



Curtailing the conventional dose of Folltropin-V for superstimulation and embryo recovery in Sahiwal cattle (*Bos indicus*)

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ABSTRACT

High cost of the follicle stimulating hormone (FSH) impacted embryo transfer technology (ETT) work in India. The experiment evaluated effects of reduced (200 mg vs 400 mg) FSH (Folltropin-V) dose on superovulation and embryo recovery in Sahiwal cattle. Animals treated with conventional dose of Folltropin, i.e. 400 mg (G1, n=6) were compared with another group administered with half dose (i.e. 200 mg) of Folltropin (G2, n=6). Superstimulatory response (number of follicles >8 mm) during estrus; unovulatory follicles, superovulatory response (one day prior to embryo flushing) using trans-rectal ultrasonography were recorded. Embryos were collected non-surgically on day-7 post insemination and total recovered and transferable embryos were noted. Results revealed that number of follicles during estrus and subsequent ovulations were significantly higher in the G1 compared to G2 (30.0 ± 1.37 and 26.3 ± 0.99 vs. 20.3 ± 0.21 and 17.7 ± 0.42 , respectively). Unovulatory follicles per animal were lower (2.6 ± 0.21 vs. 3.7 ± 0.49) in G2 than G1. The group receiving lower dose of Folltropin (G2) had significantly higher average embryo recovery compared to G1 (12.0 ± 0.63 vs 8.7 ± 0.76 , respectively). Additionally, average number of transferable embryos were non-significantly higher in G2 (7.0 ± 0.37) than G1 (5.7 ± 0.88). The study indicated that lower dose of Folltropin-V (200 mg compared to 400 mg) is more effective in superovulation and embryo production in Sahiwal cows.

Keywords: Embryo, Follicles, Folltropin-V, Sahiwal, Superovulation

Amongst all *Bos indicus* cattle breeds, Sahiwal has been considered as the best milch animal in tropical countries including India (Ilatsia *et al.* 2012). With increased rate of global warming (@ 0.17°C per decade, NOAA, 2018) the importance of Indian milch cattle breeds over exotic ones is well understood. Unlike the serious adverse affect of hot and humid climate on the production and reproduction potential of exotic or crossbreds; Sahiwal cattle are scantily influenced (Deb *et al.* 2014). Additionally, Sahiwal can be reared on comparatively limited feed resources in hot-humid climate and have better resistance to ecto-parasitic infestation and other diseases. This has encouraged farmers to adopt and rear this breed from last few years (Ilatsia *et al.* 2012).

Currently, there is shortage of elite Sahiwal cows with 3,500–4,000 kg lactation yield. Reproductive biotechnology, viz. multiple ovulation embryo transfer technology (MOET) (Misra *et al.* 2005) could be utilised to increase desired cattle population having better productive and reproductive efficiency. The embryo transfer technology (ETT) have widely been accepted in developed

countries with North-America alone accounting about 50% of ETT activities world-wide while Brazil, France and Germany are other major players (IETS 2016). However, complete data about ETT activities in India is still meagre, although ETT have been undergoing in India since 1980s. High cost of Follicle Stimulating Hormones (FSH, viz. Folltropin, Stimufol) required in ETT is a hindrance for wide exploitation and acceptance of ETT in India, and thus limited the application of ETT for improving the genetics of indigenous dairy cattle. Hence, the present need of reducing the dose of FSH for inducing optimum superovulation in Sahiwal cattle is of utmost importance. Medically, dose regimen of a drug mostly depends upon body weight and *Bos indicus* have lesser weight compared to *Bos taurus*. Furthermore, *Bos indicus* possess higher sensitivity to exogenous gonadotropin than the *Bos taurus* (Randel 1984). Hence, we hypothesised that lower FSH dose could be effective in *Bos indicus* and FSH dose requirement in Sahiwal cattle to achieve superstimulatory effect could be lesser. The superstimulatory efficacy of lower Folltropin dose (200 mg instead of 400 mg) in Sahiwal cattle under tropical Indian climatic conditions was not reported earlier. Thus, present study was conducted to evaluate the superstimulation and embryo recovery using conventional (400 mg) and half dose (200 mg) of Folltropin-

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V in Sahiwal cattle and prospects of using the lower FSH dose.

MATERIALS AND METHODS

The present research work was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, Ministry of Environment, Forest and Climate Change, GoI, which complies with the ethical principles in animal research. The present study was conducted on Sahiwal cattle maintained by Livestock Dairy Farm of the GADVASU university and no other special permission was mandatory since it involved no endangered or protected animal species.

Experimental animals: A total of 12 Sahiwal cows aged from 5 to 8 years; parity 2nd-4th and with BCS 3.5–4.0 were selected for this study. All the animals were having apparently normal genitalia, estrous cyclicity, cervical patency during the diestrus period and atleast 60 days postpartum without any metabolic or reproductive problems. The enrolled cows were having productivity above the minimum standard protocol laid by government of India and were tested negative for tuberculosis, paratuberculosis and brucellosis. All animals were provided with similar environmental conditions of shelter, *ad lib*. green fodder and water; concentrates and mineral mixture were given according to body weight and milk production.

Experimental groups: Sahiwal cows (12) were randomly divided in two groups. In Group 1 (n=6) animals were treated for superstimulatory protocol using the conventional total dose of Folltropin-V @ 400 mg (G1=400 mg) while in another group (Group 2, n=6) half dose (G2=200 mg) of Folltropin was used.

Superstimulatory protocol: The total dose was administered intramuscularly by dividing into 8 tapering doses as 80:80; 60:60; 40:40; 20:20 mg and 40:40; 30:30; 20:20; 10:10 mg, respectively for G1 and G2 at 12 h interval for each dose. In both the groups, Prostaglandin F2 α analogue (500 μ g, Estrumate) was administered 48 and 60 h after first FSH treatment followed by 100 μ g gonadotrophin releasing hormone (GnRH analog, Buserelin) at 12 h after the last FSH treatment. The cows were fixed timed and inseminated twice at 12 and 24 h of the GnRH treatment. Trans-rectal B mode ultrasonography was performed daily during the treatment schedule to record the size and number of follicles and superovulatory response (Fig. 1).

Embryo recovery and assessment: Embryos were collected non-surgically by flushing each uterine horn separately using Worrlie catheter on 7th day post-insemination (Misra *et al.* 1990). After restraining the donor animal in chute, fecal material was removed by back-racking. Epidural anaesthesia (2% lignocaine hydrochloride; 2–5 ml) in sacro-coccygeal space was administered to prevent straining during the flushing procedure. After thorough cleaning and drying the perineal region, a two-way Worrlie catheter (covered with sanitary vaginal sheath) was introduced upto cervix where the

sheath was torn apart. Then the catheter was forwarded towards anterior one-third of one uterine horn. Cuff of catheter was fixed near to tip of uterine horn by inflating the cuff with few ml of air depending on diameter of horn. Each uterine horn was flushed several times using Dullbecco's Phosphate Buffer Saline (dPBS) containing 0.1% bovine serum albumin (BSA). Each time the horn was filled tensed by media using gravitational method and inflow channel was closed. Tapping/ gentle massaging or both were done to manipulate the horn to dislodge the embryos from endometrium into flushing medium. Outflow channel was opened to collect the flushing medium. The procedure was repeated many times until 350–400 ml of media was used in flushing of each uterine horn. Flushed medium was collected and concentrated in EmCon filter and searched under stereo-zoom microscope at magnification between 10 $\times$ to 15 $\times$ to retrieve embryos. Recovered embryos were placed in embryo holding medium (dPBS plus 0.4% BSA; Fig. 2) and graded into two categories, i.e. transferrable and non-transferrable grade embryos as per the standard guidelines of IETS (Seidel and Seidel 1991). Excellent and good quality embryos were categorized as transferrable while others were judged as non-transferable embryos.

Parameters evaluated and statistical analysis: Superstimulatory response in terms of total number of follicles (sized >8.0 mm) during trans-rectal ultrasonography on day of superestrus was recorded. One day prior to flushing, total number of corpora lutea from both ovaries in each cow was recorded to assess the ovulation number or superovulatory response. The total number of recovered and total number of transferrable embryos was evaluated. Data obtained were analysed by using suitable statistical method (chi-square test and one way ANOVA) for rate of embryo recovery and results of laboratory evaluation. The level of significance was evaluated at P<0.05.

RESULTS AND DISCUSSION

The present study compared the effects of two different doses (conventional 400 mg dose compared to a lower dose of its half) of FSH (Folltropin-V) to estimate its optimal usage for superstimulation, and their effects in Sahiwal cattle. In the literature searched, no suitable documentation on comparative study of 200 mg versus 400 mg of Folltropin in Sahiwal cattle is available, therefore, this is a novel study on the aspect. The results recorded for superstimulatory



Figs 1–2. 1. Ultrasonogram of superovulated Sahiwal cow showing multiple corpus lutea over ovary, 2. Picture showing recovered embryos from Sahiwal donor cattle.

response in terms of average number of follicles (>8 mm) during superestrus, number of ovulations, unovulated follicles (UOF) and total number of recovered and transferable embryos are depicted in Table 1.

Sahiwal cows that were superovulated with 400 mg Folltropin (G1) yielded significantly greater ($P<0.05$) number of follicles (>8 mm) on superestrus and the subsequent ovulations compared with 200 mg dose group representing 30.0 and 20.3 and 26.3 and 17.7 in group 1 and group 2, respectively (Fig. 1).

Alternatively, Baruselli *et al.* (2003) in *Bos indicus* Nellore cattle studied ovulatory response with three different doses of Folltropin-V (100, 133 and 200 mg) and observed no significant differences in superstimulatory and superovulatory parameters. Comparative evaluation of ovarian response in terms of number of follicles developed and ovulated using different doses of FSH (200, 240, 280, 320 and 360 mg) indicated 320 mg dose as most effective in Bangladeshi cattle (Ali *et al.* 2012).

Previous studies on *Bos indicus* under Indian climatic conditions showed lesser superstimulatory response in Sahiwal cattle compared to results of current study (Mishra *et al.* 1997), Ongole (Kasiraj *et al.* 2000), Nellore (Silva *et al.* 2009), and in a recent study in Rathi (Purohit *et al.* 2013) cattle. Similarly, lower superstimulatory response and ovulations (12.2 and 9.4, respectively) were observed by Barati *et al.* (2006) using 200 mg Folltropin and there exists no beneficial effect on further reducing the dose to 160 or 120 mg while 250 mg of FSH resulted in higher incidence of anovulatory follicles. No significant differences in superstimulatory parameters was observed in a Thailand study (Nilchuen *et al.* 2011) using 200 mg and 250 mg FSH. In our study, both the treatments resulted in higher superstimulatory and superovulatory response above 20 and 17, respectively which are similar to our previous studies (Singhal *et al.* 2017 and Singh *et al.* 2018). Eventually

higher response could be due to difference in breed, donors' genotype, individual variation or may be owing to environmental conditions (Velazquez, 2018).

In the present study, mean number of unovulatory follicles (UOF) were 3.7 ± 0.49 (range 3–6) and 2.6 ± 0.21 (range 2–3) in cows treated with 400 mg and 200 mg Folltropin, respectively. Likewise, unovulated follicles ranging from 1.75 to 2.5 were observed using 400 mg Folltropin in *Bos indicus* Kankrej cows (Sahatpure and Mehta 2004). Complementing the present observation, increase in number of unovulatory follicles with an increase in superstimulatory treatment dose were reported in *Bos indicus* (De Armas 2001), crossbred (Deshmukh *et al.* 2010) and North Omani cattle (Hussein *et al.* 2017). As observed in the present study, average number of UOF increased with a increased FSH dose (2.0 using 200 mg and 3.17 with 250 mg) in Sahiwal $\times$ Jersey crossbred cows (Reddy *et al.* 2015). Increasing dose of FSH during superstimulation led to increased UOF which might be due to inadequate secretion of endogenous LH surge (Kumar and Sait 2011) and or overstimulation of ovaries (Barati *et al.* 2006). Results of the current study indicated that Sahiwal cattle with more UOFs have lesser number of total and transferable grade embryos, which could be due to overall increase in the size of ovary by UOFs making infundibulum unable to hold all the ovulating follicles. Also, altered hormonal milieu caused by estradiol secretion from UOF create irrhythmic oviductal contractions leading to blockage of embryos within the oviduct, thus the reduced embryo recovery.

This research showed significantly (Table 1, $P<0.05$) higher number of embryos recovered (12.0 in G2 compared to 8.7 in G1) in lower dose treatment group compared to higher dose group (Table 1, Fig. 2). Moreover, there was numerical non-significant higher recovery of transferable embryos in G2 compared with G1 (7.0 vs 5.7, respectively). Success of MOET depends on the number of transferable grade embryos recovered and not on non-transferable and total embryos recovered. It is accepted generally that the *Bos indicus* differ from *Bos taurus* in body weight, physiology, endocrinology, metabolism and sensitivity to exogenous gonadotropins and thus the FSH requirement for superovulation must be lesser in *Bos indicus* (Sartori *et al.* 2016). Recommended conventional dose of Folltropin for superstimulation in *Bos taurus* is 360–400 mg whereas *Bos indicus* breeds may require 250–280 mg (25–30% less) Folltropin (Lewis, 1992). In our previous study, 200 mg FSH superstimulated cattle with 21 follicles, average 18 ovulations and recovery of 9 embryos per cow (Singhal *et al.* 2017). Similar to this study, a lower optimal dose (200 mg) for superovulation in *Bos indicus* Nellore cow (Barros *et al.* 2003) and Brahman pluriparous cows (Krininger III *et al.* 2003) were recorded. In this aspect another study reported similar total embryo recovery of 10.4 in Brahman cattle (Sophon *et al.* 2003). Similar other studies reported that 200 mg FSH resulted in mean embryo and transferable embryo recovery of about 8.2 and 4.3 (Barati *et al.* 2006) and 10.6 and 6.5 (Baruselli *et al.* 2006), respectively. It

Table 1. Parameters during superstimulation using 400 and 200 mg of Folltropin-V in Sahiwal (*Bos indicus*) cattle

Parameter	Group1 (400 mg Folltropin)	Group2 (200 mg Folltropin)
Number of follicles (> 8 mm) at superestrus	30.0 $\pm$ 1.37*	20.3 $\pm$ 0.21*
Number of ovulations	26.3 $\pm$ 0.99*	17.7 $\pm$ 0.42*
Number of unovulated follicles (UOF > 10 mm)	3.7 $\pm$ 0.49 (r = 3–6)	2.6 $\pm$ 0.21 (r = 2–3)
Number of total embryos recovered (TRE)	8.7 $\pm$ 0.76* (r = 7–11)	12.0 $\pm$ 0.63* (r = 11–14)
Number of transferrable embryos (TE)	5.7 $\pm$ 0.88 (r = 3–8)	7.0 $\pm$ 0.37 (r = 6–8)

*Values marked differ significantly within each row at 5% ($P<0.05$) level.

may be hypothesized that limited number of receptor for FSH over the ovaries get occupied by lower dose (say 200 mg) of Follitropin and further administering the higher dose (say 400 mg) would not improve superovulatory response and embryo recovery (Mishra *et al.* 1996).

It may be concluded that the novel concept of FSH (Follitropin-V) administration at a dose rate of 200 mg instead of 400 mg can be effectively utilised for inducing superstimulation, by reducing number of unovulated follicles and increasing total and transferrable embryos in Sahiwal (*Bos indicus*) donor cattle.

REFERENCES

- Ali M S, Khandoker M A M Y, Afroz M A and Bhuiyan A K F H. 2012. Ovarian Response to different dose levels of follicle stimulating hormone (FSH) in different genotypes of Bangladeshi cattle. *Asian Journal of Animal Science* **25**(1): 52–58.
- Barati F, Niasari Naslaji A, Bolourchi M, Sarhaddi F, Razavi K, Naghzali E and Thatcher WW. 2006. Superovulatory response of Sistani cattle to three different doses of FSH during winter and summer. *Theriogenology* **66**: 1149–55.
- Barros C M, Porto L P C and Nogueira M F G. 2003. Dose-response trial in *Bos taurus* vs. *Bos indicus* cows superovulated with FSH, associated with controlled LH surge and fixed time artificial insemination. *Theriogenology* **59**: 524 (abstr.)
- Baruselli P S, Marques M O, Reis E L, Nasser L F T, Silva R C P, Menegatti J A, *et al.* 2003. Adequacy of dose of FSH (Follitropin-V) em protocolos de superovulação de vacas nelore (*Bos taurus indicus*) com inseminação artificial em tempo fixo. *Acta Scientiae Veterinariae* **31**: 244–45.
- Baruselli P S, Sa 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.
- De Armas R, Aparicio N and Nader C. 2001. Superovulatory response in *Bos indicus* breeds, borders or lack of knowledge. *Arch Tierz Dummerstorf* **44**:163.
- Deb R, Sajjanar B, Singh U, Kumar S, Singh R, Sengar G and Sharma A. 2014. Effect of heat stress on the expression profile of Hsp90 among Sahiwal (*Bos indicus*) and Frieswal (*Bos indicus* × *Bos taurus*) breed of cattle: A comparative study. *Gene* **536**: 435–40.
- Deshmukh S G, Pawshe C H, Sahatpure S K and Patil R K. 2010. Superovulatory response with once and twice daily injection of Follitropin-V in crossbred cows. *Indian Journal of Animal Sciences* **80**(9): 851–53.
- Hussein A M, Al-Shakaili Y O, Al-Ismaily A N and Al-Alawi H H. 2017. Effect of different doses of FSH on superovulation, production and quality of embryo in North Omani Cattle breed. *Indian Journal of Animal Research* **51**(1): 8–14.
- IETS. 2016. International Embryo Transfer Society. https://www.iets.org/pdf/comm_data/IETS_Data_Retrieval_Report_2016_v2.pdf
- Ilatsia E D, R Roessler, Kahi A K, Piepho H P and Zarate V. 2012. Production objectives and breeding goals of Sahiwal cattle keepers in Kenya and implications for a breeding programme. *Tropical Animal Health and Production* **44**(3): 519–30.
- Kasiraj R, Rao M M, Ranga Reddi N S, Misra A K and Pant H C. 2000. Superovulation and embryo recovery in Ongole cows using FSH or equine chorionic gonadotropin (eCG). *Indian Journal of Animal Sciences* **70**: 251–53.
- Krinninger III C E, Block J, Al-Katanani Y M, Rivera R M, Chase Jr C C and Hansen P J. 2003. Differences between Brahman and Holstein cows in response to estrous synchronization, superovulation and resistance of embryos to heat shock. *Animal Reproduction Science* **78**: 13–24.
- Kumar P and Sait S F. 2011. Luteinizing hormone and its dilemma in ovulation induction. *Journal of Human Reproduction Science* **4**(1): 2–7.
- Lewis I. 1992. Programming donors and recipients. *Embryo transfer and pregnancy diagnosis*. NSW: Post Graduate Committee in Veterinary Science, University of Sydney; 1992. pp. 69–88.
- Mishra U K, Mishra O P and Khahj J R. 1996. Estrus synchronization, superovulation and non-surgical embryo transfer in Sahiwal cows (*Bos indicus*). *Indian Journal of Animal Sciences* **66**: 1271–73.
- Mishra U K, Mishra O P and Khan J R. 1997. Effect of repeated Superovulation on superovulatory response and embryo recovery in Sahiwal cows. *Indian Journal of Animal Reproduction* **8**:18–20.
- Misra A K, Prasad S and Taneja V K. 2005. Embryo transfer technology (ETT) in cattle and buffalo in India: A review. *Indian Journal of Animal Sciences* **75**(7): 842–57.
- Misra A K, Joshi B V, Agarwal P L, Kasiraj R, Siviah S, Rangareddy N S and Siddique M U. 1990. Multiple ovulation and embryo transfer in Indian buffaloes (*Bubalus bubalis*). *Theriogenology* **33**: 1131–42.
- NOAA. 2018. National Oceanic and Atmospheric Administration. <https://www.ncdc.noaa.gov/sotc/global/201813>.
- Nilchuen P, Rattanabattimong S and Chomchai S. 2011. Superovulation with different doses of follicle stimulating hormone in Kamphaeng Saen beef cattle. *Songklanakarin Journal of Science and Technology* **33**(6): 679–83.
- Purohit G N, Barolia Y, Solanki K, Kumar P, Joshi R and Mehta J S. 2013. Superovulatory responses and embryo recoveries in Rath (i>Bos indicus) cattle with different doses of Follitropin-V. *Theriogenology Insight* **3**(2): 89–94.
- Randel R B. 1984. Seasonal effects on female reproductive on female reproductive functions in the bovine (indian breeds). *Theriogenology* **21**: 170–85.
- Reddy Y V P, Veerabramhaiah K, Mouli Krishna K and Reddy Y R. 2015. Superovulatory response in Sahiwal × Jersey crossbred cows with different doses of FSHp. *Indian Journal of Animal Reproduction* **39**(2): 61–63.
- Sahatpure S K and Mehta V M. 2004. Total cholesterol level in relation to superovulatory response in Kankrej cows. *Intas Polivet* **5**(2): 226–28.
- Sartori R, Monteiro Jr. P L J and Wiltbank M C. 2016. Endocrine and metabolic differences between *Bos taurus* and *Bos indicus* cows and implications for reproductive management. *Animal Reproduction* **13**(3): 168–81.
- Seidel Jr E G and Seidel M S. 1991. Training manual for embryo transfer in cattle. Food and Agricultural Organization of the United Nations, 51–83.
- Silva J C C, Alvarez R H, Zanega C A and Pereira G T. 2009. Factors affecting embryo production in superovulated Nelore cattle. *Animal Reproduction* **6**: 440–45.
- Singh N, Singhal S, Malik V S, Singh H, Kumar A and Brar P S. 2018. Establishment of pregnancy from Sahiwal embryo in surrogate cross-bred cattle. *The Haryana Veterinarian* **57**(1):

- 99–100.
- 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 Journal of Animal Reproduction* **38**(2): 37–39.
- Sophon S, Tasripoo K and Jintana R. 2003. *Embryo transfer in Brahman cattle: Effect of protein level in concentrates on efficiency of embryo production*. Proceedings of the 41th Kasetsart University Annual Conference, Bangkok, Thailand, pp. 96–102.
- Velazquez A, Horacio Alvarez, Villasenor F and Michael E Kjelland. 2018. Effect of follicle-stimulating hormone administered during different time intervals in superovulation protocols in beef cattle. *Reproduction Fertility and Development* **31**(1): 223–24.