Pvt. Ltd.) on Day 6 and 6.5 of the protocol i.e. with 5th and 6th dose of FSH. The intravaginal progesterone releasing device TRIU-B was removed on 7^{th} day i.e. at the time of last FSH injection. Luitenizing hormone; Lutropin 12.5 mg (Bioniche Animal Health, Inc., Belleville, On, Canada) was administered at 12 hours after the last FSH Injection in both the groups. All the cows were inseminated twice i.e. at 12 and 24 hour after LH injection using frozen thawed semen (0.25 ml/per straw) of high fertility Sahiwal bulls.

Observations

Trans-rectal ultrasonography was carried out daily using ultrasound scanner (Exago, ECM, France) equipped with B- mode linear array transrectal probe using 7.5 MHz frequency to record the location, number and size (≤7 mm, >7-10 mm, >10 mm) of the follicles and corpus luteum (CL) beginning from the start till the end of experiment. Ovulation was determined through scanning by the disappearance of a large follicle. The superovulatory response was assessed by counting number of corpora lutea (CL) prior to embryo collection on day 15 of protocol using transrectal ultrasonography.

Embryos were collected by flushing the uterus of cows non-surgically on day 7 by two way Worrlein catheter (Minitub GmbH, Germany) using Dulbecoo's phosphate buffered saline supplemented with 0.1 percent bovine serum albumin (BSA) and gentamicin @50 µg/ml. Ova/embryos were searched under stereomicroscope (Olympus, SMZ7, having magnification of 8X to 56X) and categorized as total number of ova/embryo recovered and number of transferable embryos. The grading of embryos was done at 100X magnification using Diaphot-300 (Nikon, Japan) according to the Manual of International Embryo Society (IETS) by Robertson and Nelson (1998).

Statistical analysis

The data was analyzed for means and standard errors (SE) for all variables. After confirming the normality of data and homogeneity of variance, Student`s t test (two tailed) was applied to compare mean values of number of follicles in different size categories, CLs, embryos recovered and transferable embryos in Excel computer programme. The proportions of transferable embryos were compared using Chi square test.

RESULTS AND DISCUSSIONS

The average number of small follicles (≤ 7 mm) present at the start of FSH treatment did not differ significantly in the 100 mg and 200 mg group (23.3±3.93 and 22.3±1.17, respectively) which was the prerequisite to compare efficacy of two different FSH doses (Table-2). The administration of FSH at 12 hourly interval supported the growth of small follicles which otherwise would had undergone atresia due to secretion of inhibin and estradiol by dominant follicle during normal estrous cycle. Following superstimulatory treatment, increase in average number of medium & large follicles were observed as the small follicles ≤ 7 mm kept growing under the influence of exogenous FSH administration.

On the day of estrus i.e. Day 8, the number of follicles reaching ovulatory size (>10 mm) were 18.0±4.04 and 19.2±1.11 in the group-1 and the group-2, respectively, the difference was statistically non-significant. Similar number of ovulatory follicles at the time of estrus were recorded by Baracaldo *et al.*, (2000) following ablation of 2 largest follicles or all follicles >5 mm before gonadotrophin

Table 2: Effect of two different FSH doses (100 vs. 200 mg NIH-FSH-P1) on follicular dynamics in Sahiwal

Parameter			Average number of follicles	
Day		**Follicle size**	**100 mg Group**	**200 mg Group**
Day- 4 (Day 1 of FSH)	a.	≤7 mm	23.3±3.93	22.3±0.81
	b.	>7-10 mm	0.5±0.34	0.7±0.33
	c.	>10 mm	0.7±0.33	0.3±0.21
		Total	24.5±4.49	23.3±1.31
Day- 5 (Day 2 of FSH)	a.	≤7 mm	23.2±4.24	22.5±1.36
	b.	>7-10 mm	1.3±0.61	1.5±0.56
	c.	>10 mm	0.5±0.34	0.8±0.65
		Total	25.0±4.75	24.8±1.78
Day- 6 (Day 3 of FSH)	a.	≤7 mm	17.2±2.63	14.7±1.31
	b.	>7-10 mm	8.0±2.49	8.2±1.17
	c.	>10 mm	3.2±1.87	3.8±1.22
		Total	28.3±5.69	26.7±1.36
Day- 7 (Day 4 of FSH)	a.	≤7 mm	8.2±2.34	5.5±1.18
	b.	>7-10 mm	10.0±2.31	11.2±0.98
	c.	>10 mm	9.2±2.27	9.8±1.54
		Total	27.3±5.25	26.5±0.76
Day- 8 (Day of Estrus)	a.	≤7 mm	5.2±1.22	3.2±1.33
	b.	>7-10 mm	6.4±1.73	5.3±1.20
	c.	>10 mm	18.0±4.04	19.2±1.11
		Total	29.5±5.34	27.7±3.17

Values did not differ significantly at 5% confidence interval in different treatment groups (P>0.05).

treatment. Baruselli *et al.,* (2011) reported that injecting the total dose of FSH either in 3 divided doses or in 8 divided doses did not adversely affect the number of ovulatory follicles of >8 mm size (15.8 ± 0.9 vs. 16.1 ± 1.1). Whereas, the administration of the total dose only in 2 divided doses significantly reduced the number of follicles of > 8 mm size (8.9 ± 0.5). Nasser *et al.,* (2011) also noticed similar number of follicles measuring > 8 mm to the present study at super estrus in Nellore (Ongole) cows.

Ovulatory response

Based on ultrasonography, the average number of ovulation in the 100 mg and the 200 mg were 15.0±3.51 and 16.8±1.66, the difference was non-significant (P>0.05). The percent ovulations recorded were 88.8 and 84.3 in the group 1 and the group 2, respectively (P>0.05).

Similar ovulatory response in cows responding to superovulation treatment was reported by Arosh *et al.* (2000), Kharche *et al.* (2001), Bhuyan *et al.* (2012) (100%) and Mutha Rao *et al.* (2011) (94.83%). However, the superovulatory response observed in the present study was higher than the other studies in Sahiwal; 50% (Siddiqui *et al.,* 2011), 57% (Ranjna *et al.,* 2004), 82.6% (Babu Rao *et al.,* 2005), 87.5% (Kasiraj *et al.,* 2000) and 82-91% (Sharma *et al.,* 2002). Baruselli *et al.,* (2006) reported the mean ovulations of 13.0, 12.1 and 14.9 using 100 mg, 133 mg and 200 mg dose of Folltropin-V, respectively, with no significant difference in ovulation rates between the dose schedules. Mapletoft (2002) reported that ovulation rates continued to increase upto 400 mg NIH-FSH-P1 (40 mg Armour) and did not increase beyond that dose.

Interval from AI to ovulation

Higher percentage of ovulations were recorded within the period from >24 to 48 hours in the 100 mg group compared to the 200 mg group (57.9 vs. 51.04, P>0.05), whereas, higher percentages of ovulations were recorded in > 48 -72 hours in the 200 mg group compared to the 100 mg group (39.5 vs. 29.5, respectively). None of the Sahiwal cows superstimulated using 100 mg or 200 mg NIH-FSH-P1 ovulated within 24 hours of insemination in both the groups. The average number of anovulatory follicles in the

100 mg group was higher than the 200 mg group, however, the difference was non-significant (3.3±1.38 vs. 2.67±1.12, respectively, P>0.05). Baracaldo *et al.* (2000) and Mutha Rao *et al.* (2005) reported 1.31 to 2.2 anovulatory follicles in superovulated cows. The presence of anovulatory follicles might be due to an inadequate endogenous LH surge during superovulation (Schallenberger *et al.,* 1990). However, supplementation of exogenous GnRH at super-estrus has neither increased ovulations rate nor reduced the number of anovulatory follicles in cattle (Wubishet *et al.,* 1986 and Savage & Liptrap 1987). It remains to be determined if those anovulatory follicles were biochemically aberrant (Callesen *et al.,* 1986).

Number of Corpora lutea (CLs)

In the present study, all the Sahiwal cows responded to the superstimulatory treatment by developing >2 corpora lutea suggesting that both the doses under study were effective in inducing superovulation (Table-3). Results of the study showed that the average number of corpora lutea did not differ between the group 1 and the group 2 (16.00±3.44 and 16.17±1.35, respectively, P>0.05) indicating that the 100 mg NIH-FSH-P1 dose of FSH was equally effective in inducing superovulatory response as 200 mg NIH-FSH-P1 and had no negative effect on number of ovulations or formation of CL.

In a similar study, Mishra *et al.,* (2002) superovulated Sahiwal cows with 200, 300, 400 mg NIH of FSHp for 3 days in tapering dose schedule and observed 5.16 ± 0.47, 10.0 ± 1.06 and 4.16 ± 0.06 number of corpora lutea, respectively, the response was higher in 300 mg-NIH-FSH dose compared to 400 and 200 mg NIH-FSH-P1. In our study, the superovulatory response following use of 100 and 200 mg NIH FSH P1 was higher than the superovulatory response observed by Mishra *et al.,* (2002). Ovarian response observed in our study was higher than that reported by Tasdemir *et al.* (2012) in Anatolian black cattle, Veerabramhaiah *et al.,* (2012) in Punganur cows and by Duddukari (2015) in Ongole cows. Similar results to the present study were reported by Baruselli *et al.* (2006) who observed mean ovulation rate of 13.0, 12.1 and 14.9 with 100 mg, 133 mg and 200 mg of FSH (Folltropin-V) with no significant difference in ovulation rate between the different doses used. Barati *et al.,* (2006) reported that 100 mg of FSH (8.2 CL) was as efficacious as 160 mg (9.6 CL) or 200 mg (9.4 CL) in inducing superovulatory response. Mutha Rao *et al.* (2005) stated that 200 mg FSH produced a mean ovarian response of 13.53 to 16.17 CL in Ongole cows.

Embryo recovery

The average number of embryo+ova recovered was 9±4.02 and 10.67 ±2.78 in the 100 mg group and the 200 mg group, respectively (Table-3). In the present study, the average numbers of embryos recovered in the 100 mg as well as in the 200 mg group were higher compared to the average number of embryos obtained by Baruselli *et al.* (2006) in Nelore, Mutha Rao *et al.* (2005) and Duddukuri (2015) in Ongole cows. Duddukuri (2015) recovered 1.83±0.65 and 3.83±1.72 embryos following superovulation using 100 mg and 130 mg NIH-FSH-P1 in Ongole cows, respectively. Bo *et al.* (1991) used total dose of 400 mg NIH-FSH given intramuscularly vs. subcutaneously from 8^{th} or 9^{th} day of cycle in cows and reported mean number recovery of 5.60±1.10 and 3.40±0.50, respectively. Similarly, Walsh *et al.* (1993) reported that more number of embryos was recovered when FSH was administered twice daily by intramuscular route (9.60±1.10) than once daily subcutaneous route (4.90± 1.10). Totey *et al.* (1991) studied the effect of season using 28 mg of FSHp and obtained 5.94±0.95 vs. 8.5±1.88, 3.64±0.64 vs. 2.0±0.0 and 3.84±0.50 vs. 0.0±0.0 total embryos during rainy, winter and summer seasons, respectively.

Embryo recovery rate of 56.25 & 65.97 percent was achieved in the 100 mg and the 200 mg group, respectively, which was higher than the 25% reported by Babu Rao *et al.* (2005) but was similar to 53.6% reported by Kasiraj *et al.* (2000). Higher embryo recovery rate compared to the present study had been reported by Hasler *et al.* (1983), Otoi *et al.* (1998) and Mapletoft *et al.* (2002).

Fertilization rate

Out of the total ova + embryos collected, 79.6 and 96.9 percent was fertilized in the 100 mg group and the 200 mg group, respectively (Table-4). Similar fertilization rates were also reported by Baracaldo *et al.* (2000) and Mutha Rao *et al.* (2011). The average number of unfertilized ova was 1.83±0.98 in the 100 mg group and 0.40±0.24 in the 200 mg group (Table-4), whereas, Baruselli *et al.* (2006) reported 0.6-0.8 unfertilized ova in an embryo transfer programme involving fixed timed AI which was lower compared to the 100 mg group of the present study. Similar non-transferable/degenerated embryos recovery to the present study has been reported in the Bos *indicus* by Shaw and Good (2000), Kasiraj *et al.* (2 000) and (Babu Rao *et al.,* 2005). On the contrary, Shaw and Good (2000) and Andrade *et al.* (2002) reported higher number of unfertilized ova. The variation in fertilization rate between different studies could be due to various factors viz. nutritional status of

Table 3: Effect of two different FSH doses (100 vs 200 mg) on ovulatory response and embryo recovery in Sahiwal cows.

SN	Parameters	100 mg Group	200 mg Group
1	On the day estrus (D-8)		
	Follicles≤7 mm	5.2±1.22	3.2±1.33
	Follicles>7-10 mm	6.4±1.73	5.3±1.20
	Follicles>10 mm	18.0±4.04	19.2±1.11
	Total follicles	29.5±5.34	27.7±3.17
2	Ovulation rate (%)	88.8	84.3
3	Average no. of CLs (day 7 post AI)	16.0±3.44	16.2±1.35
4	Average No. of embryos + ova recovered	9.0±4.02	10.7±2.78
5	Embryo recovery rate (%)	56.2	66.0

Values did not differ significantly at 5% confidence interval in different treatment groups (P>0.05).

Table 4: Effect of two different FSH doses (100 mg vs. 200 mg) on embryo quality parameters in Sahiwal cows

SN	Parameter	100 mg Group	200 mg Group
1	Average No. of embryos + ova recovered	9.00±4.02	10.67±2.78
2	Average Transferable embryos	3.5±1.26	3.33±1.84
3	Average degenerated embryo	3.67±2.23	7±3.5
4	Average unfertilized ova (UFO)	1.83±0.98	0.40±0.24
5	Embryo recovery rate (%)	56.25	65.97
6	Fertilization rate	79.6	96.9
7	Percent Transferable Embryos	38.9	31.2

Values did not differ significantly at 5% confidence interval in different treatment groups (P>0.05).

donor, type and dose of gonadotrophin used, timing of AI and fertility of bull used.

Transferable embryos

The average number of transferable embryos recovered was similar in the group-1 and the group 2 (3.5±1.26 vs 3.33±1.84, respectively, P>0.05, Table-4). Bo *et al.*, (1995) used a total dose of 400 mg NIH- FSH-P1(Folltropin-V) twice daily in decreasing dose given over 4 days and obtained 14.10 ± 2.70 mean transferable embryos in cross-bred cows, which was higher than achieved in the present study. Sarvaiya *et al.* (1992) superovulated Jersey x Kankrej Crossbred cattle with a total dose of 35 and 44 mg of FSH in decreasing doses and obtained 3.33 ± 3.30 and 3.25 ± 0.63 mean number of good quality embryos, respectively, which was similar to the present study.

Baruselli *et al.* (2006) reported 7.7 ± 7.4 and 5.6 ± 4.1 transferable embryos with 100 mg and 133 mg FSH respectively in Nelore cows where as Mutha Rao *et al.*, (2005) recovered 6.8 ± 1.74 to 9.08 ± 2.3 viable embryos in Ongole cows superovulated using 200 mg of FSH. Calder and Rajamahendran (1992) reported that mean number of transferable embryos in dairy cattle super-ovulated with 35 mg of FSHp during mid luteal phase (day 9-13) and early part (day 2-6)of estrous cycles was 4.30±0.66 and 0.17±0.85 respectively. The numbers of transferable embryos achieved in these studies were higher than the present study. In another study, Mishra *et al.* (1996) reported recovery of 2.0±1.63 and 5.33±4.19 in mean transferable embryos using 24 mg and 28 mg of FSH p given twice daily from 9-12 days of oestrous cycle in Sahiwal cows, respectively. In the present study, the percentage of transferable embryos recovered were 38.9 and 31.2 in the group 1 and the group 2, respectively, the difference was non-significant (P>0.05). Mapletoft (2002) conducted several experiments with the LH-reduced Folltropin®-V utilizing several different total doses, ranging from 100 to 900 mg of NIH-FSH-P1 activity and concluded that there was no evidence of detrimental effects of the dose on embryo quality.

CONCLUSIONS

It was concluded from the findings of the study that 100 mg NIH-FSH-P1 in 8 tapering dosess is equally effective to 200 mg NIH-FSH-P1 for superstimulation in the Sahiwal cows in terms of superstimulatory response, number of corpora lutea, number of embryos recovered, fertilized embryos and the number of transferrable embryos. Therefore, it is recommended to use 100mG FSH in 8 tapering doses for effective results in superstimulation and embryo production programme in Sahiwal cows.

ACKNOWLEDGEMENTS

The financial assistance under the RKVY project provided by Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India is duly acknowledged.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Andrade, J. C. O., Oliveria, M. L., Lima, P. F., SantosFilho, A. S. and Pina, V. M. R. (2002). Use of steroid hormone treatments prior to superovulation in Nelore donors. *Anim. Reprod. Sci.,* **69**: 9-14.

Arosh, J. A., Sakila, K., Devanathan, T. G. and Kathiresan, D. (2000). Follicle stimulating hormone profile and its correlation with ovarian response, embryo recovery and quality of embryos in PGF2α and Synchromate-B with Folltropin induced superovulated crossbred cows. *Indian J. Anim. Sci.,* **70**: 679-683.

BabuRao, K., MuthaRao, M. and Raju, M. S. (2005). Superovulation and embryo recovery in Ongole cows using different FSH preparations. *The Indian J. Anim. Reprod.,* **26**: 123-25.

Baracaldo, M. I., Martinez, M. and Adams, G. P. (2000). Superovulatory response following transvaginal follicle ablation in cattle. *Theriogenology,* **53**: 1239-50.

Barati, F., Niasari-Naslaji, A., Bolourchi, M., Sarhaddi, F., Razavi, K., Naghzali, E., Thatcher, W. W. (2006). Superovulatory response of Sistani cattle to three different doses of FSH during winter and summer. Theriogenology, **66**: 1149–55

Baruselli, P. S., de Sá Filho, M. F., Martins, C. M., Nasser, L. F., Nogueira, M. F. G., Barros, C. M. and Bo, G. A. (2006). Superovulation and embryo transfer in *Bos indicus* cattle. *Theriogenology,* **65**: 77-88.

Baruselli, P. S., Ferreina, R. M., Sales, J. N. S., Gimenes, L. U., SaFilho, M. F., Martins, C. M., Rodrigues, C. A. and Bo, G. A. (2011). Timed embryo transfer programs for management of donar and recepient Cattle. *Theriogenology,* **76**: 1583-93.

Bhuyan, S., Chakravarty, P., Deka, B. C., Sarmah, B. C. and Borah, S. (2012). Recovery of Embryos from superovulated cows and its correlation with progesterone concentrations. *Indian Vet. J.* **89**(April): 11-13.

Bo, G. A., Hockley, D., Tribulo, H., Trofre, F., Tribulo, R., Busso, N., Barth, A. D. and Mapletoft, R. J. (1991). The effect of dose schedule and route of administration on Superovulatory response to Folltropinin the cow. *Theriogenology, 35*: 186.

Calder, M. and Rajamahendran, R. (1992). Follicular growth, ovulation and embryo recovery in dairy cows given FSH at the beginning or middle of the estrous cycle. *Theriogenology,* **38**: 1163-74.

Callesen, H., Greve, T. and Hyttel, P. (1986). Preovulatary endocrinology and oocyte maturation in superovulated cattle. *Theriogenology,* **25**: 71-86.

Duddukuri, Y. (2015). Effect of lower doses of FSH on Superovulatory responses in ongole cattle: MVSc thesis submitted to Sri Venkateswara Veterinary University, Tirupati.

Hasler, J. F., McCauley, A. D. and Schermerhorn, E. C. (1983). Superovulatory responses of Holstein cows. *Theriogenology,* **5**: 83-9.

Kasiraj, R., MuthaRao, M., Ranga Reddy, N. S., Mishra, A. K. and Pant, H. C. (2000). Superovulation and embryo recovery in Ongole cows using FSH of equine Chorionic gonadotrophin. *Indian J. Anim. Sci.,* **70**: 251-53.

Khan, M. S., Rehman, Z. U., Khan, M. A. and Ahmad, S. (2008). Genetic resources and diversity in Pakistani cattle. *Pak. Vet. J.,* **28**: 95-102.

Kharche, S. D., Sharma, G. T., Agarwal, S. K., Majumdar, A. C., Sanwal, P. C. and Sharma, N. C. (2001). Pretreatment effect of human chorionic gonadotropin and estradiol-17β on ovarian response, embryo production and endocrine profile in cattle superovulated with FSH-P. *Indian J. Anim. Sci.,* **71**: 644-649

Lewis, I. (1992). Programming donors and recipients.In embryo transfer and pregnancy diagnosis. *Post Graduate Committee in Veterinary Science, University of Sydney, Sydney*, Australia.69-88.

Mapletoft, R. J., Bennett-Steward, K. and Adams, G. P. (2002). Recent advances in the superovulation of cattle. *Reprod. Nutri. Develop.,* **42**: 601–11.

Mishra, O. P., Khan, J. R. and Awasthi, M. K. (2002). Estrus synchronization with cloprostenol and effect of Folltropin treatment on superovulatory response and embryo Recovery in Sahiwal cows. *The Indian J. Anim. Reprod.,* **23**: 193-5.

Mishra, U. K., Mishra, O. P. and Khan, J. R. (1996). Estrus synchronization, superovulation and non-surgical embryo transfer in Sahiwal cows. *Indian J. Anim. Sci.,* **66**: 1271-73.

MuthaRao, M., Mahesh, U. and Naidu, K. V. (2011). Ovarian response and embryo yield in Superovulated Ongole cows. *Indian Vet. J.,* **88**: 18-9.

MuthaRao, M., Mahesh, U. Y. and BabuRao, K. (2005). Periovulatory steroid profile in Relation to superovulatory responses in native Ongole cows. *The Indian J. Anim. Reprod.,* **26**: 87-90.

Nasser, L., Adams, G. P., Bó, G. A., Mapletoft, R. J. (2011) Ovarian superstimulatory response relative to follicular wave emergence in heifers. *Theriogenology,* **4**: 713-724.

Otoi, T., Koyama, N., Yamamoto, K. and Tachikawa, S. (1998). Superovulatory response in beef cows following removal of the largest ovarian follicle. *Vet. Record,* **42**: 402-403.

Randel, R. D. (1984). Seasonal effects on female reproductive functions in the bovine (Indian breeds). *Theriogenology,* **21**: 170-85.

Ranjna, S. Cheema, Mathroo, J. S. and Singh, P. (2004). Blood plasma hormones in relation to superovulatory response in crossbred cattle. *The Indian J. Anim. Reprod.,* **25**: 1-7.

Robertson, I. and Nelson, R. E. (1998). Certification and identification of the embryo. Manual of the International Embryo Transfer Society (Stringfellow, D.A.; Seidel, S.M.; eds). IETS, Savoy, IL: 103-134.

Sarvaiya, N. P., Chauhan, F. S. and Mehta, V. M. (1992). Comparative efficacy of different Gonadotrophin preparation on superovulation and embryo recovery in Jersy x Kankrej (JxK) crossbred cows. *The Indian J. Anim. Reprod.,* **13**: 120-3.

Savage, N. C. and Liptrap, R. M. (1987). Induction of ovulation in cyclic mares by Administration of a synthetic prostaglandin, fenprostalene, during oestrus. *J. Reprod. Fertil.,* **35**: 239-43.

Schallenberger, E., Wagner, H. G., Papa, R., Hart, P. and Tenhumberg, H. (1990). Endocrinological evaluation of induction of the superovulation with PMSG in water buffalo (*Bubalus bubalus*). *Theriogenology,* **34**: 379-392.

Sharma, R. K., Siddiqui, M. V., and Gorani, S. (2002). Superovulation and post superovulatory Fertility of Holstein cows under tropical conditions. *Indian J. Anim. Sci.,* **72**: 31-4.

Shaw, D. W. and Good, T. E. (2000). Recovery rates and embryo quality following dominant follicle ablation in superovulated cattle. *Theriogenology,* **53**: 1521-28.

Siddiqui, M. U., Panchal, M. T. and Kavani, F. S. (2011). Circulating ovarian steroids in relation to superovulatory response and embryo recovery in Sahiwal cows and heifers. *The Indian J. Anim. Reprod.,* **32**: 12-16.

Singhal, S., Singh, N., Malik, V. S., Kumar, A. and Brar, P. S. (2017). A preliminary study on superstimulatory response using low dose FSH and subsequent embryo transfer in Sahiwal cattle during summer season. *The Indian J. Anim. Reprod.,* **38**(2): 37-39.

Singh, N., Singhal, S., Malik, V.S., Singh, H., Kumar, A., Brar, P.S., (2018). Establishment of pregnancy from Sahiwal embryo in surrogate crossbred cattle. *Haryana Vet.*, **57**(1): 99-100.

Tasdemir, U., Satilmis, M., Karashin, T., Kizil, S. H., Kaymaz, M. and Imai, K. (2012). The effect of single epidural plus intramuscular injection of FSH in superovualtory response in Anatolin Black cow. *Ank. Univ. Vet. Fak.,* **59**: 211-216.

Totey, S. M., Singh, Gurpreet, Taneha, M., Pawshe, C. H., Singh, G. and Chillar, R. S. (1991). Effect of season on superovulation and embryo recovery in Sahiwal and Crosses of Holstein x Sahiwal donor cows. *Indian J. Anim. Sci.,* **12**: 179-81.

Veerabramhaiah, K., Rao, V. H. and Naidu, V. K. (2012) Superovulatory response in endangered punganur cows with FSHp. *Tamilnadu J. Vet. Anim. Sci.,* **8**: 241-44.

Walsh, J. H., Martovani, R., Duby, R. T., Overstrom, E. W., Dobrinsky, J. R., Enright, W. J., Roche, J. F. and Boland, M. P. (1993). The effects of once or twice daily injections of FSH on superovulatory responses in heifers. *Theriogenology,* **40**: 313-32.

Wubishet, A., Graves, C. N., Spahr, S. L., Kelser, D. J. and Favero, R. J. (1986). Effect of GnRH treatment on superovulatory responses of dairy cows. *Theriogenology,* **25**: 423-7.