Unlocking the Potential of Multiple Ovulation and Embryo Transfer (MOET) in Water Buffaloes

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Abstract: Water buffaloes (Bubalus bubalis) are essential to agricultural economies across Asia and other regions, providing milk, meat, and draught power. Despite their importance, reproductive efficiency remains lower than in cattle due to species-specific constraints, including limited ovarian reserves, poor superovulatory responses, and suboptimal uterine conditions. Multiple Ovulation and Embryo Transfer (MOET) offers a promising strategy for accelerating genetic improvement, particularly in swamp buffalo-dominated areas seeking the infusion of riverine germplasm. While MOET is widely successful in cattle, its application in buffaloes is challenged by anatomical, endocrine, and physiological differences. This review synthesizes current knowledge on MOET development in water buffaloes, highlighting historical milestones, reproductive limitations, protocol refinements, hormonal strategies, embryo recovery innovations, and the role of international collaborations. By addressing biological and logistical barriers, MOET can become a strategic tool to enhance genetic gain, improve reproductive outcomes, and support sustainable buffalo production systems.

Keywords: ART, MOET, embryo collection, reproductive biotechnology, superovulation.

1. INTRODUCTION

Water buffaloes (*Bubalus bubalis*) play a vital role in livestock production systems across Asia, South America, and parts of Europe. They contribute significantly to food security and rural livelihoods [1, 2], particularly in low-input systems where they are valued for milk, meat, and draught power [3]. Despite their economic importance, buffaloes exhibit lower reproductive efficiency than cattle due to delayed puberty, prolonged postpartum intervals, seasonal breeding tendencies, and reduced ovarian activity. These constraints limit genetic progress and slow herd productivity [4-11].

With the increasing human population and demand for milk and meat, the desire to produce riverine types in swamp buffalo-dominated areas increased, and reproductive biotechnologies proved the best tools [12]. Through *in vitro* embryo production, it was proven that riverine buffalo embryos can develop full-term not only in riverine buffalo recipients [12] but also in swamp buffalo recipients [13] and even resulted in the birth of dizygotic twins [14]. Given their economic and ecological relevance, the need for advanced breeding and reproductive biotechnology programs requires adopting strategies such as MOET to harness their genetic potential and enhance global productivity fully.

Multiple Ovulation and Embryo Transfer (MOET), a cornerstone reproductive biotechnology in cattle [15, 16], offers a pathway to accelerate genetic improvement in buffaloes [17-20]. However, species-specific anatomical and physiological characteristics reduce its efficiency [21]. Recent advances in hormonal protocols, synchronization strategies, and embryo recovery techniques have improved outcomes [22, 23], but challenges remain. This review examines the development, limitations, and scientific advances of MOET in buffaloes, highlighting opportunities to improve protocols and develop sustainable breeding programs.

2. MATERIALS AND METHODS

This narrative review synthesizes published research on MOET in water buffaloes. Relevant literature was identified through targeted searches of Web of Science, Scopus, and Google Scholar using keywords such as “buffalo,” “MOET,” “superovulation,” “embryo transfer,” and “reproductive biotechnology.” Backward citation tracking was used to identify additional sources. An initial search yielded 295 records. After screening titles, abstracts, and full texts for relevance to buffalo reproductive physiology, MOET

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protocols, and technological innovations, 90 articles were retained for analysis.

3. RESULTS AND DISCUSSION

3.1. Comparative Reproductive Physiology Relevant to MOET

Buffaloes exhibit several reproductive characteristics that differ markedly from cattle and directly constrain MOET efficiency [24, 25]. Their smaller ovaries, reduced antral follicle population, and fewer follicular waves limit the pool of recruitable follicles for superovulation [24]. Lower sensitivity to exogenous gonadotropins and higher baseline progesterone due to persistent corpora lutea further complicate synchronization and ovulation control. Subtle estrus signs and greater oocyte susceptibility to oxidative stress further complicate achieving consistent superovulatory responses. Additionally, delayed endometrial remodeling and reduced expression of implantation-related genes impair uterine receptivity, underscoring the need for buffalo-specific MOET protocols.

Anatomical constraints further reduce efficiency. Buffaloes possess a more rigid mesosalpinx and mesovarium, limiting infundibular mobility and reducing the ability to capture ovulated oocytes [24]. Combined with a narrower pelvic inlet and diminished fimbrial activity, these features contribute to lower oocyte recovery rates during superovulation [19, 24]. Empirical evidence supports this: only 30-40% of ovulated oocytes are recovered in superstimulated buffaloes, compared with 70-80% in cattle [15, 26]. This discrepancy has been linked to infundibular rigidity, delayed ovulatory cascade, and asynchronous hormonal signaling.

Seasonality exacerbates these physiological and anatomical limitations. During the non-breeding season, buffaloes exhibit reduced hormonal responsiveness, resulting in weaker follicular recruitment and poorer outcomes in assisted reproductive protocols. Compounding factors such as poor thermoregulation and altered oviductal secretions may further compromise oocyte viability and transport [27].

Although these studies collectively highlight key physiological barriers, many rely on small sample sizes or single-breed populations, limiting generalizability. Mechanistic understanding of oviductal transport and endocrine-ovarian interactions remains incomplete. Table **1** presents the comparative reproductive physiology between buffalo and cattle.

Table 1: Comparative Reproductive Physiology: Buffaloes Vs. Cattle

Ovarian Anatomy	Smaller ovaries with fewer antral follicles	Larger ovaries with higher follicle density	[26] Baruselli *et al*., 2011
Follicular Wave Pattern	1-2 waves per estrous cycle	Typically 2-4 waves per cycle	[27] de Rensis *et al*., 2015
Gonadotropin Sensitivity	Lower sensitivity to exogenous FSH and LH due to reduced receptor density	Higher responsiveness to gonadotropin stimulation	[28] Ty *et al*., 1989
Corpora Lutea Activity	Persistent corpora lutea; elevated progesterone levels	More defined luteolytic cycles; cyclic progesterone profile	[26] Baruselli *et al*., 2011
Estrus Signs	Subtle or silent estrus; harder to detect visually	More overt behavioral and physical signs of estrus	[26] Baruselli *et al*., 2011
Endometrial Receptivity	Delayed remodeling; altered expression of implantation markers	Prompt endometrial changes supportive of implantation	[28] Ty *et al*., 1989
Embryo Viability Post-Transfer	Lower retention rates due to uterine and oocyte factors	Higher success in implantation and gestation	[28] Ty *et al*., 1989
Pelvic Structure	More circular and oblique pelvic inlet; fused symphysis pubis; narrower cervix	Elliptical pelvic inlet; wider cervix; variable pubic fusion	[29] Carvalho *et al*., 2014
Ovulation	Lower ovulation rate	Higher ovulation rate	[30] Dobson & Kamonpatana, 1986
Estrous Cycle	Longer, less overt estrus; seasonal breeders	Shorter cycle; pronounced estrus; non-seasonal breeders	[25] Bertoni *et al*., 2020
Oviductal Anatomy	Rigid mesosalpinx and mesovarium; reduced infundibular mobility	Flexible ligaments; efficient oocyte transport	[29] Carvalho *et al*., 2014
Thermoregulation	Poor heat dissipation; affects hormonal balance	Better thermoregulation; stable endocrine function	[25] Bertoni *et al*., 2020

Future research priorities should include detailed mapping of oviductal biomechanics in buffaloes, molecular profiling of oocyte-oviduct interactions, and season-specific hormonal strategies to stabilize MOET outcomes.

3.2. Historical Milestones in Buffalo Embryo Transfer

Buffalo embryo transfer (ET) marked a breakthrough in livestock reproductive biotechnology [31], beginning with the first successful non-surgical transfer by Drost and Cripe in 1983, which resulted in the birth of “Herman” from a day-7 blastocyst [6]. This early success demonstrated the feasibility of ET in buffaloes but also exposed significant species-specific challenges. Throughout the 1980s and 1990s, attempts to adopt cattle ET protocols across Asia and Europe yielded inconsistent, often low embryo yields due to buffaloes’ unique ovarian dynamics and lower ovulatory responses [6, 26, 32-35].

A major regional milestone in Southeast Asia was achieved by Cruz *et al*. [36], who initiated programs to upgrade swamp buffaloes using riverine embryos produced both *in vivo* and *in vitro*. These efforts established the foundation for national embryo laboratories and demonstrated successful births from fresh, vitrified, and IVEP-derived embryos [12-14]. This period highlighted the potential of ET for genetic improvement but also underscored the need for buffalo-specific protocols.

Progress accelerated in the 2000s as researchers such as Baruselli *et al*. [26] and Campanile *et al*. [24] refined superovulation strategies using follicle-stimulating hormone (FSH) and equine chorionic gonadotropin (eCG), increasing viable embryo yields from fewer than one to approximately 2.5-3.0 embryos per flush. Concurrently, ultrasound-guided transvaginal techniques improved oocyte recovery efficiency and reduced invasiveness [34], marking a shift toward more precise and animal-friendly approaches.

By the 2010s, the integration of *in vitro* embryo production (IVEP) and ovum pick-up (OPU) emerged as the preferred alternative to MOET in buffaloes, particularly those with poor superovulatory response. These advancements allowed for wider application of reproductive technologies and bolstered satellite laboratory networks in countries such as India and the Philippines [12, 20, 33].

While historical progress demonstrates steady innovation, much of the early work lacked standardized reporting and involved small sample sizes, limiting direct comparison across studies. The transition toward IVEP and OPU reflects both technological advancement and recognition of MOET’s inherent limitations in buffaloes.

Overall, the evolution of ET in buffaloes has mirrored both scientific perseverance and adoptive innovation, demonstrating the species’ potential as a viable target for genetic improvement through assisted reproductive technologies. Table **2** summarizes the major milestones in buffalo ET with emphasis on MOET achievements.

Table 2: Historical Milestones in Buffalo Embryo Transfer Emphasizing MOET Achievements

1983	First live buffalo birth via ET	Drost & Cripe, Univ. of Florida	Non-surgical transfer of Day-7 blastocyst resulted in the birth of "Herman"	[6] Drost *et al.* (1983), Theriogenology 20:579-584.
1990	Launch of buffalo ET in Philippines	Cruz *et al.*, Philippine Carabao Center (PCC)	MOET initiated using river buffalo embryos to upgrade swamp buffaloes	[36] Cruz *et al.* (1991)
1999	Species-specific MOET protocol development	Misra & Tyagi, ICAR, India	Adoptation of cattle protocols with low recovery in buffaloes (<1 embryo/flush)	[37] Misra & Tyagi (1999)
2001	Transition of MOET to IVF	Hufana-Duran *et al.* PCC, Philippines	Use of *in vitro* embryo production to support buffalo embryo transfer	[12] Hufana-Duran *et al.*, (2004)
2008	Advancement in non-surgical flushing methods	Singh *et al.*, IVRI, India	Use of ultrasound-guided transvaginal techniques improved oocyte recovery	[34] Singh *et al.* (2009)
2009	FSH/eCG MOET protocol optimization	Baruselli *et al.*, Brazil	Embryo recovery improved to ~2.5-3.0 embryos per flushing	[24] Campanile *et al.* (2010)
2021	Comparative review of MOET vs. IVEP	Ohashi *et al.*, Brazil & India	Emphasized limitations in MOET and transition to IVEP in buffaloes	[38] Ohashi *et al.* (2022)

3.3. Advancements in MOET

3.3.1. Hormonal Protocols and Superovulation Strategies

Advances in hormonal protocols have greatly improved superovulation outcomes in cattle, and many of these developments offer potential, though still limited, benefits for water buffalo [35, 36]. However, buffalo physiology continues to constrain the predictability and efficiency of these protocols.

3.3.2. FSH/eCG Optimization

In cattle, refined FSH and equine chorionic gonadotropin (eCG) regimens, including long-acting recombinant bovine FSH (bscrFSH), have produced more consistent ovarian responses and higher embryo yields while reducing the need for multiple injections [39]. Synchronizing follicular wave emergence using GnRH or estradiol has further enabled fixed-time AI (FTAI) and improved timing accuracy in cattle MOET programs [24, 39].

In buffaloes, however, superovulatory responses remain highly variable due to lower follicular reserves, higher atresia, and reduced gonadotropin sensitivity [19, 40]. Adoption of cattle-derived protocols has produced incremental improvements: descending-dose FSH regimens combined with eCG can enhance follicular recruitment and luteal support [39, 41]. Comparative studies in Murrah buffaloes show that PMSG + FSH and FSH alone treatments yield similar ovulatory responses, suggesting that cattle protocols can be applied with careful dose calibration [40]. Synchronization strategies such as Ovsynch have also improved consistency when gonadotropin treatments follow controlled follicular wave emergence [19].

A practical example illustrates both the potential and the limitations: in Murrah buffaloes treated with porcine FSH (n=7) or PMSG (n=4), ovulation rates averaged 2.86±1.57 and 1.5±1.0, respectively, yielding 14 embryos from FSH-treated animals and none from PMSG-treated animals. Only one calf (HV Jr.) resulted from four transferred embryos [36]. This underscores the high variability and low efficiency typical of buffalo MOET.

Evidence supporting FSH/eCG optimization in buffaloes is promising but limited by small sample sizes, breed-specific responses, and inconsistent environmental conditions. More controlled, multi-location trials are needed to determine optimal hormone combinations and timing.

3.3.3. Estrus Synchronization Techniques

Estrus synchronization has transformed reproductive management by enabling FTAI, improving conception rates, and reducing dependence on estrus detection. Protocols such as Ovsynch, Co-Synch, and CIDR-based regimens manipulate the luteal and follicular phases using GnRH, $PGF_2\alpha$, and progesterone to synchronize ovulation [18, 33, 42]. In cattle, these protocols integrate well with superovulation regimens, improving follicular wave control and embryo recovery [39].

In buffaloes, estrus synchronization is complicated by silent heat, seasonal anestrus, and variable ovarian responsiveness. Nonetheless, cattle-derived protocols such as Ovsynch have achieved conception rates of up to 60% during the breeding season [42]. During the non-breeding season, progestagen-based regimens (CIDR/PRID with estradiol, eCG, and $PGF_2\alpha$) show superior efficacy in inducing cyclicity and improving fertility outcomes [10, 44]. Emerging approaches, including ultrasound-guided follicular ablation and estradiol-based wave synchronization, further improve ovulatory precision and embryo quality [10]. These findings indicate that while buffaloes require more tailored hormonal strategies, the core principles of cattle synchronization can be effectively adopted with appropriate modifications.

Although synchronization protocols improve management efficiency, their effectiveness in buffaloes is strongly influenced by season, body condition, and ovarian status. More buffalo-specific synchronization models, especially those tailored for non-breeding seasons, are needed.

Table **3** remains applicable for comparing synchronization strategies between cattle and buffalo.

3.3.4. Response Variability and Dose Considerations

Buffaloes show marked variability in hormonal responsiveness due to seasonal anestrus, silent estrus, and reduced GnRH receptor expression during the non-breeding season. These limitations necessitate modified protocols incorporating estradiol and eCG to stimulate follicular recruitment and improve synchronization [13, 44]. In contrast, cattle exhibit predictable endocrine responses to GnRH and $PGF_2\alpha$, enabling precise control of follicular dynamics and reliable integration of fixed-time AI with superovulation regimens. FSH dosing can be fine-tuned according to

Table 3: Comparative Table on Estrus Synchronization Techniques in Water Buffalo Vs. Cattle

Estrus Detection	Often exhibits silent heat, making detection unreliable	Easier detection due to pronounced behavioral signs	[42, 43,] Perry & Salverson (2020);Purohit *et al.* (2019);
Breeding Season Dependency	Seasonally polyestrous with lower cyclicity during non-breeding season	Year-round breeding capability	[44,10] Neglia *et al.* (2003); Bhat & Dhaliwal (2023)
Standard Protocols Used	Ovsynch, CIDR/PRID with estradiol or eCG combinations	Ovsynch, Co-Synch, CIDR-based regimens	[24,43] Baruselli *et al.* (2010); Purohit (2019)
Hormonal Responsiveness	Variable response; often requires eCG to support follicular growth during non-breeding season	High GnRH and PGF2α responsiveness	[44,10] Neglia *et al.* (2003); Bhat & Dhaliwal (2023)
Follicular Wave Synchronization	Emerging techniques like ultrasound-guided ablation and estradiol-based synchronization show promise	Controlled using GnRH or estradiol; highly effective for superovulation timing	[39,10] Mapletoft & Bo (2012); Bhat & Dhaliwal (2023)
Fixed-Time AI Success	Moderate success during breeding season; lower conception rates in anestrous buffaloes	High success rates; widely adopted for herd-level management	[43,10] Purohit (2019); Bhat & Dhaliwal (2023)
Integration with Superovulation	Adoptation required; timing of FSH critical due to variable follicular dynamics	Well-established protocols combining GnRH and FSH	[24,19] Baruselli *et al.* (2010); Palanisammi *et al.* (2020)

breed, body weight, and ovarian status, resulting in consistently high embryo yields [18, 24, 39]. Superovulation outcomes often require individualized dose adjustments and close ultrasonographic monitoring of follicular waves [45]. Emerging strategies, such as ultrasound-guided follicular ablation combined with estradiol priming, aim to reset follicular dynamics and improve synchrony, partially emulating the predictability of cattle [19, 39].

As demonstrated in Figure **1**, during the breeding season, robust luteal activity supports higher progesterone levels, which correlate with increased responsiveness to FSH and eCG and enhance follicular growth and ovulatory efficiency. In contrast, the non-breeding season is associated with reduced luteal tone, decreased circulating progesterone, and impaired folliculogenesis, necessitating elevated doses and extended hormone protocols to achieve reproductive targets [46]. These findings emphasize the importance of season-adopted hormone dosing and luteal phase diagnostics when designing estrus synchronization and superovulation protocols for buffaloes. By aligning stimulation regimens with seasonal endocrine landscapes, MOET efficiency may approach cattle benchmarks.

3.4. Embryo Collection and Transfer Techniques

3.4.1. Surgical vs. Nonsurgical Methods

Embryo collection and transfer techniques in buffaloes have evolved significantly as researchers seek more efficient, scalable MOET approaches. Historically, surgical methods, including laparotomy and flank incisions, were widely used due to anatomical challenges such as the buffalo's narrow cervix and deep uterine horns. These techniques provide direct access to the uterus. They can yield relatively high embryo recovery rates (up to four viable embryos per flush). Still, they also carry significant drawbacks, including post-operative adhesions, prolonged recovery, and potential reductions in future fertility [47].

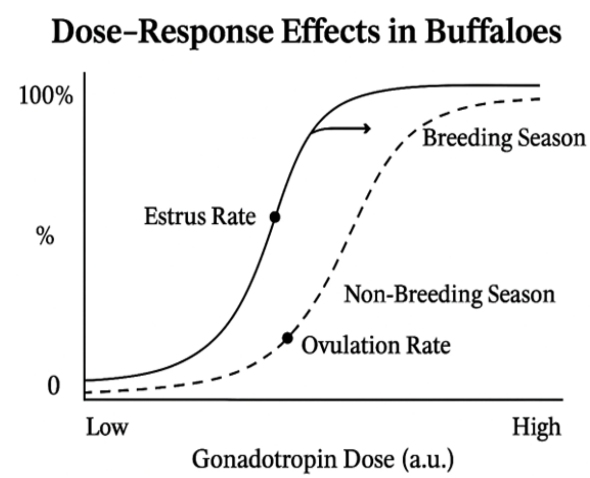

Figure 1: Dose-Response Dynamics and Seasonal Modulation of Gonadotropin-Induced Estrus and Ovulation in Buffaloes. Differential dose-response curves of gonadotropin administration on estrus and ovulation rates (%) in *Bubalus bubalis* during the breeding and non-breeding seasons. The solid curve depicts the breeding season, in which lower gonadotropin doses elicit steeper increases in estrus and ovulatory responses. The dashed curve represents the non-breeding season and shows a blunted response even at higher doses. Overlaid atop both curves is the seasonal variation in luteal activity, depicted by shaded regions beneath the curves.

In contrast, nonsurgical methods, now standard in cattle, remain more difficult to implement in buffaloes because of species-specific anatomical and physiological constraints. However, refinements in catheter design, cervical manipulation techniques, and synchronization protocols have enabled successful transcervical deep-uterine embryo transfer, achieving pregnancy rates comparable to surgical approaches while minimizing trauma to donors and recipients [20]. Although nonsurgical flushing still produces lower embryo recovery rates, ongoing improvements in hormonal stimulation and procedural technique are gradually narrowing this gap [37, 48-51]. Overall, the field is shifting toward less invasive, more commercially scalable methods that support herd-level genetic improvement.

3.4.2. Ultrasound-Guided Recovery and Transfer

Ultrasound technology has become an essential tool for enhancing the precision and success of embryo collection and transfer procedures. In buffaloes, ultrasonography plays an even more critical role due to their greater anatomical variability and less predictable ovarian response. In cattle, ultrasonography improves donor selection, optimizes flushing timing, and enhances catheter placement by providing real-time assessment of ovarian response and uterine tone [50,51]. These refinements have increased the yield of transferable embryos and reduced procedural trauma.

Transrectal ultrasonography is routinely used to monitor follicular development, confirm ovulation following superovulation, and assess uterine readiness before flushing. During transcervical embryo recovery, ultrasound guidance helps ensure accurate catheter placement and detect fluid accumulation or abnormalities in uterine tone [52]. Post-transfer, ultrasonography supports early pregnancy diagnosis and monitoring of embryonic development, thereby strengthening the entire MOET cycle.

Table **4** presents the comparative observations on the ultrasound-assisted flushing and embryo transfer in buffaloes vs. cattle.

3.4.3. Embryo Viability and Quality Outcomes

Embryo viability and quality in buffalo MOET programs remain limited by species-specific physiological and seasonal factors [54]. Compared with cattle, buffaloes typically yield 1.45-2.5 embryos per flush, with only 1-2 transferable embryos per donor. These modest outcomes reflect restricted follicular development, higher rates of follicular atresia, and reduced oocyte competence [35, 37, 55].

Ultrasonography has improved embryo transfer precision by enabling accurate embryo deposition into the ipsilateral uterine horn to the corpus luteum. Doppler ultrasonography, in particular, has emerged as a valuable tool for assessing luteal blood perfusion, a strong indicator of luteal functionality and uterine receptivity [53]. Selecting recipients with high luteal vascularization increases pregnancy success rates and supports more efficient fixed-time embryo transfer (FTET) programs.

Seasonal effects are pronounced: winter flushing consistently yields higher-quality embryos than summer, likely due to improved luteal function and reduced heat stress. Parity also influences outcomes, with younger buffaloes (first or second lactation) producing more viable embryos than older animals. Advances in hormonal stimulation, especially FSH combined with timed GnRH, have modestly improved

Table 4: Ultrasound-Assisted Flushing and Embryo Transfer in Buffalo Vs. Cattle

Timing of Uterine Flushing	Day 5-6 post-estrus due to faster embryonic development [49]	Day 7 post-estrus for optimal embryo recovery
Ultrasound Role in Flushing	Similar use; more critical due to anatomical variability and lower response [50]	Pre-procedural assessment of ovarian response and uterine tone
Embryo Recovery Technique	Adopted from cattle; requires gentler handling [51]	Non-surgical flushing via Foley catheter guided by rectal palpation
Embryo Transfer Precision	Essential due to deeper cervix and smaller uterus [52]	Ultrasound-guided deposition into ipsilateral uterine horn
Luteal Function Assessment	Increasing use of Doppler to select high-perfusion recipients [53]	Doppler ultrasound used to assess luteal blood flow
Embryo Viability & Yield	Lower yield; often 2-4 embryos per flush due to species-specific constraints [53]	5-7 transferable embryos per flush typical
Challenges	Poor superovulatory response; protocols still under refinement [19]	Variable superovulatory response; manageable with FSH protocols

embryo morphology and superovulatory response, with some protocols achieving up to 2.2 transferable embryos per donor [32]. However, developmental arrest and degeneration remain common, often due to asynchrony between donor hormonal status and uterine receptivity.

The integration of Doppler ultrasonography to evaluate luteal perfusion in both donors and recipients is emerging as a promising strategy to enhance embryo selection and improve transfer success. Although buffalo MOET yields remain lower than cattle, ongoing refinements in stimulation protocols, donor selection, and recipient assessment continue to strengthen the feasibility of genetic improvement programs. Table **5** summarizes key studies on buffalo MOET, highlighting embryo yield, ovulation rates, and pregnancy outcomes.

3.5. Ovulation Response Variations

Data from Table **5** clearly demonstrate the high inter-study variability in ovulatory response among buffalo donors, reflecting the combined influence of intrinsic factors (age, parity, body condition, AMH levels, breed) and extrinsic factors (photoperiod, heat stress, nutrition). Seasonal anestrus and reduced GnRH responsiveness, well-documented in buffaloes, further contribute to inconsistent follicular recruitment and ovulation [35, 60]. Endocrine status also plays a central role: mid-luteal progesterone levels influence follicular wave emergence, while GnRH pulsatility modulates follicular sensitivity to exogenous gonadotropins [18, 62]. Selection of donors with higher AMH and use of low-LH FSH preparations have been associated with improved responses [63].

Across studies, modified FSH protocols consistently outperform conventional regimens, largely due to more stable gonadotropin exposure and reduced follicular asynchrony. Slow-release FSH formulations and adjuvant treatments such as eCG or GnRH analogs help synchronize follicular waves and enhance luteal support [18, 64]. This is reflected in the exceptional ovulatory response reported by Perera *et al.* [56]. Although the study did not fully detail all protocol refinements, the combination of optimized FSH timing and multiple inseminations likely contributed to this outcome, underscoring the potential of protocol customization [7].

Dose-dependent effects of GnRH pre-treatment further illustrate the complexity of buffalo endocrine

Table 5: Key Studies on Buffalo MOET with Emphasis on Embryo Yield, Ovulation Rates, and Pregnancy Outcomes

Authors	Breed Type	Superovulation Protocol	Ovulatory Follicle (# of Cl)	Embryo Yield (%)	Viable Embryos (Pregnancy Outcome)
Perera *et al*., 2024 [56]	Murrah buffalo n=10	Modified superovulation protocol	(2)	18 (78.0)	(2)
Singhal *et al*., 2021 [57]	Murrah buffalo (n=9)	600 mg FSH in 10 decreasing doses at 12 hourly interval for five days with 06 µg FSH pre-treatment 2.5 days prior.	8.4 ± 0.72	3.0 ± 0.71	2.33 ± 0.64
Sing *et al*., 2015 [57]	Water buffalo	Estradiol based super stimulation protocol	15.5 ± 1.24	5.83 ± 0.86	3.67 ± 0.93
Patel *et al*., 2010 [59]	Pandharpuri buffaloes (n=8)	FSH (400 mg)	4.13 ± 0.52	2.38 ± 0.42	1.41 ± 0.32
Zambrano-Varon & Bondurant, 2007[60]	Cyclic Water buffaloes (n=10:5 normo-karyotype and 5 hybrids)	PGF2α (25 mg, IM) + FSH twice daily, morning and evening, for four days at decreasing doses	0	0	0
Techakumphu *et al*., 2001 [61]	Water buffalo (n=28)	FSH+GnRH (0 hr) FSH	7.38 ± 4.84 (3.88 ± 4.0) 10.18 ± 5.96 (4.5 ± 2.68)	2.00 ± 3.0 (51.5) 1.91 ± 2.7 (42.4)	1.63 ± 2.77 1.36 ± 1.69
Cruz *et al*, 1991[36]	Riverine Buffalo	FSH (decreasing doses), n=7 PMSG (decreasing dose), n=4	2.86 ± 1.57 1.5 ± 1.0	14 0	1 0

regulation. Pasha *et al.* [65] showed that 6 μg GnRH produced better results than 10 μg, supporting a bell-shaped response curve and suggesting that excessive GnRH may downregulate pituitary receptors or induce premature luteinization.

The superiority of FSH over PMSG/eCG, observed consistently across studies, including the zero-response PMSG group in Cruz *et al.* [36], reflects fundamental pharmacological differences. FSH's shorter half-life allows precise control of follicular growth, whereas PMSG's prolonged LH-like activity increases the risk of premature luteinization [66].

3.6. Embryo Recovery Efficiency

Embryo recovery rates in Table **5** vary widely, highlighting the influence of both hormonal and technical factors. The 78% recovery rate reported by Perera *et al.* [56] represents a major improvement over historical averages and may reflect advances in flushing technique, media formulation, and optimized collection timing. Conversely, the complete absence of embryos in Zambrano-Varon & Bondurant [60] underscores the sensitivity of buffalo MOET to donor selection, protocol design, and breed differences.

Hormonal refinements also influence embryo quality. Singh *et al.* [58] reported that LH treatment yielded 73.3% transferable embryos, outperforming GnRH (48.5%). This supports the idea that direct LH administration provides a more physiologically appropriate ovulatory signal in buffaloes, where GnRH-induced LH surges may be suboptimal.

Timing of ovulation induction is another critical determinant. Techakumphu *et al.* [61] and Singhal *et al.* [57] showed that administering GnRH 8-12 hours after standing heat significantly increased transferable embryo rates (up to 81.5%), likely by allowing additional time for final oocyte maturation.

Despite these improvements, the highest reported transferable embryo yield, 3.67 embryos per donor [58], remains at the lower end of cattle benchmarks, reflecting inherent biological constraints, such as lower ovarian reserves and stronger dominant follicle suppression in buffaloes.

3.7. Pregnancy Outcomes

Pregnancy outcomes mirror the variability observed in ovulation and embryo recovery. The 78% pregnancy rate reported by Perera *et al.* [56] with fresh embryos demonstrates that buffalo embryos, once viable, can achieve pregnancy rates comparable to those of cattle. However, pregnancy rates for IVP embryos remain low (0-26%), significantly below those of *in vivo* embryos (66-78%). This gap reflects persistent limitations in buffalo IVP systems, including suboptimal culture conditions and species-specific embryonic developmental requirements.

Recipient management is equally critical. Improved synchronization protocols and melatonin supplementation have been shown to enhance pregnancy rates, emphasizing that recipient physiology and seasonality are major determinants of ET success.

3.8. Protocol Effectiveness

Across all metrics, ovulation rate, embryo recovery, transferable embryos, and pregnancy outcomes, modified FSH-based protocols consistently outperform conventional regimens. Key refinements include: synchronization of follicular wave emergence (estradiol or GnRH pre-treatment), direct LH administration for ovulation induction, strategic $PGF_2\alpha$ timing relative to FSH, and multiple AI at optimized intervals [55, 57]. The clear advantage of FSH over PMSG/eCG, despite the latter's convenience, reinforces the need for precision-based stimulation strategies in buffaloes.

3.9. Challenges in Implementing MOET in Buffaloes

3.9.1. Limited Ovarian Reserves

Buffaloes possess inherently lower ovarian reserves than cattle, characterized by fewer primordial and antral follicles and a higher incidence of follicular atresia. These constraints directly limit the number of follicles recruitable for superovulation and contribute to the consistently low embryo yields reported across studies [19, 24, 67]. Variability in FSH responsiveness is common, with some donors failing to mount an adequate ovarian response despite standard stimulation protocols [9, 35, 68]. Elevated progesterone levels and persistent corpora lutea further disrupt follicular wave emergence and synchronization, reducing the predictability of ovulation timing [24, 69]. Collectively, these physiological limitations highlight the need for buffalo-specific MOET strategies, including precise follicular wave synchronization and tailored gonadotropin regimens.

3.9.2. Poor Response to Superovulation

Conventional FSH-based protocols in buffaloes often result in low ovulation rates, a high proportion of anovulatory follicles, and modest embryo recovery. Ovulation rates as low as 5.6%, with only ~20% of animals successfully ovulating, have been reported despite visible follicular recruitment [68]. Hormonal imbalances, particularly elevated estradiol and insufficient luteal support, are negatively associated with embryo quality and recovery [23, 69].

A comparative study in Italian Mediterranean buffaloes demonstrated that exogenous FSH can disrupt steroidogenesis and oviductal function, leading to reduced oocyte recovery and impaired cumulus expansion [60, 70]. Downregulation of key oviductal genes (PGR, ER1, VEGF, FLK1) further suggests compromised ovum capture mechanisms (71). These findings reinforce the need for refined superovulation protocols that minimize endocrine disruption and improve follicular synchrony.

Table **6** presents the hormonal and follicular dynamics during poor superovulatory response in buffaloes

3.9.3. High Progesterone Levels and Corpora Lutea Dynamics

Buffaloes exhibit distinctive corpus luteum (CL) dynamics, producing larger and more persistent CLs that secrete higher baseline progesterone than cattle [72, 73]. While progesterone is essential for pregnancy maintenance, premature elevation during superovulation can suppress LH surges, impair follicular maturation, and reduce ovulatory synchrony [71]. Persistent CLs are frequently associated with asynchronous follicular development, poor ovulation, and compromised embryo recovery [9, 71]. Seasonal fluctuations, particularly during cooler months, further complicate synchronization, as elevated progesterone may mask estrus and disrupt the timing of gonadotropin administration [74, 75]. These endocrine characteristics underscore the importance of precise luteal monitoring and season-adjusted hormonal regimens in buffalo MOET programs.

3.9.4. Low Uterine Receptivity and Embryo Retention

The buffalo endometrium exhibits less complex feto-maternal interdigitation and fewer branched villi, potentially limiting nutrient exchange and implantation success [76]. These demonstrate lower uterine receptivity than cattle, driven by species-specific differences in endometrial architecture, hormonal profiles, and maternal-embryonic signaling [77]. High rates of early embryonic mortality are frequently linked to inadequate luteal support, delayed uterine preparation, and altered cytokine expression [60]. Reduced expression of implantation-related genes, LIF, HOXA10, and IGFBP1, further compromises embryo attachment [75]. Postpartum uterine infections and retained fetal membranes exacerbate endometrial inflammation, diminishing receptivity in subsequent cycles [78].

These limitations highlight the need for buffalo-specific endometrial priming strategies, improved luteal support, and molecular-guided recipient selection to enhance embryo retention and overall MOET success.

3.10. Recent Research Insights and Breakthroughs

3.10.1. Refinement of Superovulation Protocols

Recent advances in buffalo MOET have focused on refining superovulation protocols through species-specific hormonal strategies. Synchronization approaches using GnRH and CIDR have improved ovulatory response, while tailored FSH regimens, such as split-dose or slow-release formulations, have

Table 6: Hormonal and Follicular Dynamics during Poor Superovulatory Response in Buffaloes

Follicular Recruitment	Reduced antral follicle count; lower cohort formation	[19] Palanisammi *et al*., 2020
Follicular Atresia Rate	Increased atresia; high proportion of anovulatory follicles	[60] Zambrano-Varón & BonDurant, 2007
Ovulation Rate	As low as 5.6%; only ~20% successful ovulation	[60] Zambrano-Varón & BonDurant, 2007
FSH Responsiveness	Poor maturation despite recruitment	[19] Palanisammi *et al*., 2020
Estradiol Levels	Elevated without corresponding ovulation	[68] Heleil *et al*., 2010
Progesterone Profile	Inadequate luteal phase support; reduced P_4 concentrations associated with poor embryo quality	[68] Heleil *et al*., 2010
Corpora Lutea Formation	Low number of functional corpora lutea formed post-superovulation	[60] Zambrano-Varón & BonDurant, 2007
Embryo Recovery Efficiency	Poor embryo recovery due to asynchronous follicle growth and hormonal imbalance	[68] Heleil *et al*., 2010; [37] Gasparrini, 2007

enhanced follicular recruitment and reduced premature luteinization [33]. Improvements in non-surgical embryo recovery, including ultrasound-guided flushing and epidural anesthesia, have increased embryo yield while improving donor welfare.

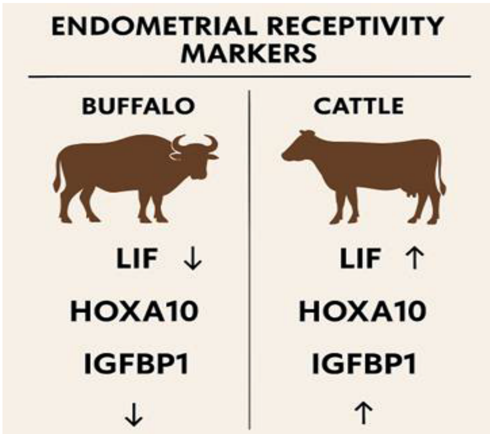

Figure 2: Comparative expression of key endometrial receptivity markers in buffaloes and cattle. LIF, HOXA10, and IGFBP1 are expressed at lower levels in buffaloes (↓), reducing blastocyst adhesion, uterine preparation, and trophoblast invasion. Cattle exhibit comparatively higher expression (↑), supporting superior implantation efficiency.

Luteal support with GnRH post-ovulation has been shown to strengthen CL function and improve embryo survival [79]. Given buffalo's strong reproductive seasonality, melatonin supplementation during the non-breeding season has improved superovulatory outcomes by mitigating oxidative stress. Complementary *in vitro* innovations, such as low-oxygen embryo culture**,** have enhanced mitochondrial integrity and blastocyst development [80].

At the molecular level, transcriptomic profiling has identified markers, MAPK14, CPT2, and BMP15, associated with improved meiotic progression and metabolic competence, offering potential tools for donor selection and embryo viability prediction [81]. Collectively, these advances highlight the growing precision of buffalo-specific superovulation strategies, though further optimization remains necessary to achieve consistent, high-level responses.

3.10.2. Endometrial Remodeling and Implantation Markers

Progress in understanding buffalo endometrial remodeling has opened new avenues for improving implantation success in MOET programs. Endometrial receptivity depends on coordinated hormonal signaling, cellular remodeling, and molecular communication within the uterine microenvironment. Key markers; HOXA10, LIF, integrin αvβ3, are consistently upregulated during the window of implantation (WOI), underscoring their role in embryo-maternal cross-talk [82].

Emerging evidence highlights the diagnostic potential of non-coding RNAs, including miR-30d and the miR-200 family, which can be detected in uterine fluid or plasma and may serve as non-invasive indicators of receptivity [83]. Endometrial receptivity assays (ERA), based on RNA sequencing, are increasingly used to personalize embryo transfer timing, particularly in cases of displaced WOI or recurrent implantation failure [84].

At the cellular level, stromal decidualization, angiogenesis, and immune cell recruitment, regulated by progesterone and local cytokines, are essential for synchronizing embryo development with endometrial maturation. Integrating these molecular and cellular markers into MOET protocols through donor screening, optimized transfer timing, and uterine environment monitoring offers promising opportunities to improve pregnancy outcomes in buffaloes.

3.10.3. Genetic and Epigenetic Considerations

Genomic and epigenetic research is beginning to clarify the biological foundations of reproductive performance in buffaloes. Comparative genomic analyses reveal distinct selection signatures between river and swamp buffaloes, particularly in genes associated with heat tolerance and immune function, traits crucial for reproductive resilience in tropical environments [85]. These insights support the development of breed-specific superovulation and embryo transfer protocols.

Population-level studies have identified high-frequency alleles with sex-specific fitness effects, suggesting that Y-chromosomal distorter-suppressor systems may influence reproductive traits through epigenetic pathways, especially under environmental stressors such as drought [86]. Epigenetic modulation, linked to parental body condition and pre-birth rainfall, indicates that environmental cues can shape reproductive outcomes through heritable molecular mechanisms [87]. Integrating genomic selection with environmental and epigenetic profiling may enhance donor selection, hormonal responsiveness, and embryo viability in buffalo MOET programs.

3.10.4. Current Limitations and Future Directions

Small sample sizes constrain the interpretation of buffalo MOET research, often fewer than 10 animals per treatment group (e.g., Singh *et al.* [58]), limiting statistical power and increasing the likelihood that observed differences reflect random variation. Inconsistent reporting of statistical significance further complicates cross-study comparisons.

Substantial protocol heterogeneity, including variation in FSH preparation, total dose, dosing schedule, and timing relative to the estrous cycle, creates a complex parameter space that hinders reproducibility. The long-time span of available studies (1983-2024) introduces additional confounders, as improvements in flushing techniques, media composition, and synchronization methods may contribute to better outcomes independent of hormonal protocols.

Despite these limitations, MOET remains a promising tool for genetic improvement in buffaloes, provided that future research adopts more rigorous statistical designs, standardized reporting, and systematic protocol optimization.

3.10.5. Protocol Optimization

Buffaloes differ markedly from cattle in ovarian responsiveness, estrus expression, follicular synchrony, and luteal stability, resulting in inconsistent embryo yields and lower pregnancy rates [15]. The absence of standardized hormonal regimens, particularly for FSH dosing, GnRH timing, and luteal support, limits reproducibility across farms and research institutions. Anatomical constraints, such as a narrow cervix and deep uterine horns, further complicate nonsurgical flushing and embryo transfer.

Future progress requires seasonally adjusted, breed-specific protocols validated across diverse production systems. Integration of real-time ultrasonography, Doppler-based luteal assessment, and genomic selection tools can improve donor and recipient management. Establishing harmonized frameworks similar to those used in cattle will be essential for scaling MOET in both commercial and conservation contexts.

3.10.6. Field-level Adoptation and Sustainability

Implementation of MOET in smallholder and extensive systems is limited by inadequate veterinary infrastructure, restricted access to hormonal agents, and shortages of trained personnel [88]. Seasonal declines in reproductive efficiency, particularly during the non-breeding period, further hinder field application [87].

To support sustainable adoption, future strategies should prioritize low-cost, breed-adopted superovulation and synchronization protocols compatible with family-based systems. Smart farming tools, such as wearable estrus detectors and mobile reproductive tracking, can enhance decision-making and reduce labor demands. Embedding MOET within community-based breeding and conservation programs may help preserve indigenous buffalo genetics while improving productivity in marginal environments [88].

3.10.7. International Collaboration and Capacity Building

Global partnerships remain essential for overcoming technical and logistical barriers. Initiatives led by organizations such as FAO and ICAR have supported protocol refinement, training programs, and genetic resource exchange across South and Southeast Asia [86,89,90]. These collaborations facilitate the development of climate-resilient breeding strategies, improved cryopreservation methods, and genomic tools tailored to buffalo physiology.

Sustained interdisciplinary cooperation, policy support, and inclusive capacity-building will be critical to ensuring that MOET becomes an accessible, adoptable, and ecologically sound biotechnology for buffalo development.

4. CONCLUSION

Modified FSH protocols that incorporate precise control of follicular wave emergence, optimized prostaglandin timing, and strategic ovulation induction consistently outperform conventional approaches in buffalo MOET. Intermediate GnRH pre-treatment doses enhance superovulatory response more effectively than higher doses. At the same time, direct LH administration yields a greater proportion of transferable embryos than GnRH, underscoring its advantages for final oocyte maturation. Across studies, FSH preparations reliably outperform PMSG/eCG despite the logistical convenience of single-dose PMSG protocols. The timing of ovulation induction remains critical: administering GnRH or LH 8-12 hours after standing heat consistently improves embryo quality compared with induction at the time of standing heat.

Pregnancy outcomes further highlight the disparity between embryo sources. *In vivo*-derived embryos achieve substantially higher pregnancy rates than IVP embryos, reflecting persistent limitations in buffalo IVP systems. Recipient synchronization, luteal support, and seasonal management strongly influence pregnancy success, underscoring that program efficiency depends not only on donor protocols but also on coordinated management across the entire MOET pipeline.

Although MOET has demonstrated its potential to accelerate genetic improvement in buffaloes, its widespread adoption remains constrained by species-specific reproductive physiology, seasonal anestrus, anatomical challenges, and field-level logistical barriers. Nevertheless, advances in hormonal strategies, genomics, cryopreservation, and embryo handling, supported by expanding international collaborations, are steadily improving the reliability and scalability of buffalo MOET. The incremental nature of these gains suggests that continued refinement of existing technologies, rather than disruptive innovations, will drive the most meaningful improvements in superovulatory efficiency.

International partnerships are emerging as key enablers of progress, fostering capacity building, harmonization of species-specific protocols, and data sharing across agroecological zones. These networks support the development of climate-resilient breeding strategies and integrated reproductive programs that combine assisted reproductive technologies with genetic conservation and environmental adoptation. As buffalo production systems evolve toward greater sustainability, MOET is positioned to become a central tool in precision breeding and long-term genetic improvement.

5. RECOMMENDATION

Based on the above review, the actionable recommendations for advancing buffalo reproductive programs are as follows: Standardize buffalo-specific hormonal protocols, particularly for FSH dosing, GnRH/LH timing, and luteal support, and validate them across breeds and seasons. Adopt season-adopted superovulation strategies, including melatonin supplementation and enhanced luteal monitoring during the non-breeding period. Strengthen donor and recipient selection using AMH profiling, Doppler-based luteal assessment, and genomic markers linked to reproductive resilience. Expand field-ready, low-cost MOET packages tailored for smallholder systems, integrating mobile estrus monitoring and simplified synchronization protocols. Invest in capacity building and international collaboration to harmonize protocols, share genetic resources, and support regional centers of excellence. Integrate MOET within broader breeding frameworks, combining ARTs with genomic selection, conservation strategies, and climate-adopted management.

This integrated approach will help transform MOET from a promising technology into a scalable, reliable, and transformative pillar of buffalo genetic improvement.

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