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Optimization of Reproductive Technologies in Water Buffalo: A Review of Current Practices

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Abstract This study provides a comprehensive overview of the biological and physiological basis of water buffalo reproduction, including reproductive anatomy, seasonal breeding, and hormonal regulation. It critically examines current reproductive technologies such as artificial insemination, estrus synchronization, in vitro fertilization, embryo transfer, and sex-sorted semen, while also exploring emerging technologies like genome editing, precision breeding, and reproductive biomarkers. A detailed case study highlights the application of embryo transfer technology for genetic improvement, emphasizing practical outcomes and lessons learned. Key factors influencing the adoption of these technologies, including socio-economic, infrastructural, and regulatory considerations, are discussed, alongside challenges such as biological constraints and technological barriers. Finally, the study proposes strategies for improving reproductive efficiency through training, infrastructure development, and policy support. This study underscores the importance of optimizing reproductive technologies to achieve sustainable water buffalo production and suggests future research directions to further enhance these efforts.
Keywords Water buffalo; Reproductive technologies; Artificial insemination; Genome editing; Embryo transfer

1 Introduction

Water buffaloes play a crucial role in global agriculture, particularly in regions such as South Asia and the Mediterranean, where they are a primary source of milk, meat, and draught power. They are well-adapted to harsh environments and can thrive on low-quality forage, making them invaluable in ecologically disadvantaged agricultural systems. In countries like Nepal, buffaloes contribute significantly to milk and meat production, underscoring their economic importance (Devkota et al., 2022). However, despite their adaptability, the reproductive efficiency of water buffaloes is often compromised due to various biological and management challenges (Bm, 2019).

Reproductive efficiency is a critical factor in water buffalo production, as it directly impacts the economic viability of buffalo farming. Challenges such as delayed puberty, silent estrus, and prolonged postpartum intervals lead to suboptimal fertility rates, causing significant economic losses for farmers (Singhal et al., 2021). The reproductive performance of buffaloes is further affected by environmental factors like climatic stress and poor nutrition, which can delay puberty and extend postpartum anoestrus (Bm, 2019). Effective reproductive management strategies, including assisted reproductive technologies (ART) and hormonal protocols, are essential to enhance fertility and improve overall herd productivity (Warriach et al., 2015; Coman et al., 2024).

This study attempts to explore the significance of reproductive efficiency in water buffalo production, discuss the challenges faced in optimizing reproductive technologies, and provide an overview of the effectiveness of various reproductive technologies and management strategies. By synthesizing recent developments and identifying areas for improvement, this review seeks to offer insights that can enhance the reproductive performance of water buffaloes and support the sustainability of buffalo farming globally.

2 Biological and Physiological Basis of Reproduction in Water Buffalo

2.1 Reproductive anatomy and physiology

Water buffaloes (*Bubalus bubalis*) are multipurpose livestock known for their milk, meat, and draught power. However, they exhibit low fecundity characterized by delayed puberty, less pronounced estrus signs, and long postpartum anestrus periods (Qg et al., 2020). The reproductive anatomy of buffaloes is similar to that of other bovines, but their physiological characteristics, such as ovarian cyclicity and estrus expression, are often influenced by environmental and management factors (Bm, 2019). The ovarian function during the estrous cycle is crucial for the application of assisted reproductive technologies (ART), which aim to synchronize follicular development and ovulation (Baruselli et al., 2018).

2.2 Seasonal breeding and its implications

Buffaloes are seasonal breeders, with reproductive activity peaking during periods of decreasing day length, typically from late summer to early autumn. This seasonality is influenced by both exogenous factors like photoperiod and climate, and endogenous factors such as hormonal regulation (D'Occhio et al., 2020). Seasonal breeding patterns can lead to challenges in maintaining consistent milk production and reproductive efficiency throughout the year. In regions like Nepal, buffaloes show active breeding from July to December, with low reproductive activity from April to June and January to March (Devkota et al., 2022). This seasonality necessitates management strategies to mitigate its impact, such as hormonal treatments to induce estrus and ovulation during the non-breeding season.

2.3 Hormonal regulation of reproduction

The hormonal regulation of reproduction in buffaloes involves complex interactions between endogenous hormones and external factors. Melatonin, produced by the pineal gland, plays a significant role in regulating reproductive seasonality by influencing gonadotropin secretion and gonadal function (D'Occhio et al., 2020). The presence of melatonin receptors, such as MT1 and MT2, and their genetic polymorphisms have been associated with variations in reproductive activity (Gunwant et al., 2018). Hormonal protocols, including melatonin implantation and estrus synchronization, have been developed to enhance reproductive performance, particularly during the non-breeding season. These protocols aim to control follicular and luteal functions, allowing for timed artificial insemination and improved conception rates (Baruselli et al., 2018).

In summary, the reproductive efficiency of water buffaloes is intricately linked to their biological and physiological characteristics, seasonal breeding patterns, and hormonal regulation. Understanding these factors is essential for optimizing reproductive technologies and improving productivity in buffalo herds.

3 Current Reproductive Technologies in Water Buffalo

3.1 Artificial insemination (AI)

Artificial insemination (AI) is a pivotal technology for genetic improvement and controlling the breeding period in water buffalo. However, AI in buffalo is more challenging than in cattle due to irregular estrous cycles, subdued estrous behavior, and reproductive seasonality, which can lead to higher rates of anestrus and embryonic mortality outside the breeding season. Recent advancements in AI protocols focus on controlling the luteal phase with prostaglandins and progesterone, and managing follicle development and ovulation using hormones like GnRH, hCG, eCG, and estradiol. These protocols facilitate fixed-timed AI, eliminating the need for estrous detection and improving reproductive efficiency (Neglia et al., 2020; Coman et al., 2024).

3.2 Estrus synchronization techniques

Estrus synchronization (ES) is often used alongside AI to enhance reproductive efficiency in buffalo. The difficulty in detecting estrus in buffaloes can lead to suboptimal timing of AI, reducing reproductive success. Various ES protocols, such as those involving prostaglandin and GnRH, have been developed to improve pregnancy rates. Studies have shown that administering ovulatory hormones like GnRH or hCG at the time of AI can significantly enhance pregnancy rates, making these protocols both effective and cost-efficient for field application (Atabay et al., 2020; Ahmad and Arshad, 2020; Du et al., 2021).

3.3 In vitro fertilization (IVF) and embryo transfer (ET)

In vitro fertilization (IVF) and embryo transfer (ET) are advanced reproductive technologies that allow for the genetic improvement of buffalo herds. These techniques involve the collection of oocytes, fertilization in vitro, and the transfer of embryos to recipient females. The combination of IVF with sexed semen can further enhance genetic gains by allowing for the selection of offspring sex. Although these technologies offer significant potential, their application in buffalo is still limited by factors such as the efficiency of oocyte retrieval and embryo development (Pellegrino et al., 2016; Baruselli et al., 2018).

3.4 Use of sex-sorted semen

The use of sex-sorted semen in AI and IVF is a promising approach to control the sex ratio of offspring in buffalo breeding. This technology involves sorting sperm to favor either X- or Y-chromosome-bearing sperm, allowing for the production of predominantly female or male calves. Studies have demonstrated the feasibility of using sexed semen in buffalo, achieving high pregnancy rates and sex accuracy. However, the success of this technology can vary based on factors such as season, technician skill, and the genetic background of the buffalo (Lu et al., 2015; Chebel and Cunha, 2020).

In summary, the optimization of reproductive technologies in water buffalo involves a combination of AI, estrus synchronization, IVF, and the use of sex-sorted semen. These technologies, when effectively applied, can significantly enhance reproductive efficiency and genetic improvement in buffalo herds. However, challenges such as estrous detection, seasonality, and the efficiency of advanced reproductive techniques need to be addressed to fully realize their potential.

4 Emerging Technologies in Water Buffalo Reproduction

4.1 Genome editing and its potential in reproductive enhancement

Genome editing, particularly using CRISPR/Cas9 technology, has shown significant promise in enhancing reproductive capabilities in water buffalo. This technology allows for precise genetic modifications, such as the integration of specific genes into the Y chromosome, which can be used for sex control in pre-implantation embryos. The successful generation of transgenic cloned buffalo embryos demonstrates the potential of genome editing to improve reproductive outcomes by enabling the selection of desired traits at the embryonic stage (Figure 1) (Zhao et al., 2020; Singh et al., 2020).

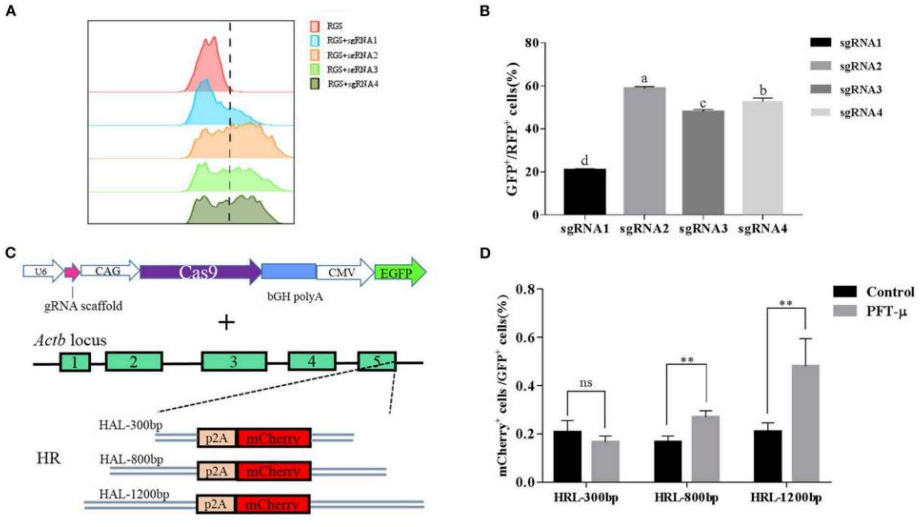

Figure 1 Evaluation of sgRNA efficiency and knock-in strategies in buffalo fetal fibroblast cells (Adapted from Zhao et al., 2020)

Image caption: (A) The RGS reporter system was used to determine the efficiencies of sgRNAs; the cleavage activities were evaluated by FACS and are presented as the ratio of GFP+/RFP+ cells to all transfected cells; (B) Histograms show the relative cleavage activity of different sgRNAs; (C) Schematic overview of the strategy used to target the Actb locus in buffalo fetal fibroblast cells; (D) Knock-in efficiencies were evaluated by FACS and are presented as the ratio of mCherry+ cells to all transfected cells. **P < 0.01, ns, no significant difference (Adopted from Zhao et al., 2020)

4.2 Precision breeding using genomic selection

Precision breeding through genomic selection is an emerging approach that leverages detailed genomic data to enhance breeding programs. This method allows for the selection of genetically superior animals, thereby accelerating genetic gain. The integration of genomic selection with assisted reproductive technologies, such as in vitro embryo production, can significantly improve the efficiency of breeding programs by ensuring that only the best genetic material is propagated (Singh et al., 2020; Currin et al., 2021).

4.3 Advances in reproductive biomarkers for fertility prediction

Recent advances in the identification of reproductive biomarkers have improved the ability to predict fertility in water buffalo. Proteomic profiling of spermatozoa has identified specific proteins associated with high fertility, which can be used as biomarkers to assess the fertilizing potential of semen before artificial insemination. This approach helps mitigate economic losses due to failed pregnancies by ensuring that only semen with high fertilizing potential is used (Karanwal et al., 2023; Andrei et al., 2024).

In summary, the integration of genome editing, precision breeding, and the use of reproductive biomarkers represents a significant advancement in optimizing reproductive technologies in water buffalo. These emerging technologies hold the potential to enhance genetic gain, improve fertility rates, and increase the overall efficiency of breeding programs (Warriach et al., 2015; Zicarelli, 2019).

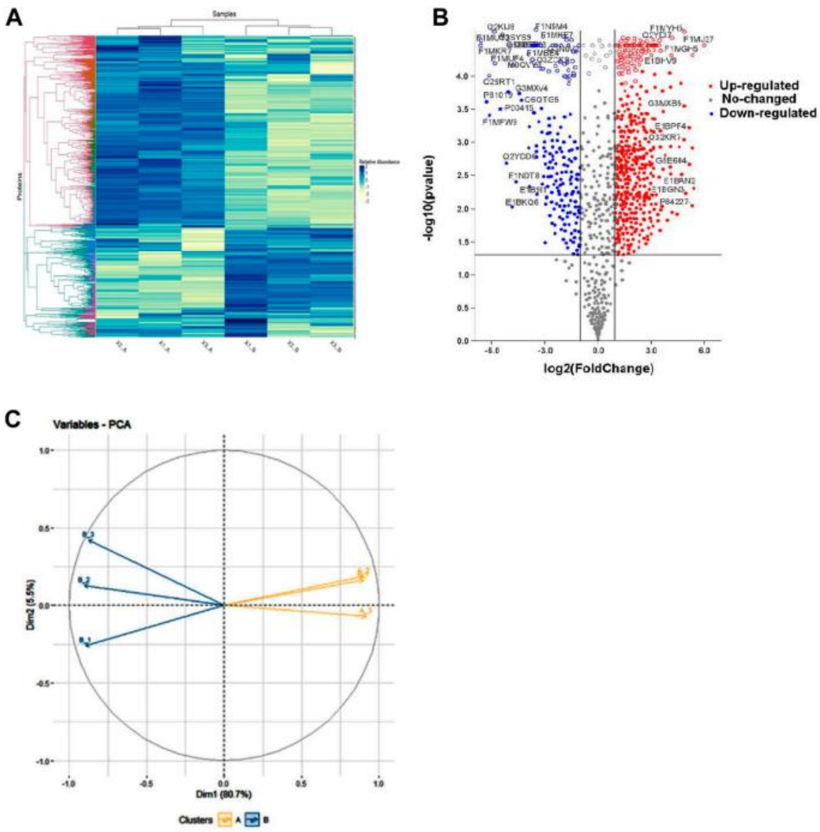

Figure 2 Representation of differentially abundant proteins (DAPs) in high and low fertile spermatozoa (Adopted from Karanwal et al., 2023)

Image caption: (A) Heat map showing differentially abundant proteins (DAPs) among the replicates of HF and LF groups. Intense blue colour represents high abundance of proteins while white colour represents low abundance of proteins. (B) Volcano plot of all DAPs identified in the proteomics data determine by log fold change vs. –log10 p-value. Red points: DAPs that were significantly high abundant in high fertile bull (fold change >2; $p < 0.05$). Blue points: DAPs that were significantly low abundant in high fertile bull (fold change <0.5; $p < 0.05$). Grey points: DAPs that showed neutral abundance. Volcano plot showing the significantly abundant proteins determine by log fold change (log fold) vs. -log10 p-value. (C) PCA plot representing the level of variances between the replicates of HF and LF samples (Adopted from Karanwal et al., 2023)

5 Case Study: Application of Embryo Transfer Technology in Improving Genetic Traits

5.1 Background and objectives of the case study

The primary objective of this case study is to explore the application of embryo transfer technology in water buffaloes to enhance genetic traits, particularly for increased milk and meat production. The Philippine Carabao Center has been at the forefront of this initiative, aiming to produce genetically superior water buffaloes through advanced reproductive biotechnologies. This involves the use of in vitro embryo production and transfer techniques to improve the genetic pool of water buffaloes in the Philippines (Duran et al., 2017).

5.2 Methods employed in the field

The methods employed include the collection of ovaries from slaughtered river buffaloes, followed by in vitro maturation and fertilization of oocytes. The embryos are then cryopreserved using vitrification and transported for non-surgical transfer into recipient buffaloes. The study also explored the use of swamp buffaloes as surrogate mothers and the potential for twinning by transferring embryos in pairs (Duran et al., 2017). Additionally, ovum pick-up (OPU) combined with in vitro embryo production (IVEP) has been utilized to exploit the genetics of high-yield buffaloes, addressing challenges such as low in vivo embryo recovery (Baruselli et al., 2018; Baruselli et al., 2020).

5.3 Results and outcomes

The results from the studies conducted demonstrated varying success rates. In one study, a 16.36% pregnancy rate and a 10.91% calving rate were achieved with river buffalo recipients. When swamp buffaloes were used as surrogates, a 12.5% pregnancy rate and a 10% calving rate were observed. The study on twinning showed a 23.1% calving rate with a 3.8% twinning rate when embryos were transferred in pairs (Duran et al., 2017). These outcomes highlight the potential of embryo transfer technology in improving genetic traits in water buffaloes, although efficiency varies based on the methods and conditions applied.

5.4 Lessons learned and recommendations

The case study underscores the importance of optimizing embryo transfer techniques to improve genetic traits in water buffaloes. Key lessons include the need for precise synchronization of follicular development and ovulation, as well as the importance of selecting appropriate surrogate mothers to enhance pregnancy and calving rates (Baruselli et al., 2018; Baruselli et al., 2020). It is recommended to further refine in vitro embryo production techniques and explore the use of genomic-assisted selection to accelerate genetic gains. Additionally, addressing factors such as environmental conditions, donor selection, and the use of advanced technologies like sex-sorted sperm cells could further improve the efficiency and outcomes of embryo transfer programs in water buffaloes (Gaddis et al., 2017; Currin et al., 2021).

6 Factors Influencing Adoption of Reproductive Technologies

6.1 Socio-economic considerations

The adoption of reproductive technologies in water buffalo is significantly influenced by socio-economic factors. Buffaloes are crucial for the economic sustenance of many farmers, particularly in developing countries, due to their contributions to milk, meat, and draught power (Bm, 2019; Nanda et al., 2019). However, the economic impact of reproductive failures can be substantial, affecting the profitability of buffalo farming systems. For instance, longer calving intervals negatively impact profitability, emphasizing the need for efficient reproductive management (Nava-Trujillo et al., 2020). The cost of implementing advanced reproductive technologies can be prohibitive for smallholder farmers, who often lack the financial resources to invest in such innovations. Therefore, socio-economic constraints play a critical role in the decision-making process regarding the adoption of these technologies.

6.2 Infrastructure and technical expertise requirements

The successful implementation of reproductive technologies in buffaloes requires adequate infrastructure and technical expertise. Many buffaloes are managed in areas with restricted access, which can limit the application of procedures like ovum pick-up and in vitro embryo production (Konrad et al., 2017). The availability of skilled personnel to perform these techniques is also a limiting factor, as the quality of support services such as artificial insemination and disease control directly influences fertility outcomes (Bm, 2019; Zicarelli, 2019). Furthermore, the adaptation of technologies from cattle to buffaloes requires a deep understanding of buffalo biology and reproductive physiology, which may not be readily available in all regions. Thus, the lack of infrastructure and technical expertise can hinder the widespread adoption of reproductive technologies in buffalo farming.

6.3 Policy and regulatory environment

The policy and regulatory environment can either facilitate or impede the adoption of reproductive technologies in buffaloes. In some regions, there may be a lack of supportive policies that encourage the use of advanced reproductive techniques. For example, the absence of incentives for genetic improvement programs or subsidies for technology adoption can slow down progress (Nanda et al., 2019). Additionally, regulatory barriers related to the use of certain hormones or genetic materials can restrict the application of these technologies (D'Occhio et al., 2020). Effective policy frameworks that promote research, development, and dissemination of reproductive technologies are essential to enhance their adoption and improve reproductive efficiency in buffaloes.

In summary, the adoption of reproductive technologies in water buffalo is influenced by socio-economic factors, infrastructure and technical expertise requirements, and the policy and regulatory environment. Addressing these challenges through targeted interventions and supportive policies can enhance the reproductive performance and economic viability of buffalo farming systems.

7 Challenges and Limitations in Optimizing Reproductive Technologies

7.1 Biological constraints specific to water buffalo

Water buffaloes exhibit unique biological challenges that impact the optimization of reproductive technologies. One significant issue is the difficulty in estrus detection, which leads to low reproductive efficiency and complicates the timing of artificial insemination (AI) (Atabay et al., 2020; Neglia et al., 2020). Additionally, water buffaloes have variable estrous cycles and reduced estrous behavior, which are further complicated by reproductive seasonality, resulting in higher incidences of anestrus and increased embryonic mortality during nonbreeding seasons. The low antral follicle populations and high levels of follicular atresia also contribute to the inefficiency of in vivo embryo production (Baruselli et al., 2020).

7.2 Technological barriers and gaps

Technological barriers in optimizing reproductive technologies in water buffalo include the limited efficiency of in vitro embryo production (IVEP) and the challenges associated with ovum pick-up (OPU) (Baruselli et al., 2018; Baruselli et al., 2020). The low blastocyst development rates in prepubertal buffaloes further constrain the widespread implementation of these technologies (Figure 3) (Currin et al., 2021). Moreover, the need for precise synchronization of follicular development and ovulation to apply assisted reproductive technologies (ART) consistently remains a challenge. The current protocols for estrus synchronization and ovulation induction, although adapted from cattle, still yield variable results in buffaloes (Warriach et al., 2015).

7.3 Economic and logistical challenges

Economic and logistical challenges also play a significant role in the optimization of reproductive technologies in water buffalo. The cost of implementing advanced reproductive technologies such as OPU and IVEP can be prohibitive, especially for smallholder farmers who dominate buffalo farming in developing countries (Warriach et al., 2015). Additionally, the need for specialized equipment and trained personnel to perform these procedures adds to the logistical complexity (Baruselli et al., 2020). The variability in reproductive outcomes due to factors such as season, age, and individual donor variation further complicates the economic viability of these technologies (Currin et al., 2022).

In summary, optimizing reproductive technologies in water buffalo is hindered by biological constraints, technological barriers, and economic and logistical challenges. Addressing these issues requires a multifaceted approach that includes improving estrus detection, enhancing the efficiency of ART protocols, and making these technologies more accessible and cost-effective for farmers.

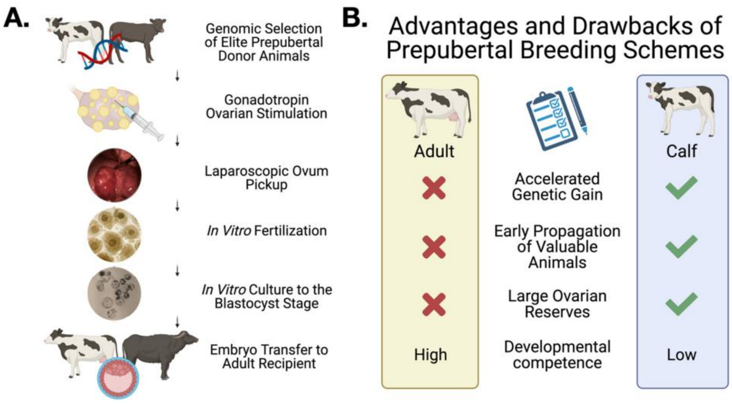

Figure 3 Overview of LOPU-IVEP in prepubertal buffalo and cattle (Adopted from Currin et al., 2021)

Image caption: (A): Flow chart showing the typical steps involved in prepubertal LOPU-IVEP programs. (B): Comparison between adult and prepubertal breeding schemes, showing the advantages and drawbacks of each. Figure created with BioRender.com, accessed on 30 July 2021 (Adopted from Currin et al., 2021)

8 Strategies for Improving Reproductive Efficiency in Water Buffalo

8.1 Training and capacity building for farmers and technicians

Training and capacity building are crucial for enhancing reproductive efficiency in water buffalo. Educating farmers and technicians on the latest assisted reproductive technologies (ART) such as estrous synchronization and artificial insemination can significantly improve outcomes. For instance, understanding the timing and administration of hormones like GnRH and hCG during AI can lead to higher pregnancy rates, as demonstrated in various studies (Atabay et al., 2020; Coman et al., 2024). Additionally, training on the management of reproductive disorders and the use of ovum pick-up (OPU) and in vitro embryo production (IVEP) can further enhance reproductive success (Baruselli et al., 2018; Qg et al., 2020).

8.2 Strengthening infrastructure for reproductive technologies

Strengthening infrastructure is essential to support the implementation of advanced reproductive technologies. This includes establishing facilities for OPU and IVEP, which are critical for improving genetic gains in buffalo herds (Qg et al., 2020). Moreover, ensuring the availability of necessary equipment and resources for cryopreservation and embryo transfer can mitigate the effects of seasonal breeding patterns and improve overall reproductive efficiency (Da Silva et al., 2021). Infrastructure improvements also involve creating environments that reduce stress and improve the welfare of buffaloes, which can positively impact fertility (Zicarelli et al., 2019).

8.3 Policy interventions and incentives

Policy interventions and incentives play a vital role in promoting the adoption of reproductive technologies. Governments and agricultural bodies can provide subsidies or financial incentives for farmers who implement ART, thereby reducing the economic burden and encouraging widespread use. Policies that support research and development in buffalo reproduction can lead to innovations that further enhance reproductive efficiency. Additionally, creating awareness and providing access to veterinary services can help address reproductive health issues and improve herd productivity (Devkota et al., 2022; Coman et al., 2024).

In summary, improving reproductive efficiency in water buffalo requires a multifaceted approach involving training, infrastructure development, and supportive policies. By focusing on these areas, the productivity and genetic potential of buffalo herds can be significantly enhanced.

9 Concluding Remarks

The optimization of reproductive technologies in water buffalo has seen significant advancements, particularly in the areas of estrus synchronization and in vitro embryo production (IVEP). Estrus synchronization protocols, such as Ovsynch and its modifications, have improved pregnancy rates by allowing precise timing for artificial insemination (AI). The integration of ovum pick-up (OPU) with IVEP has emerged as a viable alternative to traditional superovulation methods, offering a means to exploit the genetics of high-yield buffaloes despite challenges in embryo recovery. Additionally, the use of antioxidants like rutin and chlorogenic acid has been shown to enhance sperm quality and fertility during cryopreservation, addressing the issue of cryoinjury in buffalo sperm.

Future research should focus on refining synchronization protocols to further enhance reproductive efficiency, particularly in non-cyclic buffaloes, where current methods show limited success. There is also a need to improve the cost-effectiveness and success rates of IVEP, making it more accessible for commercial use. Investigating the genetic and environmental factors affecting OPU/IVEP efficiency could lead to more tailored and effective reproductive strategies. Moreover, exploring the potential of emerging technologies such as genomic-assisted selection and advanced cryopreservation techniques could accelerate genetic gains and improve overall herd productivity.

Reproductive technologies play a crucial role in enhancing the genetic potential and productivity of water buffalo herds. By improving reproductive efficiency and enabling the propagation of superior genetics, these technologies contribute significantly to sustainable buffalo production. The continued development and optimization of these technologies are essential for meeting the growing demand for buffalo products, such as milk and meat, while ensuring the conservation of this economically important species. As these technologies evolve, they will not only improve productivity but also support the long-term sustainability of buffalo farming.

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Conflict of Interest Disclosure
The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References
Ahmad N., and Arshad U., 2020, Synchronization and resynchronization strategies to improve fertility in dairy buffaloes, Theriogenology, 150: 173-179.
https://doi.org/10.1016/j.theriogenology.2020.01.025

Andrei C., Posastiuc F., Constantin N., and Mitrea I., 2024, New insights into semen separation techniques in buffaloes, Frontiers in Veterinary Science, 10: 1347482.
https://doi.org/10.3389/fvets.2023.1347482

Atabay E., Atabay E., Maylem E., Encarnacion É., and Salazar R., 2020, Enhancing prostaglandin-based estrus synchronization protocol for artificial insemination in water buffaloes, Buffalo Bulletin, 39: 53-60.

Baruselli P., Carvalho J., Elliff F., Silva J., Chello D., and Carvalho N., 2020, Embryo transfer in buffalo (*Bubalus bubalis*), Theriogenology, 150: 221-228.
https://doi.org/10.1016/j.theriogenology.2020.01.037

Baruselli P., Soares J., Bayeux B., Silva J., Mingoti R., and Carvalho N., 2018, Assisted reproductive technologies (ART) in water buffaloes, Animal Reproduction, 15: 971-983.
https://doi.org/10.21451/1984-3143-AR2018-0043

Bm O., 2019, Reproduction in water buffalo: comparative aspects and implications for management, Bioscientifica Proceedings, 54: 157-168.
https://doi.org/10.1530/BIOSCIPROCS.4.012

Chebel R., and Cunha T., 2020, Optimization of timing of insemination of dairy heifers inseminated with sex-sorted semen, Journal of Dairy Science, 103(6): 5591-5603.
https://doi.org/10.3168/jds.2019-17870

Coman S., Berean D., Cîmpean R., Ciupe S., Coman I., and Bogdan L., 2024, Clinical modalities for enhancing reproductive efficiency in buffaloes: a review and practical aspects for veterinary practitioners, Animals, 14(18): 2642.
https://doi.org/10.3390/ani14182642

Currin L., Baldassarre H., and Bordignon V., 2021, In vitro production of embryos from prepubertal holstein cattle and mediterranean water buffalo: problems, progress and potential, Animals, 11(8): 2275.
https://doi.org/10.3390/ani11082275

Currin L., Baldassarre H., De Macedo M., Glanzner W., Gutierrez K., Lazaris K., Guay V., Herrera M., Da Silva Z., Brown C., Joron E., Herron R., and Bordignon V., 2022, Factors affecting the efficiency of in vitro embryo production in prepubertal mediterranean water buffalo, Animals, 12(24): 3549. https://doi.org/10.3390/ani12243549

D'Occhio M., Ghuman S., Neglia G., Della Valle G., Baruselli P., Zicarelli L., Visintin J., Sarkar M., and Campanile G., 2020, Exogenous and endogenous factors in seasonality of reproduction in buffalo: a review, Theriogenology, 150: 186-192. https://doi.org/10.1016/j.theriogenology.2020.01.044

Da Silva J., Alves M., Bridi A., Bohrer R., Escobar G., De Carvalho J., Binotti W., Pugliesi G., Lemes K., Chello D., and Perecin F., 2021, Reproductive seasonality influences oocyte retrieval and embryonic competence but not uterine receptivity in buffaloes, Theriogenology, 170: 77-84. https://doi.org/10.1016/j.theriogenology.2021.04.019

Devkota B., Shah S., and Gautam G., 2022, Reproduction and fertility of buffaloes in Nepal, Animals, 13(1): 70. https://doi.org/10.3390/ani13010070

Du C., Nan L., Sabek A., Wang H., Luo X., Hua G., and Zhang S., 2021, Evaluation of Ovsynch versus modified Ovsynch program on pregnancy rate in water buffaloes: a meta-analysis, Tropical Animal Health and Production, 53: 1-12. https://doi.org/10.1007/s11250-021-02828-7

Duran D., Duran P., Duran P., and Cruz L., 2017, Production of river buffalo (*Bubalus bubalis*) calves by embryo in vitro production-vitrification and transfer techniques in the Philippines, Buffalo Bulletin, 36: 607-614.

Gaddis K., Dikmen S., Dikmen S., Null D., Cole J., and Hansen P., 2017, Evaluation of genetic components in traits related to superovulation, in vitro fertilization, and embryo transfer in Holstein cattle, Journal of Dairy Science, 100(4): 2877-2891. https://doi.org/10.3168/jds.2016-11907

Gunwant P., Pandey A., Kumar A., Singh I., Kumar S., Phogat J., Kumar V., Patil C., Tomar P., Kumar S., and Magotra A., 2018, Polymorphism of melatonin receptor (MTNR1A) gene and its association with seasonal reproduction in water buffalo (*Bubalus bubalis*), Animal Reproduction Science, 199: 51-59. https://doi.org/10.1016/j.anireprosci.2018.10.006

Karanwal S., Pal A., Chera J., Batra V., Kumaresan A., Datta T., and Kumar R., 2023, Identification of protein candidates in spermatozoa of water buffalo (*Bubalus bubalis*) bulls helps in predicting their fertility status, Frontiers in Cell and Developmental Biology, 11: 1119220. https://doi.org/10.3389/fcell.2023.1119220

Konrad J., Clérico G., Garrido M., Taminelli G., Yuponi M., Yuponi R., Crudeli G., and Sansiñena M., 2017, Ovum pick-up interval in buffalo (*Bubalus bubalis*) managed under wetland conditions in Argentina: Effect on follicular population, oocyte recovery, and in vitro embryo development, Animal Reproduction Science, 183: 39-45. https://doi.org/10.1016/j.anireprosci.2017.06.004

Lu Y., Liao Y., Zhang M., Yang B., Liang X., Yang X., Lu S., Wu Z., Xu H., Liang Y., and Lu K., 2015, A field study on artificial insemination of swamp and crossbred buffaloes with sexed semen from river buffaloes, Theriogenology, 84(6): 862-867. https://doi.org/10.1016/j.theriogenology.2015.05.022

Nanda A., Ps B., and Prabhakar S., 2019, Enhancing reproductive performance in dairy buffalo: major constraints and achievements, Reproduction (Cambridge, England) Supplement, 61: 27-36. https://doi.org/10.1530/BIOSCIPROCS.5.003

Nava-Trujillo H., Valeris-Chacin R., Morgado-Osorio A., Zambrano-Salas S., Tovar-Breto L., and Quintero-Moreno A., 2020, Reproductive performance of water buffalo cows: a review of affecting factors, Journal of Buffalo Science, 9: 133-151. https://doi.org/10.6000/1927-520x.2019.08.03.15

Neglia G., De Nicola D., Esposito L., Salzano A., D'Occhio M., and Fatone G., 2020, Reproductive management in buffalo by artificial insemination, Theriogenology, 150: 166-172. https://doi.org/10.1016/j.theriogenology.2020.01.016

Pellegrino C., Morotti F., Untura R., Pontes J., Pellegrino M., Campolina J., Seneda M., Barbosa F., and Henry M., 2016, Use of sexed sorted semen for fixed-time artificial insemination or fixed-time embryo transfer of in vitro-produced embryos in cattle, Theriogenology, 86(3): 888-893. https://doi.org/10.1016/j.theriogenology.2016.03.010

Qg S., Cm T., Jian H., Hui L., Tz Z., Yc Y., Hf X., Yb Z., Yn Q., Hj X., and Sd S., 2020, Effect of ovum pick-up frequency on quality of buffalo oocytes, Approaches in Poultry, Dairy and Veterinary Sciences, 7(4): 677-680. https://doi.org/10.31031/apdv.2020.07.000670

Singh B., Mal G., Kues W., and Yadav P., 2020, The domesticated buffalo - An emerging model for experimental and therapeutic use of extraembryonic tissues, Theriogenology, 151: 95-102. https://doi.org/10.1016/j.theriogenology.2020.04.003

Singhal S., Prasad S., Singh H., Shukla M., and Prasad J.K., 2021, Effects of pre-treatment with GnRH on the efficiency of superstimulatory protocol in water buffalo (*Bubalus bubalis*), Iranian Journal of Applied Animal Science, 11(2): 305-310.

Warriach H., McGill D., Bush R., Wynn P., and Chohan K., 2015, A review of recent developments in buffalo reproduction-a review, Asian-Australasian Journal of Animal Sciences, 28: 451-455. https://doi.org/10.5713/ajas.14.0259

Zhao X., Nie J., Tang Y., He W., Xiao K., Pang C., Liang X., Lu Y., and Zhang M., 2020, Generation of transgenic cloned buffalo embryos harboring the EGFP gene in the Y chromosome using CRISPR/Cas9-mediated targeted integration, Frontiers in Veterinary Science, 7: 199.
https://doi.org/10.3389/fvets.2020.00199

Zicarelli L., 2019, Enhancing reproductive performance in domestic dairy water buffalo (*Bubalus bubalis*), Society of Reproduction and Fertility supplement, 67: 443-455.
https://doi.org/10.7313/UPO9781907284991.034