Optimizing Synchronization Protocol in Bangladeshi Crossbred Dairy Recipients

Md. Anisur Rahman; Md. Shane Khoda
Department of Livestock Services Bangladesh, Dhaka-1215

Mohammad Musharraf Uddin Bhuiyan*; Nasrin Sultana Juyena
Jayonta Bhattacharjee; Shankar Biswas

Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202

Abstract

Embryo transfer (ET) is an important reproductive biotechnology used to accelerate genetic improvement and enhance reproductive efficiency in cattle. However, the success of embryo transfer programs is influenced by several factors related to embryo characteristics, recipient physiology, management practices, and environmental conditions. The present study evaluated the effects of various recipient- and embryo-related factors on pregnancy rate following embryo transfer in crossbred dairy cattle.

A total of 131 crossbred dairy heifers and cows were used as recipients under field conditions during winter and summer seasons. The animals were aged between 17 and 28 months, with body condition scores ranging from 2.5 to 3.5 and body weights between 310 and 430 kg. Recipient animals were synchronized using two hormonal protocols (CIDR and GnRH + $PGF_{2}\alpha$). Pregnancy diagnosis was performed approximately 60 days after embryo transfer using transrectal ultrasonography.

The overall pregnancy rate observed in this study was 24.4% (32/131). Recipients synchronized using the CIDR protocol showed significantly higher pregnancy rates (37.0%) compared with the GnRH + $PGF_{2}\alpha$ protocol (17.6%) ($P < 0.05$).These findings indicate that synchronization protocol play important roles in embryo transfer success under field conditions.

Keywords: Embryo transfer; Embryo survival; Pregnancy rate; Reproductive performance; Synchronization protocol.

Introduction

The livestock sector plays a critical role in global food security, economic development, and rural livelihoods by supplying milk, meat, and other animal-derived products essential for human nutrition. Rapid population growth and increasing demand for animal protein have intensified the need to improve livestock productivity and reproductive efficiency worldwide (Thornton, 2010). Identifying animals with high fertility potential and implementing effective reproductive management strategies are therefore essential for maintaining herd performance (Santolaria et al., 2006). Reduced fertility in high-producing dairy cows has been linked to metabolic stress, endocrine imbalance(Lucy, 2001). To overcome these limitations and accelerate genetic progress, several assisted reproductive technologies (ARTs) such as artificial insemination (AI), embryo transfer (ET), in vitro fertilization (IVF) significantly improved reproductive performance and genetic gain in cattle populations (Pontes et al., 2009; Fleming et al., 2018; Obšteter et al., 2021). Among these technologies, embryo transfer enables rapid multiplication of superior genetics by transferring embryos from elite donor cows into synchronized recipient females (Hasler, 2004).Therefore, understanding the biological and management factors influencing embryo transfer success is essential for improving pregnancy rates and maximizing the benefits of reproductive biotechnologies in cattle production systems.

Materials and Methods

Experimental Animals

A total of 131 crossbred dairy heifers and cows were used as recipients with ages

DOI: https://doi.org/10.5281/zenodo.19316034

ranging from 17 to 28 months. Body weight of the animals ranged from 310 kg to 430 kg, while the body condition score (BCS) varied between 2.5 and 3.5. Only animals showing normal estrous cycles and good reproductive health were included in the experiment. Animals suffering from reproductive disorders, systemic diseases, or poor body condition were excluded from the study.

Body Condition Score (BCS) Evaluation

Only animals with BCS between 2.5 and 3.5 at a five-point scoring system were selected as embryo recipients.

Body Weight Measurement

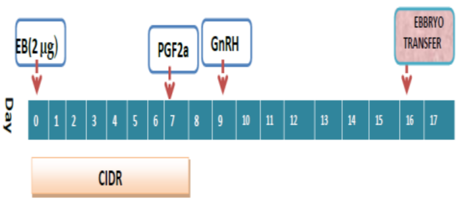
The weight of the recipient animals ranged between 310 kg and 430 kg. Body weight was included as one of the variables in this study to evaluate its potential effect on pregnancy rate following embryo transfer.

Age of Recipient Animals

The animals included in the study were between 17 and 28 months of age, representing the typical reproductive age of crossbred dairy heifers and cows. Age groups were analyzed to determine whether age influenced pregnancy success following ET.

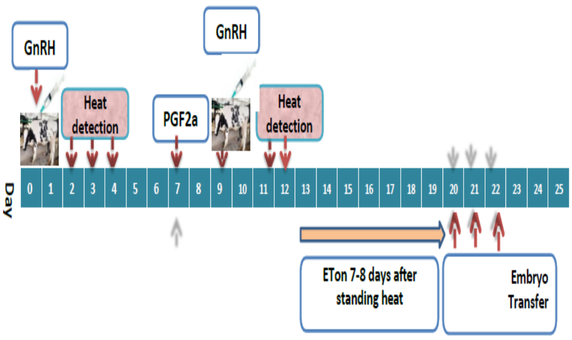
Estrus Synchronization Protocols

To ensure proper synchronization between embryo development and uterine receptivity, recipient animals were synchronized using two different hormonal synchronization protocols.



Synchronization & ET protocol (CIDR)

A. CIDR Protocol-CIDR® (Controlled Internal Drug Release) is an intravaginal progesterone-releasing device manufactured by Zoetis. Each device contains 1.38 g of progesterone incorporated into a T-shaped silicone elastomer insert designed for controlled hormone delivery. While the device remains in situ, CIDR® maintains elevated systemic progesterone concentrations, effectively suppressing estrus and ovulation. Upon removal, circulating progesterone levels decline rapidly, permitting synchronized onset of estrus and ovulation (Wiltbank et al., 2015; Menchaca et al., 2020). The CIDR device was inserted intravaginally into the recipient animals at D0. The use of CIDR in recipient cattle allows tighter regulation of ovarian activity prior to superstimulation and improves the uniformity of follicular responses among animals, thereby enhancing the predictability of superovulatory outcomes (Martins et al., 2017; Menchaca et al., 2020; Sá Filho et al., 2021). Concurrently, a Estradiol benzoite 2 µg was administered intramuscularly. The device was left in place for 7 days to maintain progesterone levels and control the estrous cycle. At D7 the CIDR device was removed , prostaglandin injection was administered to induce luteolysis and synchronize estrus. Fertagyl® (GnRH, MSD) 2 ml was injected intramascularly at day 9. Embryo was transfered at day 16.



Synchronization & ET protocol to the recipients cattle.

B. GnRH + $PGF_{2}\alpha$ Protocol-In this protocol, Fertagyl® (GnRH) 2ml was injected intramascularly at first day. Followed by Estrumate® ($PGF_{2}\alpha$) 2ml injection at day 7. Second GnRH was administered at day 9. Finally embryo was transfered at day 20, day 21 and day 22.

Fertagyl® is routinely incorporated into **e**strous and ovulation synchronization

DOI: https://doi.org/10.5281/zenodo.19316034

protocols, and multiple ovulation and embryo transfer (MOET) schemes, to optimize reproductive timing and efficiency in cattle (Pursley et al., 1995; Bó et al., 2016; Sá Filho et al., *2021).* Estrumate® ($PGF_2\alpha$) was used to induce luteolysis (Bó et al., 2019; Baruselli et al., 2020).

Embryo Collection and Quality Assessment

Embryos used in this study were obtained from donor cows through standard embryo collection procedures. The collected embryos were evaluated under a stereomicroscope (Olympus SZX2–TR30, Tokyo, Japan) for quality and developmental stage.

Embryo quality was classified according to the International Embryo Transfer Society (IETS) standards. Only embryos of Grade 1(excellent quality) and Grade 2 (good quality) were selected for transfer in order to maximize the probability of successful pregnancy establishment.

Embryo Transfer Procedure

Embryo transfer was performed using the non-surgical transcervical method, which is widely used in cattle embryo transfer programs. The embryo was loaded into a sterile embryo transfer straw and placed into an embryo transfer gun. The gun was then carefully inserted through the cervix into the uterus with the help of rectal manipulation. The embryo was deposited into the uterine horn ipsilateral to the corpus luteum, which provides the most favorable environment for embryo implantation.

Pregnancy Diagnosis

Pregnancy diagnosis was performed approximately 60 days after embryo transfer using transrectal ultrasonography. Animals that were confirmed pregnant during ultrasound examination were recorded as successful embryo transfer cases. Pregnancy rate was calculated using the following formula:

Pregnancy Rate(%)=Number of pregnant recip ientsTotal number of recipients/Total number of recipients ×100 .

Statistical Analysis

All collected data were compiled and analyzed using appropriate statistical methods. Pregnancy rates among different groups were compared using the Chi-square (χ^2) test. Statistical significance was determined at $P < 0.05$. Descriptive statistics were used to summarize pregnancy rates across different experimental variables.

Results & Discussion

The application of Embryo transfer(ET) allows the rapid dissemination of superior genetics and contributes significantly to improving productivity in both dairy and beef herds (Mueller and Van Eenennaam, 2022). Despite these advantages, pregnancy success following embryo transfer varies considerably due to the influence of multiple factors including embryo characteristics, recipient physiological status, synchronization protocols, and environmental conditions (Hasler, 2004; Hansen, 2020).

In the present study, the overall pregnancy rate following embryo transfer was 24.4%**,** which is within the range reported in many field studies under commercial conditions. Previous research has indicated that pregnancy rates after ET can vary widely depending on management practices, embryo quality, and recipient selection (Roper et al., 2018; Pérez-Mora et al., 2020). Therefore, evaluating the influence of different biological and management factors on pregnancy success remains essential for improving the efficiency of ET programs.

Effects of synchronization protocol

The synchronization protocol significantly affected embryo transfer success. Recipients synchronized using the CIDR protocol achieved a pregnancy rate of 37% (17/46)**,** whereas GnRH + $PGF_2\alpha$ protocol showed a pregnancy rate of 17.60% (15/85)**.** Statistical analysis revealed a significant difference between the two protocols ($\chi^2 = 6.07$, $P = 0.014$)**.** Suggested that CIDR, effectively regulate the estrous cycle and create a favorable hormonal environment for embryo implantation (Pérez-Mora et al., 2020).

Conclusion

The present study was conducted to evaluate the effect of synchronization protocol on pregnancy rate following embryo transfer in crossbred dairy cattle. A total of 131 crossbred dairy heifers and cows were used as recipients. The overall pregnancy rate obtained in this study was 24.4%, which is comparable to pregnancy rates reported in many field embryo transfer programs. The synchronization

DOI: https://doi.org/10.5281/zenodo.19316034

protocol had a significant effect on pregnancy rate, as recipients synchronized using the CIDR protocol showed higher pregnancy rates compared with those synchronized using the GnRH + $PGF_2\alpha$ protocol.

Overall, the findings suggest that synchronization protocol play important roles in determining embryo transfer success in crossbred dairy cattle under field conditions.

Abbreviations

ET , embryo transfer; BCS, body condition score; CIDR, controlled internal drug release; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; IETS, International Embryo Technology Society; MOET, multiple ovulation and embryo transfer; $PGF_2\alpha$, prostaglandin $F_2\alpha$; ARTs, assisted reproductive technologies; AI, artificial insemination; IVF, in vitro fertilization;

Acknowledgement

The authors are thankful to Livestock and Dairy Development Project (LDDP), Department of Livestock Services Bangladesh, for funding this research.

Conflicts Of Interest

The authors have declared no conflicts of interest.

REFERENCES

Baruselli PS, Ferreira RM, Sá Filho MF, *et al.* 2018. Control of follicular development for reproductive management of cattle. *Animal* 12(2):403–413.

Baruselli PS, Ferreira RM, Sá Filho MF. 2020. Timed embryo transfer programs in cattle. *Theriogenology* 150:237–245

Bó GA, Baruselli PS, Mapletoft RJ. 2016. Alternative approaches to synchronization of ovulation in cattle. *Theriogenology* 86(1):1–9.

Bó GA, Baruselli PS, Mapletoft RJ. 2019. Superovulation and embryo transfer in cattle: Current perspectives. *Animal Reproduction* 16(3):423–440.

Fleming A, Abdalla EA, Maltecca C, Baes CF, 2018. Reproductive and genomic technologies to optimize breeding strategies for genetic progress in dairy cattle. *Archives Animal Breeding* 61: 43–57. https://doi.org/10.5194/aab-61-43-2018

Hansen PJ, 2020. The incompletely fulfilled promise of embryo transfer in cattle—why aren't pregnancy rates greater and what can we do about it? *Journal of Animal Science* 98(11): skaa288. https://doi.org/10.1093/jas/skaa288

Hasler JF, 2004. Factors influencing the success of embryo transfer in cattle. Proceedings of the World Buiatrics Congress, Québec, Canada.

Lucy MC, 2001. Reproductive loss in high-producing dairy cattle: Where will it end? *Journal of Dairy Science* 84: 1277–1293,https://doi.org/10.3168/jds.S0022-0302(01)70158-0

Martins CM, *et al.* 2017. Use of progesterone devices to control ovarian function in cattle. *Animal Reproduction* 14(3):477–486.

Menchaca A, *et al.* 2020. Advances in progesterone-based synchronization protocols in cattle. *Animal Reproduction* 17(3):e20200045.

Mueller ML, Van Eenennaam AL, 2022. Synergistic power of genomic selection, assisted reproductive technologies, and gene editing to drive genetic improvement of cattle. *CABI Agriculture and Bioscience* 3:13.https://doi.org/10.1186/s43170-022-00080-z

Obšteter J, Jenko J, Gorjanc G, 2021. Genomic selection for any dairy breeding program via optimized investment in phenotyping and genotyping. *Frontiers in Genetics* 12: 637017. https://doi.org/10.3389/fgene.2021.637017

Pérez-Mora A, Segura-Correa JC, Peralta-Torres JA, 2020. Factors associated with pregnancy rate in fixed-time embryo transfer in cattle under humid tropical conditions of Mexico. *Animal Reproduction* 17(2): https://doi.org/10.1590/1984-3143-AR2020-0007

Pontes JHF, Nonato-Junior I, Sanches BV, Ereno-Junior JC, Uvo S, Barreiros TRR, Oliveira JA, Hasler JF, Seneda MM, 2009. Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore donor cows. *Theriogenology* 71: 690–697. https://doi.org/10.1016/j.theriogenology.2008.09.031

Pursley JR, *et al.* 1995. Synchronization of ovulation in dairy cows using GnRH and $PGF_2\alpha$. *Journal of Dairy Science* 78(8):1734–1742

Roper DA, Schrick FN, Edwards JL, Hopkins FM, Prado TM, Wilkerson JB, Saxton AM, Young CD, Smith WB, 2018. Factors in cattle affecting embryo transfer pregnancies in recipient animals. *Animal Reproduction*

DOI: https://doi.org/10.5281/zenodo.19316034

*Science*196:79–85. https://doi.org/10.1016/j.anireprosci.2018.11.001

Sá Filho MF, Meneghetti M, Peres RFG, *et al.* 2021. Hormonal strategies to optimize reproductive efficiency in cattle. *Animal Reproduction* 18(3):e20210045.

Santolaria P, López-Gatius F, García-Ispierto I, Yániz J, Nogareda C, López-Bejar M, 2006. Screening for high fertility in high-producing dairy cows. *Theriogenology* 65: 1678–1689. https://doi.org/10.1016/j.theriogenology.2005.09.017

Thornton PK, 2010. Livestock production: Recent trends, future prospects.*Philosophical Transactions of the Royal Society B* 365: 2853-2867, https://doi.org/10.1098/rstb.2010.0134

Wiltbank MC, Souza AH, Carvalho PD, *et al.* 2015. Physiological and practical effects of progesterone on reproduction in dairy cattle. *Biology of Reproduction* 92(3):1–12.

DOI: https://doi.org/10.5281/zenodo.19316034