Haryana Vet. (March, 2023) **62(SI),** 5-7

Research Article

EARLY PHASE OF ESTABLISHMENT OF MULTIPLE OVULATION EMBRYO TRANSFER IN SAHIWAL COW AT PUSA, BIHAR

SUMIT SINGHAL*, PRAMOD KUMAR, NARENDRA KUMAR, K.M. KUMAR, A.K. SINGH, A. KUNDU and A.P.S. ASWAL

Centre of Excellence on Indigenous Breed, Piprakothi

Rajendra Prasad Central Agriculture University, Pusa-848 125, India

Received: 15.03.2022; Accepted: 16.06.2022

ABSTRACT

Sahiwal cows being one of the best indigenous milch and climatic resilient breed required to be propagated at a faster rate particularly in region having limited feed resources and rain-fed area like Bihar. The study showed that multiple ovulation embryo transfer could be successful model in propagating Sahiwal germ plasm in Bihar.

Keywords: Cows, Multiple ovulation embryo transfer, Sahiwal, Stimufol

How to cite: Singhal, S., Kumar, P., Kumar, N., Kumar, K.M., Singh, A.K., Kundu, A. and Aswal, A.P.S. (2023). Early phase of establishment of multiple ovulation embryo transfer in Sahiwal cow at Pusa, Bihar. *Haryana Vet.* **62(SI)**: 5-7.

Sahiwal cow is one of the best milch indigenous breed with an adaptability to flourish under the diverse Indian climatic conditions. However, the average milk productivity of indigenous cattle is about 3-4 kg/day yet some elite Sahiwal cattle have more than 3500 kg per lactation length in 305 days (DAHD, 2021). This data itself showed the potential of Sahiwal milk production and scope of per animal productivity improvement, which could be possible by multiplying the elite germ plasm at a faster rate by utilizing the multiple ovulation embryo transfer (MOET) technology. MOET is being applied for genetic improvement of livestock with varying level of success. Since the inception of Rastriya Gokul Mission, Government of India (GoI) have incurred huge budget to popularize the MOET and other related assisted reproductive techniques for the indigenous cattle particularity for Sahiwal and one such project was started in Pusa, Bihar. In Bihar, the average milk productivity per cattle is very less compared to many states and country's average milk yield. The MOET has proved to improve the dairy (Sartori *et al.*, 2002) and meat husbandry (Carter *et al.*, 2008) in foreign countries. But the limited success of embryo transfer in indigenous cattle was reported due to variable superovulatory response, less embryo recovery and low ET conception rate (Baruselli *et al.*, 2006). Therefore, the present study was conducted to explore the potential and success of MOET in Sahiwal in Bihar.

MATERIALS AND METHODS

Selection of experimental Sahiwal cattle: A study was carried out on Sahiwal cattle at Dairy Farm, Rajendra Prasad Central Agriculture University, Pusa, Bihar. The cattle were clinically healthy and normal cycling aged between 4-9 years. Their history and genitalia were thoroughly checked to avoid any reproductive problems, like abortion, dystocia, endometritis, metritis and pyometra etc. Gynaeco-clinical examinations of animals revealed functional ovaries, normal uterine horns and patent cervix during diestrus. All the Sahiwal cattle were kept under uniform conditions of feeding and managemental practices.

Estrus induction and detection of base heat: The animals were either natural cyclic or induced to estrus with 2 ml intramuscular injection of Cloprostenol Sodium (Estrumate®, MSD) in the animals having mature corpus luteum. The estrus detection was done morning and evening by close observation for external signs, such as bellowing, mucus discharge from vulva, mounting, frequent micturation, swollen and edematous vulva and confirmed by rectal palpation.

Experimental Design and Superovulatory Treatment: Sahiwal cattle (n=12) were selected and subjected for the superstimulatory protocol as per the protocol given in Table 1.

Insemination of donors during superovulatory estrus: During the superovulatory estrus, the cattle were artificially inseminated with frozen semen of Sahiwal bull at fixed time 48 hr after the prostaglandin injection for three times at 12 hr interval.

Embryo Collection: The embryos were collected non-surgically on day-7 of first artificial insemination using 18 G Rusch catheter (75 cm length) as per detail methodology (Singhal *et al.*, 2020). Briefly, per-rectal back racking, epidural anaesthesia (2% Lignocaine hydrochloride), cleaning of perineal area was done to avoid straining & contamination. About 400 ml of flushing medium (Euroflush®, IMV) was used for flushing of each uterine

*Corresponding author: drsinghalvet@gmail.com

Table 1

Protocol for superstimulation in Sahiwal cattle using Stimufol

Day	Donor	Recipient
0	Cow in Heat	PG Estrumate 2ml
10	Stimufol 1ml ME (Morning and Evening)	
11	Stimufol 0.75 ml ME	PG Estrumate 2ml
12	Stimufol 0.5 ml ME + PG Estrumate 2ml M	
13	Stimufol 0.25 ml ME	
14	AI ME	
15	AI Morning only	
21	Embryo Flushing	Embryo Transfer

horn. Following the process of embryo flushing, 30 ml Gentamicin was injected into the uterine horns to protect against any possible infection and Cloprostenol Sodium (@ 2ml; Estrumate®, MSD) was administered for luteolysis to bring back animal in cycle. After flushing, the media collected into the Emcon filter was transferred to 94 × 15 mm sterile Petridish. Each petridish was searched thoroughly under stereozoom microscope (SMZ-745 T, Nikon, Japan) at 15-20x magnification for the embryo.

RESULT AND DISCUSSION

The superovulatory response was assessed manually by per rectal examination of both the ovaries on day of embryo flushing. There was wide variation in the number of corpus luteum (CL) present over the ovaries which showed that superstimulatory response is highly unpredictable and variable. In our study, the number of CL found varied from 0-32 examined per-rectally. Unpredictability during MOET were reported in buffalo (Misra and Tyagi, 2007), *Bos taurus* (Bo and Mapletoft, 2014) and in *Bos indicus* Sahiwal (Nidha *et al.*, 2020) were unresponsive to superstimulatory regimen and many of *Bos indicus* didn't show superstimulatory response and particularly Sahiwal cattle are erratic responders to follicle stimulatory hormone (Nidha *et al.*, 2020). Poor response to superstimulation might be due to inherent, individual animal variability, negative energy balance, climatic condition, and many known & unknown factors (Cabrera *et al.*, 2013).

In this study, the number of embryos recovered and searched from uterine flushing ranged from 0-7 (Fig. 1). The nil embryo recovery observed were might be due to poor response, unable to pass the flushing catheter through cervix, improper fixation of catheter, low flushed out fluid recovery, sub-clinical endometritis causing infected or higher debris content in flushed medium leading to inadequate searching or due to various factors causing poor fertilization (Carvalho *et al.*, 2013).

Fig. 1. Embryos recovered from flushing

Embryos searched were classified under transferrable and non-transferrable quality based on morphological examination (IETS, 2016). Embryos were transferred in three synchronized recipients in the anterior one-third of the uterine horn ipsilateral to the ovary containing corpus luteum. All three cattle were non-return to the heat upto day-14 of embryo transfer (ET). Later, one recipient (33%) returned to heat on day-25 of ET while other two surrogates (66%) were non-return upto day-30 of ET and one was found pregnant at 2 months of embryo transfer. Similarly, in 30% cases even the most competent *in vivo* produced blastocyst failed to sustain the conception due to inadequate maternal environment (McMillan, 1998). Studies showed that heat cycle got extended by few days to weeks during ET in cattle and buffalo. Misra and Tyagi (2007) reviewed that the variations in conception and success of ET programme are due to varied expertise, environment, known and unknown factors. Non-return to heat upto day-30 ET were reported as 50% in a previous study (Singh *et al.*, 2018). Lower conception rate in ET were observed in Ongole (20%; Kasiraj *et al.*, 2000), contrarily, compared to this study slight higher conception rates were reported in Red Sindhi (38.46%; Rangasamy *et al.*, 2015) following embryo transfer of indigenous cattle.

The study concluded that the MOET could be a successful technology in faster propagation of Sahiwal cattle in Bihar.

ACKNOWLEDGEMENT

Facilities provided by the university to carry out the present investigation and financial assistance provided under Rastriya Pashudhan Vikas Yojana, GoI is duly acknowledged.

REFERENCES

Baruselli, P.S., de Sa Filho, M.F., Martins, C.M., Nasser, L.F., Nogueira, M.F.G., Barros, C.M. and Bó, G. (2006). Superovulation and embryo transfer in *Bos indicus* cattle. *Theriogenol.* **65(1)**: 77-88.

Bo, G.A. and Mapletoft, R.J. (2014). Historical perspectives and recent research on superovulation in cattle. *Theriogenol.* **81**: 38-48.

Cabrera, C., Lane, M. and Farver, T. (2013). 210 factors affecting superovulation response in cattle: a retrospective study. *Reprod. Fertil. Dev.* **26**: 219.

Carter, F., Forde, N., Duffy, P., Wade, M., Fair, T., Crowe, M.A, Evans, A.C., D.A. Kenny, D.A., Roche, J.F. and Lonergan, P. (2008). Effect of increasing progesterone concentration from day 3 of pregnancy on subsequent embryo survival and development in beef heifers. *Reprod. Fertil. Dev.* **20**: 368-375.

Carvalho, P.D., Souza, A.H., Sartori, R., Hackbart, K.S., Dresch, A.R. and Vieira, L.M. (2013). Effects of deep-horn AI on fertilization and embryo production in superovulated cows and heifers. *Theriogenol.* **80**: 1074e81.

DAHD. (2021). https://dahd.nic.in

IETS. (2016). International Embryo Transfer Society. https://www.iets.org/pdf/comm_data/IETS_Data_Retrieval_Report_2016_v2.pdf

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 J. Anim. Sci.* **70(3)**: 251-53.

McMillan, W.H. (1998). Statistical models predicting embryo survival to term in cattle after embryo transfer. *Theriogenol.* **50**: 1053-1070.

Misra, A.K. and Tyagi, S. (2007). *In vivo* embryo production in buffalo: current situation and future perspectives. *Italian J. Anim. Sci.* **6**: 74-91.

Nidha I., Brar P.S, Singh, N., Singh, H., Singhal, S. and Malik, V.S. (2020). Relationship of antral follicle count, plasma estradiol and progesterone levels with super ovulatory response and embryo production in Sahiwal Cows. *Int. J. Curr. Microbiol. App. Sci.* **9(6)**: 352-359.

Rangasamy. S., Sridevi. P., Saravanan, D. and Sarath, T. (2015). A rare incidence of high superovulatory response and embryo recovery in a Red Sindhi cow and its conception rate. *Indian Vet. J.*, **92(5)**: 78-79.

Sartori, R., R. Sartor-Bergfelt, S.A. Mertens, J.N. Guenther, J.J. Parrish and M.C. Wiltbank. (2002). Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. *J. Dairy Sci.* **85**: 2803-2812.

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. *Haryana Vet.* **57(1)**: 99-100.

Singhal, S., Singh, N., Singh, H., Malik, V.S. and Brar, P.S. (2020). Curtailing the conventional dose of Folltropin-V for super-stimulation and embryo recovery in Sahiwal cattle (*Bos indicus*): a novel concept. *Indian J. Anim. Sci.* **90(11)**: 1471-1475.

CONTRIBUTORS MAY NOTE

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of 'The Haryana Veterinarian' for manuscript writing/ submission.
- Please pay processing fee of Rs. 1000/- online in the account of Dean, College of Veterinary Sciences, along with each article.
- After revision, please return the revised manuscript and rebuttal at the earliest.
- Please mention your article reference number in all correspondence for a quick response.
- We solicit your co-operation.
- All correspondence should be addressed to 'The Editor', Haryana Veterinarian, Department of Veterinary Parasitology, College of Veterinary Sciences, LUVAS, Hisar-125004.

Editors