The Haryana Veterinarian (June, 2024) **63(1),** 1-3 ISSN 0033-4359 (P) **Research Article**

FROZEN THAWED SEMEN FERTILIZED AND *IN VITRO* PRODUCED EMBRYO TRANSFER IN COWS

R.M. MEHAR, M.B. AMLE*, K.P. KHILLARE, M.N. RANGNEKAR, A.B. MALI[1], B.P. SANGALE[3], V.B. JAWANE[3] and V.S. DHAYGUDE[2]
Department of Animal Reproduction, Gynaecology and Obstetrics,
[1]Department of Veterinary Clinical complex, [2]Department of Veterinary Pathology
Krantisinh Nana Patil College of Veterinary Science, Shirwal-412801, Maharashtra, India
[3]IVF Laboratory, Bull Mother Farm, Animal Husbandry (Govt. of Maharashtra), Tathawade, Pune

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ABSTRACT

The rationale of research work was to study the efficiency of oocytes retrieved through ovum pick up from non-stimulated donor cows, IVF using frozen thawed semen and IVC to cultivate embryos and resultant pregnancies in recipient cows. The results revealed that cleavage and blastocyst rates of *in vitro* fertilized oocytes using conventional semen were 31.62±5.39 and 6.62±2.35%, respectively. The IVE of codes 1, 2, 3, 4 were found as (mean±SE) 0.83±0.40, 1.50±0.43, 2.00±0.26 and 6.00±1.12, respectively. The overall pregnancy rates were 12.5%, upon transfer of *in vitro* produced conventional semen fertilized embryos.

Keywords: Conventional semen, *In vitro*, Pregnancy Rate, Recipients

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In vitro embryo production is one of the most important and latest assisted reproductive technologies in cow breeding and management. In vitro produced embryo transfer technology enables the faster propagation of genetically modified and possibly productive herds than conventional genetic techniques. Large-scale embryo production in domestic mammals particularly in cattle has been possible for the aim of producing vast number of embryos for research, producing genetically superior offspring and as a bridge to other reproductive technologies. Breeding strategies utilizing IVEP combination of OPU and conventional semen offer possible options to improve dairy cattle genotypes and more efficient production with delivery of improved germ plasm. It is essential to investigate the suitability of sperm for *in vitro* production of dairy embryos for subsequent transfer into recipient cows and resultant conception.

The proportion of follicular aspirated oocytes from non-stimulated donor cows developing to the blastocyst stage, the quality of these blastocysts and recipients are important in determining success of *in vitro* embryo transfer. The most practical method for expanding the number of recipient animals is to adopt procedures that allow for embryo transfer without the requirement for estrus detection, which is usually referred to as fixed time embryo transfer (FTET) (Marinho *et al.*, 2012). Therefore, the aim of the present study was to investigate and compare the suitability of frozen thawed semen/spermatozoa for production of *in vitro* embryos and subsequent transfer to recipient cows and evaluation of pregnancy rate.

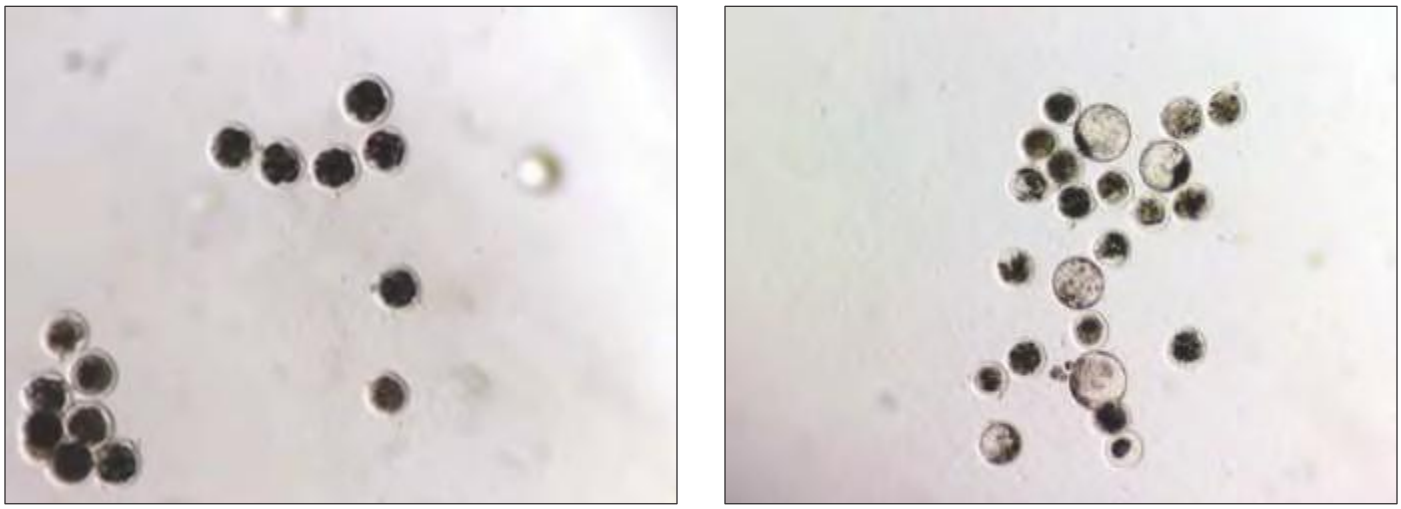
*Corresponding author: mukundamle@mafsu.in

MATERIAL AND METHODS

The research work was conducted on cows at IVF Laboratory, Bull Mother Farm, Animal Husbandry (Govt. of Maharashtra), Tathawade, Pune. In this study, cumulus oocyte complexes (COCs) were aspirated using ultrasound guided OPU technique from ovarian follicles of unsynchronized donor cows at every two weeks. A total 3 donor cows were enrolled in the IVF programme submitted to total of 6 OPU sessions. Oocytes aspirated were subjected for *in vitro* maturation (IVM) followed by *in vitro* fertilization (IVF) with the conventional sperm and cultured (IVC) for *in vitro* embryo production (IVEP).

The morphological development of the IVPE was assessed through assessment of cleavage rate on day 4 and blastocyst rate on day 7. Cleavage rate was determined based on number of oocytes cleaved *in vitro* divided by number of oocytes fertilized. The embryo with more than 3 blastomeres was considered as cleaved. Blastocyst rate was assessed on day 7 based on number of blastocysts produced *in vitro* divided by number of oocytes fertilized. Embryos produced from donor cows were evaluated for two criteria *viz*. stage and grade according to the IETS guidelines (Bo and Mafletoft, 2013). A stage was assigned for each embryo: Stage 3-Early morula, Stage 4-Morula, Stage 5-Early Blastocyst, Stage 6-Blastocyst, Stage 7-Expanded Blastocyst. The codes for embryo grade ranges from 1 to 4 as excellent or good, fair, poor or dead and degenerating, respectively.

A total 8 recipient cows selected for *in vitro* produced embryo transfer were synchronized for induction of estrus on the day of retrieval of ova from donor. The experimental



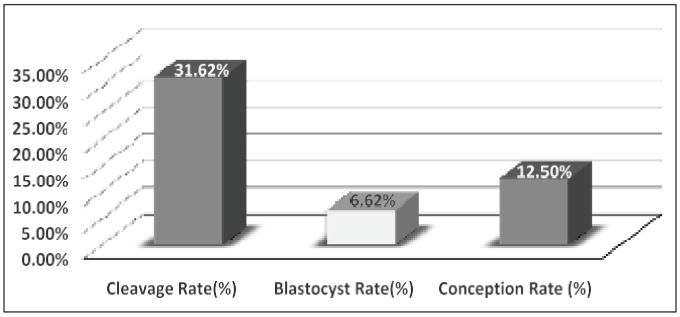
Cleavage (Day 4) and blastocyst (Day 7) of in vitro fertilized oocytes utilizing frozen thawed Semen

recipients were synchronized using double Prostaglandin (500 µg of Cloprostenol intramuscularly) in cattle having corpus luteum on ovary detected by transrectal palpation. Second dose (500 µg) was administered 11 days apart 48-72 h before the day of oocyte retrieval from donor cows. All the cows were observed for estrus response, intensity of estrus, signs and duration of induced estrus. Fixed time ET protocol was implemented for estrus synchronized recipients. Before embryo transfer (day 7) the ovaries were examined using ultrasonography (with 6-10 MHz linear multi-frequency transducer) (Ultrasound machine veterinary, Make-IMV, France, Model-Exago, Serial number 1909EX01), to confirm the presence and size of CL. Only recipients with a CL greater than or equal 20 mm in diameter received an embryo during IVEP programme.

The diameter of the CL and the volume of luteal tissue were addressed by transrectal ultrasonography. A quality score as excellent, good and fair quality were applied. Corpus Luteum volume was calculated by volume of sphere: [$V = 4/3\pi (D/2)^3$ (Spell *et al.*, 2001), where D is the maximum luteal diameter]. The relationship between luteal development and CL volume on day 7 of *in vitro* embryo transfer corresponding to pregnancy rate in recipient cows was investigated. Recipients were assessed between 30-45 days by USG after embryo transfer to determine pregnancy status. The conception rate was calculated by dividing the total number of pregnant cows by number of cows transferred with IVPE multiplied by 100.

RESULTS AND DISCUSSION

An embryonic development of *in vitro* fertilized IVF oocytes were assessed in IVC and overall cleavage rate of in vitro fertilized IVF oocytes using frozen thawed conventional semen were observed as 31.62 ± 5.39 per cent. Reports concerned with higher cleavage rate percentage when oocytes were fertilized *in vitro* in cows by Presicce *et al.* (2019) (49.5±5.4%) and Nogueira *et al.* (2021) (61.19



Cleavage, blastocyst and conception rate of *in vitro* produced conventional semen fertilized embryos

%). Whereas lower cleavage rate was also reported by Harkal (2019) (22.7%). In the present study, average cleavage rate of the IVF embryos may be due to donor cows used for oocyte collection were not given exogenous hormones. The cleavage rate may be influenced by not selecting the oocytes prior to IVF or possibly utilization of frozen semen of different sires in IVF system.

In the present study, the blastocyst rate observed was 6.62±2.35%. Higher IVF blastocyst rates in cows were reported by Harkal (2019) 15% and Nogueira *et al.* (2021) 21.13% than the results of the present study. The reason behind the low blastocyst rate in the study might be due to the intrinsic quality of the oocyte is the key factor that determines oocyte competence up to the blastocyst stage (Nogueira *et al.*, 2021).

A total 62 embryos were obtained from 6 sessions of OPU which were fertilized with the conventional frozen thawed semen. Overall 10.33±1.80 number of embryos were produced per session. The IVE of codes 1, 2, 3, 4 were found to be 0.83±0.40, 1.50±0.43, 2.00±0.26 and 6.00±1.12, respectively. Code 3 and 4 IVPE were significantly higher ($p<0.01$) than embryos of code1 and 2. The quality depends on breed and age of donor at oocyte collection, nutritional and hormonal status and frequency of oocyte collection (Nogueira *et al.*, 2021).

The estrus response parameters of recipient cows the such as estrus response and onset of estrus following double PG schedule for synchronization was obtained as 75% and 55.87±4.93 h, respectively. The percentage of weak, normal and intense estrus intensity obtained were 25, 50 and 25 per cent, respectively. The results of estrus synchronization following double PG were in accordance to other study reported by Reddy *et al.* (2001) and Mani *et al.* (2021).

On the day of ET the mean ovarian CL dimensions (length, width) for pregnant and non-pregnant cows were 21.1, 25.7 mm and 22.30, 21.27 mm, respectively. The mean CL diameter in non-pregnant and pregnant recipient cows were 21.7, 90.85 mm and 23.4, 02.30 mm, respectively. The results of present study for CL dimeter in pregnant cows were similar with study of Gonella Diaza *et al.*, 2013 (>20 mm). Spell *et al.* (2001) noticed higher CL diameters, 24.1 mm and 24.0 mm in pregnant and non-pregnant cows compared to present study. The pregnancy rate with a mean diameter of corpus luteum was higher in recipients that became pregnant compared with non-pregnant. The present study confirmed 12.5% conception rate in pregnant recipient cows associated with the CL in the ovary ranging between 22 mm and 24 mm on day 7 post-estrus. The higher pregnancy rates (33%) achieved when the pregnant recipient cows were associated with an ovarian CL diameter ranging between 17 and 22 mm was presented by Jaskowski *et al.* (2021).

The observed luteal volume in pregnant and non-pregnant recipients in the present study receiving IVPE as 8.86 cm^3 and 7.30 cm^3, respectively. The pregnancy rate with a mean luteal volume of corpus luteum was higher in pregnant recipients that received an embryo on day 7^{th} after estrus compared with non-pregnant ones. Spell *et al.* (2001) determined the mean luteal volume in pregnant and non-pregnant recipients was reported as 7.1 cm^3 and 6.8 cm^3, respectively. Our values are higher than the Spell *et al.* (2001) which might be due to the effect of estrus synchronization treatment in recipient cows.

The conception rate in recipient cows subjected to transfer of conventional semen fertilized embryos (n=8) was recorded as 12.5%. Several researchers reported higher conception rate in cows as (17.5%), Ambrose *et al.* (1999), (33.3%) Veira *et al.* (2016). The recipient cattle receiving IVF derived embryos in good body condition and well-defined corpus luteum tend to have increased pregnancy rate under field condition (Marinho *et al.*, 2012).

The pregnancy rates achieved when *in vitro* embryos of stage 6 (blastocyst) were transferred recorded as 33.33 per cent when transferred to recipient cows. No pregnancies were established when stage 3 (early morula), stage 4 (compact morula) and stage 5 (early blastocyst) embryos. Transfer of code 1 (excellent) embryos resulted in 25 per cent pregnancy rate. A total of 3 recipient cows received transfer of embryos in left uterine horn and 5 cows received embryos in right horn with conception rate of 20 per cent. The majority of IVPE were in stage 6 with declining numbers in stages 5, 4 and 3, respectively. The embryos at stage 6 tended to yield the higher pregnancy rate followed closely by stage 5. Similarly, also code 1embryos were transferred to recipient cows compared to code 2 embryos in the present study. The stage, grade and transfer side of *in vitro* produced embryos were non-significant ($p > 0.01$) factors for the pregnancy rate. Benyei *et al.* (2006) reported slightly higher (42.0%) resultant pregnancy rates of stage 6 *in vitro* embryo.

The fresh *in vitro* produced frozen thawed semen embryos may yield the better pregnancy results provided that the embryos are exposed to least amount of time during transport and transfer. Based upon the pregnancy results in this study, every effort should be made to transfer majority of IVPE achieving developmental stage 5 (early blastocyst) or 6 (blastocyst) and embryo grade 1 or 2 and while transferring them at day 7 to recipient cows.

REFERENCES

Ambrose, J.D., Drost, M., Monson, R.L., Rutledge, J.J., Leibfried-Rutledge, M.L., Thatcher, M.J. and Thatcher, W.W. (1999). Efficacy of timed embryo transfer with fresh and frozen *in vitro* produced embryos to increase pregnancy rates in heat-stressed dairy Cattle. *J. Dairy Sci.* **82(11)**: 2369-2376.

Harkal, S.B. (2019). Effect of different vacuum pressures on oocyte recovery and *in vitro* maturation of oocytes collected by ultrasound guided trans vaginal follicular aspiration in cattle. M.V.Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India.

Jaśkowski, B.M., Bostedt, H., Gehrke, M. and Jaśkowski, J.M. (2021). Ultrasound characteristics of the cavitary Corpus Luteum after oestrus synchronization in heifers in relation to the results of embryo transfer animals. *Animals.* **11(6)**: 1706.

Mani, P., Mani, S., Kaliannan, R. and Angamuthu, R. (2021). Pattern of induced estrus during superovulatory programme in Kangayam donor cows. *Pharma Innov. J.* **10(4)**: 5-8.

Nogueira, B.G.R., de Souza, L.F.A., Puelker, R.Z., Giometti, I.C., Firetti, S.M.G., Dias, T.S.D.S.B. and Castilho, C. (2021). Factors affecting the *in vitro* production of bovine embryos in a commercial program. *Res. Soc. Dev.* **10(2)**: e16110212264.

Presicce, G.A., Neglia, G., Salzano, A., Padalino, B., Longobardi, V., Vecchio, D. and Gasparrini, B. (2020). Efficacy of repeated ovum pick-up in Podolic cattle for preservation strategies: a pilot study. *Italian J. Anim. Sci.* **19(1)**: 31-40.

Reddy, S.M., Bandopadhyay, S.K., Gupta, R.D., Haldar, S.K. and Dass, P.K. (2001). Synchronisation of estrus by single and double dose of PGF_2 alpha in Crossbred heifers in superovulation protocols. *Indian J. Anim. Reprod.* **22**: 14-16.

Spell, A.R., Beal, W.E., Corah, L.R. and Lamb, G.C. (2001). Evaluating recipient and embryo factors that affect pregnancy rates of embryo transfer in beef cattle. *Theriogenol.* **56(2)**: 287-297.

Vieira, L.M., Rodrigues, C.A., Netto, A.C., Guerreiro, B.M., Silveira, C.R.A., Freitas, B.G. and Baruselli, P.S. (2016). Efficacy of a single intramuscular injection of porcine FSH in hyaluronan prior to ovum pick-up in Holstein cattle. *Theriogenol.* **85(5)**: 877-886.