RESEARCH ARTICLE

Effect of Oxidative Stress on Estrus Synchronization and Pregnancy Rate after *In-Vitro* Produced Embryo Transfer in HF Crossbred Cattle

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Abstract

The present research involved 60 normal cycling recipients, including 30 heifers and 30 cows, synchronized using a double PGF2α protocol. A total of 140 oocytes were collected from 11 elite donors, averaging 12.72 oocytes per ovum pick-up (OPU) session at the Government Bovine Breeding Farm in Tathwade. Total 46 blastocyst-stage developed embryos were transferred to various farms in Western Maharashtra. Estrus response and ovarian corpora lutea (CL) development was higher in synchronized heifers (86.66%) than in cows (83.33%). Pregnant heifers had larger CL diameters and luteal areas than non-pregnant ones, while CL diameters in cows were similar regardless of pregnancy status. Total 24 (80.00%) heifers and 22 (73.33%) cows were suitable for embryo transfer. Overall, 76.66% of recipients received *in-vitro*-produced embryos, with conception rates of 50.00% in heifers and 31.82% in cows, resulting in an overall rate of 41.30%. Malondialdehyde (MDA) levels were higher in non-pregnant recipients compared to pregnant ones, suggesting that elevated MDA levels may be a useful selection criterion in embryo transfer technology.

Key Words: Cow, Conception rate, *IVP* embryo transfer, Heifer, Oxidative stress (MDA).

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Introduction

Crossbreeding local cattle with exotic breeds has been adopted in India to meet rising milk demands, helping the country become the world's leading milk producer, contributing 24.76% of global supply. However, milk production per animal remains low, making reproductive biotechnology crucial for enhancing yields. Assisted reproductive technologies (ARTs) like artificial insemination, ovum pickup (OPU), and *in-vitro* fertilization (IVF) are vital for improving productivity among crossbred cattle. OPU and *in-vitro* embryo production (IVEP) are increasingly used in India, involving the collection and fertilization of oocytes with genetically superior semen.

For successful embryo transfer, recipient cows need good physical condition and healthy reproductive systems, though they may not be genetically superior. High-producing lactating cows may yield poorer results in embryo transfer due to increased liver metabolism of estradiol and progesterone, which reduces estrus expression and pregnancy rates (Sangsritavong *et al.,* 2002). It's better to use cows in their first or second lactation for better results. Additionally, while antioxidants are important for fertility, their specific roles in oocyte and embryo development remain unclear. The oxidative stress markers like MDA (Malondialdehyde), SOD (superoxide dismutase), and GSH-Px (glutathione peroxidase) are potential biomarkers during estrus synchronization and post-embryo transfer in crossbred heifers and cows. This study evaluated MDA as potential biomarker during estrus synchronization, at *in-vitro*-produced embryo transfer, and post-embryo transfer in crossbred heifers and cows along with the conception rates.

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Materials and Methods

Estrus Synchronization of Recipients

A total of 60 experimental HF crossbred cattle were screened gynaecologically by performing rectal and ultrasound examinations on the day of treatment/selection of animals.

Reproductive ultrasonography was carried out in all animals to evaluate the ovarian status, endometrial wall thickness, and echotexture of the endometrium. A real-time B-mode ultrasonography (IMV, France, Model-Exago, Serial number 1909EX01) instrument was used with a linear array of 6-10 MHz multi-frequency transducer. All the selected recipients were divided into two groups (Heifers and Cows), thirty each, and were synchronized using double PGF2α protocol. Inj. Cloprostenol sodium 500 µg was given intramuscularly 11 days apart, with heat detection on day 14 and fixed time ET on day 21 in both heifers and cows.

Estrus Monitoring

The assessment of all cows and heifers involved visual observations aimed at documenting externally noticeable signs of estrus. These signs encompassed cervico-vaginal mucous (CVM) discharge, mounting activities, the inclination to be mounted, vocalizations such as bellowing, and swelling of the vulvar region (tumefaction).

Evaluation and Grading of *In-Vitro* Produced Embryo

The *in-vitro*-produced embryos were evaluated and assessed for quality on Day 7th under a stereo zoom microscope at lower magnification (40x) 3-4 times. The embryos were examined, evaluated, and graded according to the International Embryo Transfer Society (IETS) criteria.

Corpus Luteum (CL) Evaluation of Recipient Cows

Evaluation of the presence of the corpus luteum in the ovary and its dimensions was carried out by USG a day before embryo transfer.

Transfer of *IVP* Embryos in Recipient Cows

After evaluation and grading, *in-vitro* produced fresh embryos were aspirated into a 0.25 mL straw by using a micro tip and tuberculin syringe/embryo loader. The embryos were uniquely loaded in the straw, in 5 columns: (i) embryo holding medium, (ii) air, (iii) embryo holding medium + embryo, (iv) air, and (v) embryo holding medium. The embryos aspirated into 0.25 mL straws were loaded into the embryo transfer gun. The gun was covered by the side-delivery embryo transfer sheath, followed by covering it with a plastic sheath. Freshly *in-vitro* produced embryos were then transferred into the recipient.

Evaluation of Conception after Embryo Transfer

Pregnancy status of recipients was determined using transrectal ultrasonography (7.5 MHz linear transducer) on Day 30 to 40 post-embryo transfer and reconfirmed through a rectal examination on Day 60.

Estimation of Oxidative Stress by Lipid Peroxidation

Blood samples were collected on the day of PG-I and PG-II injection, the day of embryo transfer, and the day of pregnancy diagnosis (approximately the 30th day of embryo transfer) for estimation of lipid peroxidation in the serum using thiobarbituric acid reactive substances (TBARS) and was expressed as Malondialdehyde (MDA) produced in µmol/dL.

Statistical Analysis

Data on oxidative stress, measured by MDA values, was collected for pregnant and non-pregnant individuals. Comparative analyses were performed on corpus luteum characteristics and pregnancy rates in heifers and cows. Statistical methods included ANOVA, the Student's T-test for MDA values, the chi-square test for pregnancy rates, and correlation tests. Data analysis was conducted using Microsoft Excel and SPSS to determine significance, with $p<0.05$ considered statistically significant (Ridgman, 1990).

Results and Discussion

Estrus Response in Synchronized Recipient

In this study, recipients synchronized using the double PGF2α protocol showed an estrus response of 86.66% in heifers (26 out of 30) and 83.33% in cows (25 out of 30) (Table 1). These findings aligned with Gugssa *et al.* (2016), who reported 85.7% estrus expression in crossbred heifers, while Galma (2020) noted a 90% response in Boran and crossbred heifers. In contrast, Sahatpure and Patil (2008) and Malik *et al.* (2018) documented higher estrus rates (100%) using the double PGF2α protocol. Other studies reported varied responses, such as Ahlawat *et al.* (2015) at 83%, Krishana *et al.* (2022) at 81.3%, Gugssa *et al.* (2016) at 87.2%, and Deshmukh *et al.* (2013) at 90% among cows. This difference may stem from the delayed resumption of ovarian cyclicity postpartum in cows and lower estradiol levels due to the metabolic clearance rate in suckling cows (Galma, 2020).

Variations in estrus responses could be attributed to the hormones used, the stage of estrus, seasonal factors, management practices, and the genetic traits of recipients. Additionally, the rate of ovarian corpus luteum (CL) development was low in cows, potentially due to the presence of cystic ovarian conditions and anovulation-like symptoms observed in the recipient cows, as detailed in Table 2.

Table 1: Effect of synchronization protocols on estrus response (%), ovarian CL development rate (%), and conception rate (%)

Attributes	Gr-I (heifers)	Gr-II (cows)
No. of animals synchronized	30	30
Estrus response rate (%)	86.66 % (26)	83.33 % (25)
Ovarian CL development rate (%)	83.33 % (25)	76.66 % (23)
No. of recipients suitable for, and performed ET	24 (80.00 %)	22 (73.33 %)
Recipients conceived after ET (%)	12 (50.00)	7 (31.82)
Overall conception rate (%)	41.30 (19/46)	

Corpus Luteum Characteristics with Pregnancy in Recipient

The CL width and CL luteal area exhibited numerical differences between pregnant and non-pregnant heifers and cows; however, all values were statistically non-significant (p>0.05; Table 2). Pérez-Mora *et al.* (2020) and Say *et al.* (2021) found similar findings in their studies, noting no correlation between CL diameter, CL luteal area, and the status of embryo-transferred pregnant and non-pregnant heifers and cows. Thomson *et al.* (2021) determined suitability of a potential ET recipient by observing an appropriately timed estrus and a detectable CL, regardless of size or quality of CL.

Oxidative Stress Measured by Plasma Lipid Peroxidation

As per the result, the values of MDA levels during each sampling stage were found to be higher in cows compared to heifers, with statistically significant difference in MDA1 (first PG injection) and MDA4 (day of PD) (p≤0.005) only, but not at second PG or day of ET. However, it did not differ statistically between pregnant and non-pregnant heifers as well as cows at any of the stages of estrus synchronization and embryo transfer process (Table 3).

Isobe *et al.* (2024) highlighted that repeat-breeder cows had significantly higher MDA levels than repeat-breeder heifers, and that the pregnancy rate from embryo transfer (ET) in repeat breeder cows decreased during summer due to increased oxidative stress. Yazlik *et al.* (2022) noted a negative correlation between malondialdehyde (MDA) levels and fertilization rates in human IVF studies, which also affected embryo quality. Sheldon and Dobson (2004) reported that non-pregnant cows experience hormonal fluctuations and inflammation, activating immune cells that release reactive oxygen species (ROS) and increase oxidative stress. Bernabucci *et al.* (2005) indicated that cows undergo metabolic changes during lactation, resulting in elevated oxygen consumption and ROS production. Spears and Weiss (2008) pointed out that cows often lack antioxidants like vitamin E, selenium, and zinc during early lactation, impairing their ability to combat oxidative damage. Energy imbalance is significant in cows as it leads to free radical generation and higher MDA levels, primarily due to post-parturient stress, milk production, and metabolic changes.

Pregnancy Rates in HF Crossbred Recipients

The pregnancy rate observed was 50.00% in synchronized recipient heifers and 31.82% in cows, as shown in Table 1. In this study, the overall conception rate was 41.30% (19/46). Many earlier researchers have also noted higher conception rates in recipient heifers compared to cows. Fukaya *et al.* (2024) reported a pregnancy rate of 53.20% in heifers, 45.82% in primiparous cows and 46.14% in multiparous cows. Similarly, Ferraz *et al.* (2016) found rates of 42% in heifers, 37% in primiparous cows, and 36% in multiparous cows. The findings of the present study to some extent aligned with these reports. In contrast, Pérez-Mora *et al.* (2020) reported

Table 2: Comparative characteristics of CL and Pregnancy in HF crossbred recipients (Mean ± SE)

Groups		CL parameters			
		Length (mm)	Width (mm)	Diameter (mm)	Luteal area (mm^2)
Group I	Pregnant heifers	18.57±0.44	16.48±0.82	17.50±0.63	242.37±17.24
	Non-pregnant heifers	18.26±0.73	15.98±0.56	17.12±0.64	231.04±15.88
	P value	0.723^{NS}	0.624^{NS}	-	0.635^{NS}
Group II	Pregnant cows	20.33±1.18	16.53±1.30	18.43±1.24	297.80±32.46
	Non-pregnant cows	19.34±0.71	17.64±0.59	18.49±0.65	284.99±18.71
	P value	0.468^{NS}	0.383^{NS}	-	0.722^{NS}

NS=statistically non-significant

Table 3: MDA values at the time of synchronization, embryo transfer, and pregnancy diagnosis (Mean ± SE)

Group		MDA1 (µmol/dL)	MDA2 (µmol/dL)	MDA3 (µmol/dL)	MDA4 (µmol/dL)
Group I (n=20)		4.59±0.31	5.42±0.46	5.80±0.44	5.93±0.29
Group II (n=20)		6.46±0.50	7.23±0.86	7.94±0.66	7.40±0.39
P value		0.003^{**}	0.74^{NS}	0.011^{NS}	0.005^{**}
Group I (n=20)	Pregnant	4.55±0.51	5.30±0.57	5.64±0.67	5.67±0.40
	Non-pregnant	4.65±0.35	5.57±0.79	6.00±0.582	6.26±0.44
	P value	0.885^{NS}	0.780^{NS}	0.694^{NS}	0.339^{NS}
Group II (n=20)	Pregnant	6.64±0.64	7.57±0.71	8.49±0.26	7.20 ±0.83
	Non-pregnant	6.38±0.68	7.09±1.21	7.71±0.91	7.49±0.460
	P value	0.820^{NS}	0.808^{NS}	0.601^{NS}	0.738^{NS}

**Highly significant, NS=statistically non-significant.

higher pregnancy rates, 86% in heifers and 73.6% in cows, surpassing the results of the current study. Jaskowski *et al*. (2021) conducted embryo transfer (ET) in 300 heifers and reported only 113 confirmed pregnancies, resulting in a pregnancy rate of 37.6%, which is lower than what was observed in this study.

The lower pregnancy rates in cows may be attributed to several factors. Heifers, having never been pregnant before, are less likely to experience complications of parturition, which in turn reduces the likelihood of uterine infections. Additionally, the cows involved in the study were high-yielders, a trait that is often linked to lower conception rates following ET, as higher production levels are generally inversely related to reproductive health. Conception rates can vary based on several factors, including the quality of the embryos, the distance from the laboratory, the site of embryo deposition, the manipulation of the uterine horn during embryo transfer, the expertise of the technician, and the individual characteristics of the animals.

Conclusion

The HF crossbred heifers exhibited better reproductive performance than cows when using the double PGF2α synchronization protocol. Synchronized heifers had a higher estrus response and a better conception rate compared to cows. This suggests that HF crossbred heifers are more suitable recipients for ET. In addition, higher oxidative stress levels, indicated by increased lipid peroxidation, were found in non-pregnant recipients, suggesting a negative correlation between oxidative stress and conception success. This means oxidative stress could be a useful criterion for selecting ET recipients. Notably, no significant differences in conception rates were observed based on corpus luteum diameter or luteal area, indicating these factors may not reliably predict ET success in HF crossbreds.

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References

Ahlawat, A.R., Dongre, V.B., Ghodasara, S.N., Murthy, K.S., & Gajbhiye, P.U. (2015). Single and double dose of PGF2α for estrus induction and conception rate in Gir cows. *Intas Polivet*, *16*(1), 54-56.

Bernabucci, U., Ronchi, B., Lacetera, N., & Nardone, A. (2005). Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *Journal of Dairy Science, 88*(6), 2017-2026.

Deshmukh, S.G., Pawshe, C.H., Sahatpure, S.K., & Bankar, P.S. (2013). Synchronization of estrus with double dose of PGF2α in cows. *Indian Journal of Veterinary Sciences and Biotechnology*, *9*(2), 59-61.

Ferraz, P.A., Burnley, C., Karanja, J., Viera-Neto, A., Santos, J.E.P., Chebel, R.C., & Galvão K. N. (2016). Factors affecting the success of a large embryo transfer program in Holstein cattle in a commercial herd in the Southeast Region of the United States. *Theriogenology, 86* (7), 1834-1841.

Fukaya, S., Yamazaki, T., Abe, H., Nakagawa, S., Baba, T., Bai, H., & Kawahara, M. (2024). Characterization of conception rate after embryo transfer in comparison with that after artificial insemination in dairy cattle. *Journal of Dairy Science, 107*(11), 9516-9526.

Galma, B. (2020). Estrus response of Boran and Boran-Holstein crossed cattle to PGF2α and pregnancy rates to sexed and conventional semen. *MSc Thesis*. Addis Ababa University, Holeta, Ethiopia.

Gugssa, T., Ashebir, G., & Yayneshet, T. (2016). Effects of fixed-time AI and AI at detected estrus on conception rate in smallholder zebu and crossbred heifers and cows subjected to double PGF2α administration. *Tropical Animal Health and Production, 48*, 1209-1213.

Isobe Takashi, Yuki Kajihara, & Yuko Ikebata (2024). Influences of oxidative stress and energy balance on pregnancy rates after the transfer of embryos to repeat-breeder Japanese Black cattle. *Animal Science Journal, 95*(1), e13947.

Jaskowski, Bogdan M., Heinrich Bostedt, Michael Gehrke, & Jan M. Jaśkowski (2021). Ultrasound characteristics of the cavity corpus luteum after oestrus synchronization in heifers in relation to the results of embryo transfer. *Animals, 11*(6), 1706.

Krishana, N.V., Reddy, Y.N., Tagore, K.V.V., & Veera, K. (2022). Fertility response in postpartum anoestrous cows using Ovsynch and double PG protocols. *The Pharma Innovation Journal*, *11*(12), 2809-2813

Malik, A., Heppy, D.M., & Widaningsih, N. (2018). Comparing response of estrus synchronisation in the heifers and cows on estrus initial, duration of estrus and pregnancy rate Bali cattle. *Advances in Animal and Veterinary Sciences, 6*(6), 242-245.

Pérez-Mora, A., Segura-Correa, J.C., & Peralta-Torres, J.A. (2020). Factors associated with pregnancy rate in fixed-time embryo transfer in cattle under humid-tropical conditions of México. *Animal Reproduction*, *17*(2), e20200007.

Ridgman, W.J. (1990). *Statistical Methods*, 8th edn, by GW Snedecor & WG Cochran. Iowa State University Press, Ames, Iowa, USA, pp. 503.

Sahatpure, S.K., & Patil, M.S. (2008). Synchronization of oestrus with prostaglandin F2 alpha analogue in non-descript cow. *Veterinary World, 1*(7), 203-204.

Sangsritavong, S., Combs, D.K., Sartori, R., Armentano, L.E., & Wiltbank, M.C. (2002). High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. *Journal of Dairy Science, 85*(11), 2831-2842.

Say, E., Özmen, M.F., & Sağırkaya, H. (2021). The influence of corpus luteum size on the conception in embryo transfer recipient cows. *Livestock Studies, 61*(2), 77-81.

Sheldon, I.M., & Dobson, H. (2004). Postpartum uterine health in cattle. *Animal Reproduction Science*, *82-83,* 295-306.

Spears, J.W., & Weiss, W.P. (2008). Role of antioxidants and trace elements in health and immunity of transition dairy cows. *The Veterinary Journal, 176*(1), 70-76.

Thomson, S.P., Holmes, R.J., Landes, P.T., & Allworth, M.B. (2021). Assessment and selection of the recipient cows' corpus luteum at the time of embryo transfer, and its influence on conception rate. *Australian Veterinary Journal*, *99*(7), 288-292.

Yazlık, M.O., Kurt, S., Terzi, O.S., Kaya, U., & Olgac, K.T. (2022). Comparison of blood metabolites and GSH-Px, SOD, MDA levels as a predictor of pregnancy in primiparous cows after the presynch-ovsynch protocol. *Large Animal Review, 28*(5), 227-233.

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