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Effect of Lengthening FSH Treatment on Super Stimulatory Response and Embryo Production in Sahiwal Cattle

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ABSTRACT

The study evaluated the effect of lengthening of superstimulation protocol (4 Day vs 6 Day) on superovulatory response and embryo production in Sahiwal cows, there is no literature regarding this comparative evaluation. Sahiwal cows were randomly divided into two groups viz. short FSH group (Group1; 4D FSH; n=6) and long FSH group (Group 2; 6D FSH; n=6). Animals in both the groups were administered injection estradiol benzoate (@ 2 mg) and progesterone (@ 50 mg/animal) intramuscularly along with insertion of an intravaginal device (progesterone device) on a random day of estrous cycle (Day 0). The FSH treatments were started 4 days later (i.e on the expected day of wave emergence). A dose of 200 mg FSH and 300 mg FSH divided equally over 8 (Group 1) and 12 doses (Group 2) IM were injected at 12-h intervals in group 1 and group 2 respectively. Prostaglandin F2α (500 ug cloprostenol inj.) was injected on Day 7 morning (Group 1) and Day 9 morning (Group 2) and CIDR was removed. In both the groups, hCG (3000 I.U) was administered after 12 h of CIDR removal and AI was done at 22-24 h of hCG treatment. The embryo recovery was done non-surgically on the 7th day from first A.I (i,e Day 16 for group 1 and Day 18 for group 2). The mean number (± SEM) of >8mm follicles on the day of estrus did not differ between groups (P= 0.06) and comparatively higher number of ovulations were detected in 6-day FSH group than 4-day FSH group (71.3±11.77 vs 80.5±7.69. P= 0.27 respectively) but not statistically significant. Total number of CL's, embryos collected and transferable embryos were higher in number in the 6-day group as compared to 4 Day groups. In conclusion, extending FSH treatment resulted in a greater number of follicles acquiring the ability to ovulate with higher numbers of ovulations.

Keywords: Sahiwal, Superovulation, Follicular development, FSH, Embryo, CL

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INTRODUCTION

Bos indicus breeds are more adapted to hot conditions than Bos taurus breeds due to their better thermo-regulatory abilities (Hammond *et al.,* 1998) and thermotolerance at the cellular level (Block *et al.,* 2002; Paula-Lopes *et al.,*

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2003). Sahiwal is a native Bos indicus breed of India and is considered the best milch breed in the tropics (Rehman *et al.,* 2014). Sahiwal cows are well known for higher milk production, greater endurance for hot climates under tropics and subtropics, resistance to tropical diseases, production of synthetic material around the globe and low cost of maintenance with high feed conversion efficiency (Ilatsia *et al.,* 2011).

To propagate this breed, multiple ovulation and embryo transfer (MOET) involving superstimulation and in-vivo embryo collection is considered the best methods in cattle (Thibier, 1996). The objective of super stimulatory treatments in cattle is to obtain the maximum number of viable embryos with a high probability of producing pregnancies. Conventionally, the superovulatory protocols involve the use of purified FSH extracted from porcine or bovine pituitaries. The standard approach is to administer FSH for 4 to 5 days, but a prolongation of the treatment period to 6 to 7 days may be beneficial (García Guerra *et al.,* 2015). The comparative evaluation of 4-day versus 6-day FSH protocols for superovulatory response and embryo recovery in Sahiwal cattle is lacking. The objective of this experiment was to compare superovulatory response and embryo recovery following administering FSH for 4 day versus 6 day FSH injection protocols in Sahiwal cattle.

MATERIALS AND METHODS

Animals: Pluriparous Sahiwal cows weighing between 400 to 550 kg with a body condition score of more than 2.5 to 3.5 on a scale of 1 to 5 were selected for the study. The selected cows were non-pregnant and free from uterine abnormalities. These cows were maintained under a loose housing system and were fed with chaffed green fodder, wheat straw, concentrates, common salt, mineral mixture and *ad libitum* drinking water. The Committee for the purpose of Control and Supervision of experimental animals, Government of India have approved the use of these animals (No 25/17/2019-CPCSEA).

Grouping and synchronization of wave emergence of animals: The selected cows were randomly divided into short FSH (4 day FSH or Group 1) and long FSH (6 day FSH or Group 2), consisting of 6 animals in each group. At the start of the experiment, cine loops of both ovaries from all the animals were recorded for accurate measurements and counting of ovarian follicles. All the animals in each group were administered with estradiol benzoate (@ 2 mg) and progesterone (@ 50 mg/animal) intramuscularly on random day of estrous cycle (Day 0), along with it an intravaginal progesterone device were be placed on the same day.

Superstimulation protocol for 4 day and 6 day: Superstimulatory treatment was started on the day 4 (i.e. expected day of wave emergence) in both the groups. Group 1 (4 day FSH) was given a total dose of 200 mg (FSH, Stimufol) equally distributed (25 mg each) over 8 im injections and Group 2 (6 day FSH) was given total dose of 300 mg (FSH, Stimufol) equally distributed (25 mg each) 12 im injections in the morning and evening (12 h intervals). Prostaglandin $F_{2\alpha}$ (500 µg cloprostenol inj) was administered on Day 7 morning (i.e. at 60 h from the start of FSH) for group 1 and on Day 9 morning (i.e. at 96 h from the start of FSH) for group 2. CIDR was removed on Day 8 for group 1 and Day 10 for group 2. All animals in both group were administered hCG (Inj Chorulon @ 3000 IU) intramuscularly after 12 h of CIDR removal and the fixed time insemination was done at 22-24 h of LH treatment. The second insemination was done on next day.

Counting of follicles and CL: At the start of the experiment, all cows were examined by trans-rectal ultrasonography. The number and location of different sized follicles and corpora lutea on ovarian sketches was recorded. On Day 4, trans-rectal ovarian ultrasonography was performed to count the number of small follicles. Ovarian ultrasonography was done to count the number of follicles >8 mm at the time of hCG injection (Inj Chorulon @ 3000 IU). Ultrasonography was done to count the number of CL and not ovulated follicles on day 13 for group 1 and day 14 for group 2.

Collection of embryos: Embryos were recovered non-surgically on the 7th day from first A.I (Day 16 in group 1 and day 18 in group 2) using embryo flushing media (BoviFlush, Minitube). Embryos were evaluated and classified based on stage of development as unfertilized ova (UFO), early morula, compact morula, early blastocyst, blastocyst, expanded blastocyst, hatched blastocyst. For quality assessment the IETS recommended codes were used, given as **Code 1:** Excellent or good; **Code 2:** Fair; **Code 3:** Poor; **Code 4:** Dead or degenerating

Statistical Analysis: The number of follicles were grouped according to the size of follicular diameter under the groups: < 5 mm, 5 to 8 mm, > 8 mm. The number of CL, embryos recovered and transferable embryos between the groups were compared using t-test. Data are presented as mean ± SEM and probability of ≤0.05 was considered statistically significant.

Table 1: Effect of 4 Day and 6 Day FSH protocol on ovulatory response and embryo recovery in Sahiwal cows

SN	Parameters	4 Day FSH (n=6)	6 Day FSH (n=6)	P -values
1	Day 4 (Start of FSH treatment)			
	Follicles <5mm	20.3 ± 1.50	19.3 ± 1.91	0.345
	Follicles 5-8mm	2.7 ± 0.42	3.8 ± 1.01	0.162
	Follicles >8mm	0.2 ± 0.17	0.3 ± 0.21	0.138
2	On the day estrus			
	Follicles <5mm	2.5±1.59	1.5±9.0	0.297
	Follicles 5-8mm	10.2±1.60	9.0±2.35	0.346
	Follicles >8mm	11.7±2.14	16.8±2.17	0.060
	Total follicles	24.3±0.95	27.3±0.99	0.027
3	Total no. of CLs	8.7±1.43	13.8±2.32	0.131
4	Ovulation rate (%)	71.3±11.77	80.5±7.69	0.267
5	No. of embryos + ova recovered	5.0±2.11	11.3±4.08	0.104
6	Embryo recovery rate (%)	68.9±14.54	68±21.72	0.487
7	No. of transferable embryos	3.5±1.41	7.2±3.44	0.179
8	Percent transferable embryos (%)	70	63.2	

RESULTS AND DISCUSSION

Follicular data before and after FSH treatment: The mean (± SEM) number of follicles measuring <5 mm on Day 0 was 18.0 ± 1.06 and 18.2 ± 0.60 for 4-day and 6-day groups, respectively (P=0.44). At the start of FSH treatment, mean number of follicles <5 mm did not differ between the groups (20.3 ± 1.50 and 19.3 ± 1.91, for 4-day and 6-day group respectively; P=0.34). There was also no significant difference in the mean number of follicles 5-8 mm at the start of FSH treatment (2.7 ± 0.42 and 3.8 ± 1.01; P=0.16), or at the day of hCG/expected estrus (10.2 ± 1.60 and 9.0 ± 2.35), for 4-day and 6-day groups, respectively. The mean number of follicles >8 mm reaching ovulatory size at the time of hCG treatment or at the expected day of estrus was higher in 6-day group (16.8 ± 2.17 and 11.7 ± 2.14) but did not differ significantly.

Ovulation and embryo collection: There was a numerical difference between the mean number of CL (8.7±1.43 and 13.8±2.32; P=0.13) and ovulation rate (71.3±11.77 and 80.5±7.69; P=0.267) for 4-day and 6-day, respectively but not statistically significant. The mean numbers of total embryo (5.0±2.11 and 11.3±4.08; P=0.10) and transferable embryos (3.5±1.41 and 7.2±3.44; P=0.17) were numerically higher in 6-day FSH group than the 4-day group, difference was not statistically significant. Also, the mean percentage of transferable embryos (70 vs 63.2) did not differ between the groups.

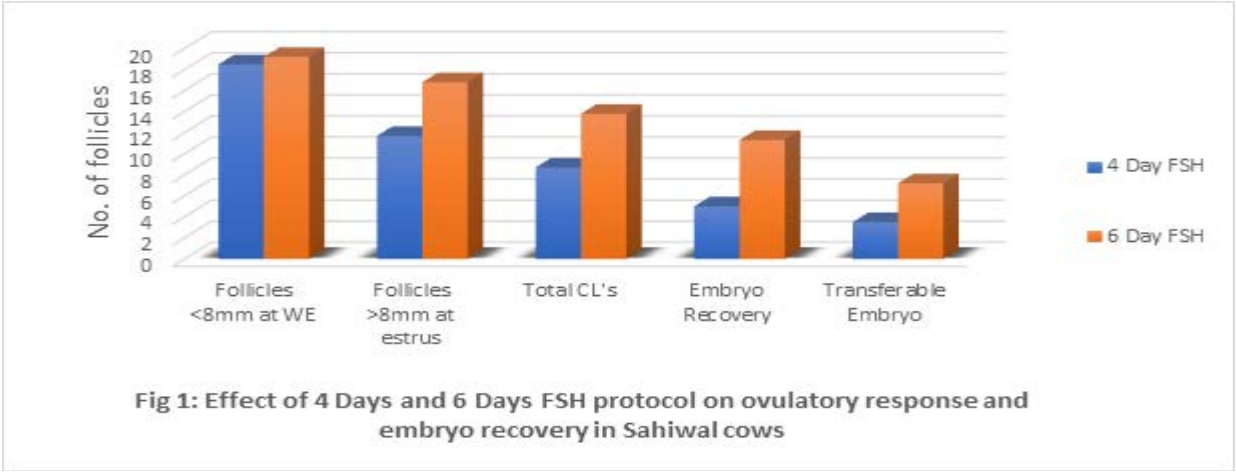

Fig 1: Effect of 4 Days and 6 Days FSH protocol on ovulatory response and embryo recovery in Sahiwal cows

The mean number (±SEM) of >8mm follicles on the day of estrus inclined to be higher (11.7±2.14 vs 16.8±2.17, P= 0.06) and higher number of ovulations were detected (71.3±11.77 vs 80.5±7.69. P= 0.27) in 6-day FSH group than 4-day FSH group, respectively. Lengthening of superstimulation protocols from 4 to 6 days resulted in a numerically greater number of ovulations and CL than the conventional 4-day protocol. In a study by Garcia Guerra *et al.* (2012) where they also compared between a 4-day and 7-day FSH superstimulatory treatment protocol in 24 beef cows, the mean (±SEM) number of >10mm follicles was higher (27.5 ± 4.1 vs 19.5 ± 2.6; P = 0.11) and higher number of ovulation (30.9 ± 3.9 vs. 18.3 ± 2.9, P = 0.01). Dias et al. (2013) also superstimulated beef cows with a constant dose of FSH (25 mg/day) for 4 days or 7 days and superstimulatory response was greater in 7-day FSH group (P<0.05) than 4-day FSH group, number of ovulations was numerically (15.4 vs 11.6) higher but not significant. The mean total number of embryos (5.0±2.11 vs 11.3±4.08, P = 0.104) and transferable embryos (3.5±1.41 vs 7.2±3.44, P = 0.179) was greater in number but not statistically significant. Garcia Guerra et al. (2012) and Dias et al. (2013) also reported that the total number of embryos collected, fertilized ova and transferable embryos were not significantly different, but a numerically higher number was seen in the 7-day treatment group. So, our hypothesis that prolongation of the FSH treatment period from 4 to 6 days will result in greater superovulatory response and number of transferable embryos was partially supported. Moreover, there was no difference in percentage of transferable or freezable embryo which also shows that lengthening of superstimulatory treatment protocol does not affect the oocytes or embryo competence. But there are also many factors which influences the superstimulatory response and embryo recovery. Several animal-related factors like the day of estrus cycle and stage of follicular wave at which stimulation is started in the donor, the variability between animals in the number of follicles at the beginning of FSH treatment, age and breed cause variations in embryo production. Technical factors like gonadotropin used, superovulation protocols, inseminations which includes timing and site of insemination, quality of semen and nutritional factors outcome of superovulation and embryo recovery.

The number of transferable embryos recovered from the extended FSH treatment group was twice that of the short-day group. The rise in the number of oocytes capable for fertilization (number of ovulations) or an increment in the proportion of oocytes capable of fertilization and development could explain the effect of 6-day treatment. The presence of more follicles >8 mm numerically at the end of the FSH treatment and a greater ovulation rate in 6-day groups supports the above statement and the longer day therapy appeared to provide follicles more time to develop the ability to ovulate.

CONCLUSION

Superstimulation protocol involving administration of FSH for 6 days increased the average number of follicles reaching ovulatory size (>8 mm), CL's and transferable embryos over the conventional 4 day FSH protocol in Sahiwal cows.

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CONFLICT OF INTEREST

None

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