Effect of CIDR on superovulatory response of FSH administration in Sahiwal cows

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Journal of Livestock Science (ISSN online 2277-6214) 16: 247-252
Received on 03/01/24; Accepted on 13/3/25; Published on 20/3/25
doi. 10.33259/JLivestSci.2025.247-252

Abstract

The present study was designed to study the superstimulation in Sahiwal cows divided into two Group I (n=6, GPG based normal super-stimulatory Protocol) and Group II (n=6, CIDR based super-stimulatory Protocol). Five out of six cows in Group I and six out of six in Group II responded to the super ovulatory treatment (≥3 ovulations). A total of 239 ovulations/CL were counted by per rectal palpation on both the ovaries. Out of which 130 ovulations/CL in Group I and 109 ovulations/CL in Group II were recorded. The total mean ovulation rate (no of CL) was 19.91 out of which 21.67±5.40 in Group I and 18.17±1.85 in Group II. The total recovered Embryo/ova were 115 out of which 71 in Group I and 44 in Group II. The mean Embryo/ova recovered per donor and recovery rate was 9.58 & 48.12 % respectively. Out of which the mean Embryo/ova recovery per donor in Group I were 11.83 ± 4.36 & 54.62% and in Group II were 7.33±1.54 & 40.37 % respectively. The super-stimulatory response, and quantity of embryos recovered in Group I was better than Group II, hence GPG based normal superovulatory protocol is better than CIDR based Protocol.

Keywords: Sahiwal cow; Bihar; Embryo transfer technology; Ov-synch protocol

Introduction

Due to fast and continuous population growth, agricultural land area goes to decreasing day by day, alarming that in near future livestock sector should have concrete foundation to support GDP of India. Embryo transfer technology (ETT) is one of the most advance technologies that can revolutionize the livestock sector of India. The Embryo transfer is a technique that can greatly increase the number of offspring from genetically superior cows. ETT has emerged as an important tool to improve livestock at a faster rate. With this technique, the genetic contribution of both the male and female are utilized simultaneously. Thus, the most important application of ETT is the production of breeding bulls from the best-proven bulls and best available cows. Superovulation is a prerequisite for the successful application of embryo transfer in species with a physiologically low ovulation rate like cattle. The in-vivo embryo production involves superstimulation of donors using eCG (equine chorionic gonadotropin) or, FSH (Follicle-Stimulating Hormone) and recovery of embryos using non-surgical approaches. The embryos are then transferred to synchronized recipients to establish pregnancies. The efficiency of superstimulation of animals is mostly dependant on the protocol that was used. Superstimulation is the basis of embryo transfer which is the biotechnology to increase the progeny per genetically superior donor cow (Bo *et al*., 2009). Superstimulatory protocols have improved greatly since the early days of embryo transfer. Traditionally the superstimulatory protocols consisted of a single administration of the hormones that were not purified. During this era gonadotrophin treatment were initiated during the mid-luteal phase 9-11 days after oestrous around the time of emergence of the second follicular wave (Mapletoft *et al*., 2011). Super stimulatory protocols have improved greatly since the early days of embryo transfer. The improvement in the protocols now ensures that the emergence of the follicular wave and timing of ovulation occur at the same time in the donors and a fixed artificial insemination is guaranteed regardless of the phase of oestrous (Baruselli *et al*., 2006; Bo *et al*., 2009). The uniformity that the protocol improvement has brought is reported to have positive effects on commercial embryo transfer and breeding programmes.

In India and other Asian countries, the demand to multiply the genetic material of valuable *Bos indicus* females has increased. Sahiwal cow is one of them which are more suitable to our climatic conditions as compared to *Bos Taurus.* Sahiwal breeds of cattle which is originated in the Punjab region alongside Indian–Pakistan border. Sahiwal cattle is renowned for its higher milk production, remarkable power of endurance for hot climate of sub–tropics, relative resistance to diseases and low maintenance cost (Dey 2017). The propagation and conservation of genetically superior germplasm of Sahiwal cow through Embryo Transfer Technology (ETT) is needed. Therefore, it necessitates multiplying these high yielding cows within a very short period into a population of Sahiwal with much higher milk yield.

Keeping in view of variability in the super stimulatory responses as well as embryo recovery rate, a comparative study on super stimulatory protocol have been designed with aim and objective to compare the super stimulatory response between mid-luteal phase and CIDR (Controlled Internal Drug Release) inserted cows with FSH administration as well as to compare the embryo quality.

Materials and Methods

The present study was carried out after the approval of the institutional ethic committee. The experiment was conducted on Sahiwal breed of cattle at Livestock Farm Complex (L.F.C) of Bihar Veterinary College, Bihar Animal Sciences University, Patna, India. The Livestock Farm Complex is located at an altitude of 170 feet above the sea level and latitude of 25^0 North and a longitude of 85^0 East. The average temperature is 26^0 C and the relative humidity range between 60 to 65 %. All the animals were kept at Instructional Livestock Farm Complex (I.L.F.C) under uniform feeding and managemental conditions throughout the experiment period.

A total of 12 Sahiwal cows divided into two Group I (n=6, GPG (Gonadotropin-Prostaglandin-Gonadotropin) based normal super-stimulatory Protocol) and Group II (n=6, CIDR based super-stimulatory Protocol). Cows were of normal oestrous cycle, aged between 3 to 6 years old with healthy body condition, between of 2^{nd} and 3^{rd} lactation free from any parasitic infestation, absence of history of any pathological reproductive condition like pyometra, metritis, endometritis etc. on the basis of previous breeding, health records, per-rectal examination of their genital organs to reveal functional ovaries, normal uterine horns and patent cervix during dioestrums were selected.

All the donor Sahiwal cows were dewormed with 100 ml of Nilzan Susupension at rate of 1 ml per 03 Kg. Body Weight (Virbac, India) sixty days prior to starting of experiment.

Feed of all the donor Sahiwal cows was supplemented with mineral mixture (Agrimin Forte, Virbac, India) at the dose rate of 50 gm per animal per day was started 30 days prior to experiment.

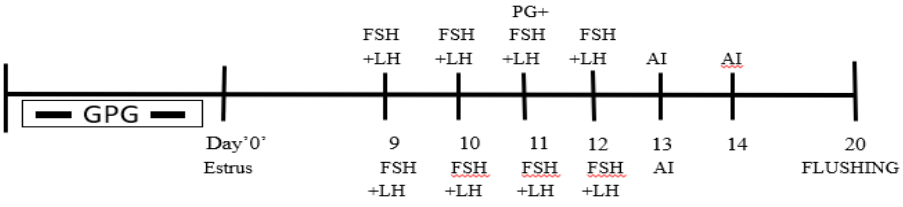

Figure: 1. Day wise treatment schedule for Group I (GPG based normal Protocol)

Group I (GPG based normal protocol) treatment (n = 6)

All the donor sahiwal cows for the Group I (GPG based normal Protocol) was synchronized by Ov-synch protocol (Pursely *et al*., 1995) (Buserelin acetate 10µg on day zero, Cloprostenol Sodium 500µg on day 7th and Buserelin Acetate 10µg on Day 9). Day 10th of the Ov-synch protocol was considered as day of estrus or, day zero. Estrus signs also were detected by visual examinations such as bellowing, mucus discharge from vulva, mounting, frequent micturition, swollen and oedematous vulva etc. The superstimulatory treatment was started on day 9 of estrous cycle for four consecutive days in eight divided dose at the regular interval of 12 hours (Morning and Evening) in tapering dose of pFSH + pLH (Stimufol®, Reprobiol SPRL) intramuscularly (Day 9th – 50 +10µg /50 +10µg, Day 10th -37.50 + 7.5µg /37.50 + 7.5µg, Day 11th – 25 + 5µg /25 + 5µg and Day 12th - 12.50 + 2.5µg /12.50 + 2.5µg ; total 250µg pFSH and 50 µg pLH).With 5th dose of pFSH and pLH injection, Cloprostenol Sodium 500µg was given intramuscularly to induce superstimulatory estrus. After 48hr of Cloprostenol Sodium 500µg administration superovulated animal was artificially inseminated thrice at the regular interval of 12 hr (two straws was used at each time of insemination). On the day of 20th embryo flushing was done in standard aseptic condition (Figure-1).

Group II (CIDR Protocol) (n = 6)

Treatment for super stimulation in all the donor Sahiwal cows of Group II (CIDR Protocol developed by Macmillan and Peterson, 1993) was started with CIDR (EAZI BREED^{TM}CIDR$^{®}$1380, DEC International NZ Ltd, New Zealand) insertion in vagina with maintaining all the aseptic measure irrespective to the day of estrous cycle, injection Oestradiol benzoate 2mg (Pregheat®, Virbac, India) and injection Hydroxyprogesterone caproate 50 µg (Duraprogen®,Vetcare, India) was given IM.

The superstimulatory treatment was started on day 4th of CIDR insertion for four consecutive days in eight divided dose at the interval of 12 hours (Morning and Evening) in tapering dose of pFSH + pLH intramuscularly (Day 4th- 50 + 10µg /50 + 10µg, Day 5th – 37.50 + 7.5µg /37.50 + 7.5µg, Day 6th- 25 + 5µg /25 + 5µg and Day 7th–12.50 + 2.5µg /12.50 + 2.5µg; total 250µg pFSH, Stimufol®).

With 6th and 7th dose of pFSH injection, Cloprostenol Sodium 500µg was given intramuscularly to induce superstimulatory estrus. CIDR was removed with 7th dose of FSH injection.

After 12 hrs. of GnRH administration, superovulated animal was artificially inseminated thrice at the regular interval of 12 hrs. (two straws was used at each time of insemination). On day 16th embryo flushing was done in standard aseptic condition (Figure-2).

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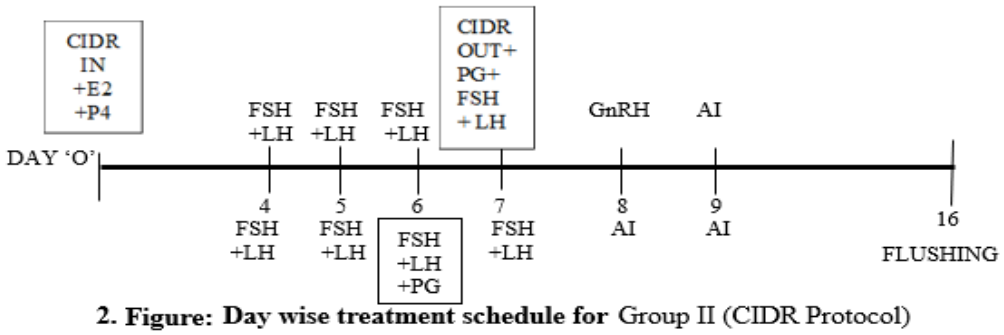

2. Figure: Day wise treatment schedule for Group II (CIDR Protocol)

Estrus detection and artificial insemination of super-ovulated cows

All donors were subjected to estrus detection twice a day 6:00 A.M morning and 6:00 P.M evening starting 24 hours after the administration of Cloprostenol Sodium. Estrus was confirmed on the basis of mucous

discharge, vulvar oedema and redness, mounting on other cow and tonicity of uterine horn confirmed by per-rectal examination. The superovulated cows were artificially inseminated thrice at 12h interval.

Embryo collection

The embryo was collected on 7th day from first day of artificial insemination, non-surgically in aseptic environment using flushing media (Euro flush, IMV Ltd) of 1000 ml per animal

Laboratory Preparation

All the necessary equipment, instruments, glass wares were washed properly and rinsed with double and triple distilled water and were. For embryo flushing a ready-made flushing media i.e., EUROFLUSH ET medium (IMV, Tech.) was used. Prior to embryo collection, the media bottles were maintained at 37^{0}C by keeping the solution in the incubator.

Evaluation of Superovulatory Response by Ovarian Palpation

Donor cows were examined per-rectally on day 6 after superpvulatory estrous to evaluate ovarian response. Superovulatory response was recorded on the basis of number of CL and anovulatory follicles. Superovulatory response was considered successful if number of CL in animal were three or more than three.

Preparation of Donor Animals for Flushing (Embryo Recovery)

The donor was fasted overnight prior to embryo collection to reduce ruminal content. After evacuation of faeces, epidural anesthesia was induced with 3-8 ml of 2% Lignocaine hydrochloride (LOX® 2%, Neon Laboratories Limited, Andheri (East), Mumbai, India) in lumbo-sacral or, in between 1st and 2nd coccygeal space.

Embryo Recovery

The sterilized Woerlein catheter (916/18 Gauge), tubing and embryo filter was rinsed with flushing media prior to embryo flushing. Woerlein catheter (75 cm length) fitted with sterile stylet was introduced into uterine horn to be flushed through the cervix of the animal. The inflatable balloon was placed in the uterine horn and stylet was removed and then balloon was inflated slowly with 8-12 ml of air according to the size of the lumen of uterine horn such that it should not damage the endometrium. The catheter was connected to a 'Y' junction of tubing, whose one end was connected to flushing medium bottle and other end to the Emcon embryo filter. After that, the flushing medium was allowed to pass into the horn lumen through the catheter.

Each uterine horn was flushed 6-7 times until about 500 ml flushing medium has been used by gravitational method. During each flushing the uterine horn was filled up with 30-60 ml of the flushing medium. The recovery was also done by the gravitational flow and passed through the embryo filter.

During flushing, the flushed out medium from the horn, recovered in the embryo filter was so adjusted that about 15-20 ml of media always remains in the filter so as to avoid sticking of embryo in the filter. The flow of flushing media in and out of the uterine horn was controlled by tweezer clamps. When flushed media turns out to clear after 6-7 flushing of one horn i.e., no uterine contents were observed, the Woerlein catheter was withdrawn after deflation of balloon from the flushed uterine horn and again inserted into the other uterine horn and the process was repeated.

After completion of flushing process, 60 ml Lenovo-AP® (Intas Pharmaceuticals Lt.) was infused 2/3rd into the uterine horn using A.I sheath and gun to protect the animal from any possible infection. After flushing, the collected filtered media was transferred to 94 x 15 mm Petri dish (Grenier Bio one, Germany). The embryo filter then washed with 15-20 ml of fresh flushing medium into another petridish to evaluate embryos. Each petridish was searched thoroughly 3-4 times under stereo zoom microscope (SMZ-2B, Nikon, Japan) at lower magnification 20-40x magnification for the presence and quality of embryos recovered.

Isolation and evaluation of embryon The embryos/ova were isolated with the help of embryo aspirator and transferred into small tissue culture dish (35 x 10 mm, Falcon Becton Dickinson, Labware, New Jersey, USA) containing 1.5-2.0 ml of embryo holding medium for further use. Embryos were given 2 washing in the holding medium (0.4 % Bovine Serum Albumin, IMV) and were evaluated under 40x magnification of stereo zoom microscope for its developmental stage as well as for its quality. The embryos were examined, evaluated and graded morphologically as Unfertilized Ova, Early Morula, Compact Morula, Early Blastocyst, Blastocyst, Expended Blastocyst, Hatched Blastocyst, Empty Zona and Degenerated with the guideline laid down by International Embryo Technology Society (Figure-3).

Statistical analysis

All the collected data was statically analysed using SPSS software version 23. Mean ± SE was determined by descriptive statistics method. Single Factor Analysis of variance (ANOVA) was used to compare the mean at different time intervals among different group and compare the mean values at different intervals with their respective base values in each group. (Snedecor and Cochran 1994).

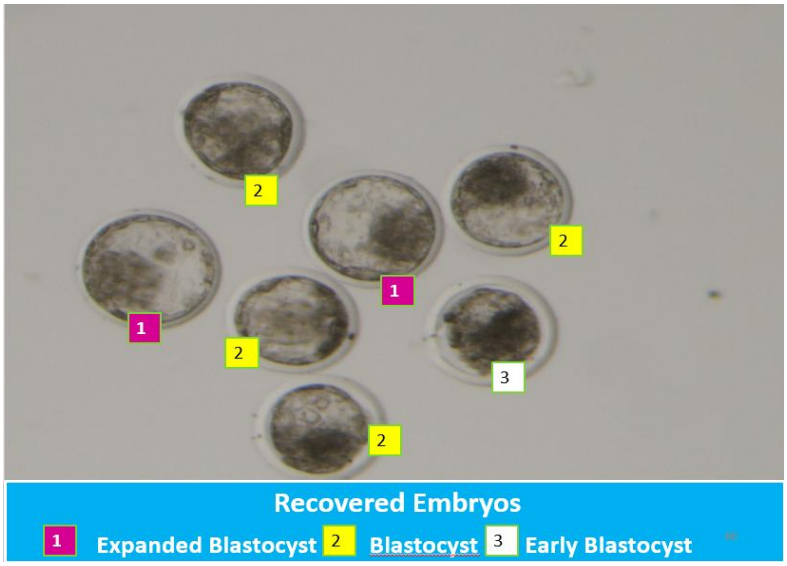

Fig 3 Recovered embryos

Results and Discussion

Super ovulatory Response and Embryo Recovery

A total of 239 ovulation/corpus luteum were counted by per rectal palpation on both the ovaries of the both groups. Out of which 130 ovulation/Corpus luteum in Group I (GPG based normal protocol) and 109 Ovulation/Corpus luteum in Group II (CIDR Protocol) recorded. The total mean ovulation rate (number of Corpus Luteum) was 19.91. Out of which 21.67±5.40 in Group I (GPG based normal Protocol) and 18.17±1.85 in Group II (CIDR Protocol). The total recovered Embryo/ova were 115 out of which 71 in Group I (GPG based normal Protocol) and 44 in Group II (CIDR Protocol). The overall mean Embryo/ova recovered per donor and recovery rate was 9.58 and 48.12 % respectively. The mean Embryo/ova recovery per donor in Group I (GPG based normal Protocol) were 11.83±4.36 and 54.62% and in Group II (CIDR Protocol) were 7.33±1.54 and 40.37 % respectively. The overall superstimulatory response was 91.67 % in present work which is lower than the report by Imtiyaz *et al*., (2021) 100% in Sahiwal cows and higher than the report by Siddiqui *et al*., (2008) 75% in Sahiwal cows; Ferreira *et al*., (2014) 85.70%. Baruselli *et al*., (2006) recorded that 20-30% donor cows were unresponsive to superovulatory treatment. Many previous studies found that about 33% animals did not respond to superovulation even after following standard superovulatory protocol (Mishra, 2002; Mishra and Pant, 2006). The mean ovulation 21.67±5.40 in Group I was found which is lesser than the report by Hassan *et al.,* (2016) in Sahiwal cows and while 18.17±1.85 in Group II of present work was lower than the report by Mahmood *et al*., (2021) 77.79±3.76 and higher than report by Purohit *et al.,* (2023) 7.13± 1.21. was higher than the report by Singhal *et al.,* (2020) in Sahiwal cows.

In the present study, the overall mean Embryo/Ova recovered per donor and recovery rate was 9.58 and 48.12 % while group I was higher value of recovered embryo per donor as well as recovery rate than group II. The present study reports higher embryo recovery per donor than the earlier report of 9.00 (Singhal *et al*.,2017 in Sahiwal cows) 6.62 (Acosta *et al*., 2016), 6.7 embryos (Ferreira *et al*., 2014), 8.4 embryos (Junior *et al*., 2008), 8.3 embryos (Neto *et al*., 2005). The lower embryo recovery rate in relation to the large no of ovulations may be due to fimbrial inability to catch the ova from enlarged superovulatory ovary. In the present study, total embryo recovery in Group II (CIDR Protocol) (7.33±1.54) was lower as compared to reported by Son *et al*., (2007) (10.0±1.4) in EB-CIDR group. The comparison between XB and HF cows revealed that the TEs production in CIDR-GnRH and CIDR-EB based superovulatory protocol enhances the transferable embryo production (Mahmood *et al*., 2021).

Conclusion

The super-stimulatory response in Group I was better than Group II, hence GPG based normal superovulatory protocol is better than CIDR based Protocol. The quality & quantity of embryos recovered in Group I was better than Group II, hence GPG based normal superovulatory Protocol is better than CIDR based protocol.

Acknowledgement

Authors are very much thankful to Vice- Chancellor, Bihar Animal Sciences University, Patna for providing funds to carryout curricular research. We wouldn't forget to thank Dean, BVC, Patna, Dr JK Prasad for his contribution in research and University official and staffs for assisting during research works.

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