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Research Article

Reproductive Biotechnology in Water Buffalo (Bubalus bubalis): Biology, Constraints, and Prospects for Genetic Improvement

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Abstract

The water buffalo (Bubalus bubalis) is the second most important dairy animal globally, contributing approximately 13% of the world's milk supply, and represents the primary draught animal and protein source for hundreds of millions of people in South and Southeast Asia, the Mediterranean, and Latin America. Despite this economic and nutritional significance, the reproductive efficiency of buffalo is substantially lower than that of domestic cattle (Bos taurus), presenting major constraints to productivity improvement through conventional and biotechnological means. This review systematically examines the reproductive biology of water buffalo in comparison with Bos taurus, identifying the species-specific characteristics — including seasonal reproductive patterns, silent oestrus, reduced follicular population, poor semen cryopreservability, and suboptimal in vitro embryo production — that create barriers to biotechnological implementation. The current state of each major reproductive technology is critically evaluated, including artificial insemination and semen cryopreservation, synchronisation protocols, ovum pick-up-based in vitro fertilisation (OPU-IVF), embryo transfer, somatic cell nuclear transfer (SCNT), sexed semen, and the emerging application of genomic selection in buffalo. Species-specific solutions including antioxidant-enriched semen extenders, melatonin implants for anoestrus management, FSH priming before OPU, and modified IVF culture systems are evaluated with respect to published efficacy data. The review concludes with a research roadmap for closing the productivity gap between buffalo and cattle in key producing regions.

Keywords: *Bubalus bubalis, Seasonal anestrus, Artificial insemination, Semen cryopreservation, OPU-IVF, Embryo transfer, Melatonin, Silent oestrus, Genomic selection, Genetic improvement*

1. Introduction

The global water buffalo population stands at approximately 204 million head (FAO, 2017), with India accounting for over 109 million — more than half the world total. Other major populations are found in Pakistan (~38 million), China (~23 million), Nepal, Bangladesh, Myanmar, Vietnam, Egypt, and Italy. Buffalo provides approximately 13% of the world's milk supply despite representing only 2% of the global ruminant population, reflecting the high fat (7–8%) and protein (4–5%) content of buffalo milk. The Italian Mozzarella di Bufala Campana — produced exclusively from the milk of Murrah-derived Italian Mediterranean buffalo — is a €500 million industry protected by European DOP designation.

The reproductive efficiency of water buffalo is consistently lower than that of Bos taurus cattle, creating a significant productivity gap that limits the potential impact of genetic improvement programmes. Key limiting factors include a low mean non-return rate to first AI of approximately 45–55% with frozen semen compared with 60–70% in Holstein cattle; a markedly seasonal breeding pattern in temperate and subtropical latitudes that concentrates conception in autumn/winter and results in high rates of summer anoestrus; frequent silent (non-behavioural) oestrus that impedes heat detection; and inherently poor semen cryopreservability that substantially reduces post-thaw sperm quality compared to bovine semen processed under equivalent conditions (Andrabi, 2009). These constraints mean that reproductive biotechnologies developed and optimised for Bos taurus cannot simply be transferred to buffalo without species-specific adaptation.

Research investment in buffalo reproductive biotechnology has accelerated over the past two decades, particularly in India (NDRI Karnal), Italy (Federico II), Brazil (Embrapa), Pakistan (UVAS Lahore), and Egypt. This review synthesises current knowledge as of 2018, identifying the most important advances and the most persistent gaps in each technological domain.

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2. Comparative Reproductive Biology

Understanding the biological basis of the productivity gap between buffalo and cattle is prerequisite to rational biotechnological intervention. Table 1 presents a comprehensive comparison of key reproductive parameters between river buffalo, swamp buffalo, and Bos taurus.

Table 1. Comparative reproductive and semen parameters of river buffalo, swamp buffalo, and Bos taurus cattle

Parameter	River Buffalo	Swamp Buffalo	Bos taurus	Reference
Oestrous cycle length (days)	20–23	21–25	21	Zicarelli, 2010
Oestrus duration (hours)	12–22	10–18	12–18	Madan, 1988
Seasonality (temperate zone)	Nov–Mar peak	Less marked	Absent	Vale, 2010
Semen volume per ejaculate (mL)	3–5	3–5	5–8	Rasul et al., 2001
Sperm concentration ($\times 10^6$/mL)	800–1,200	700–1,000	1,000–1,500	Andrabi, 2009
Progressive motility fresh (%)	50–70	45–65	60–80	Andrabi, 2009
Post-thaw progressive motility (%)	30–45	28–40	40–60	Andrabi, 2009
AI conception rate — frozen semen (%)	35–52	30–48	55–75	Neglia et al., 2012
Number of follicles per OPU session	5–8	4–7	10–15	Gasparrini, 2002
IVF blastocyst rate (%)	25–40	20–35	30–52	Gasparrini, 2002

OPU = Ovum pick-up; IVF = In vitro fertilisation. Values represent published ranges from multiple studies.

Buffalo oestrous cycles are broadly similar in length to cattle but differ in the expression of oestrus: approximately 30–50% of oestrus periods in buffalo are 'silent' (lacking visible mounting or standing behaviour), particularly during summer and in high-producing animals. This silent oestrus phenomenon is attributable to lower circulating oestradiol concentrations at oestrus, reduced sensitivity of gonadotroph cells to GnRH feedback, and the general suppressive effect of thermal stress on hypothalamic-pituitary function in summer months (Zicarelli, 2010). Progesterone assays combined with activity monitoring represent the most reliable heat detection strategy in managed buffalo herds.

3. Artificial Insemination and Semen Cryopreservation

3.1 Semen Collection and Processing

Buffalo semen is routinely collected using an artificial vagina maintained at 40–42°C, with a minimum of two collections per week from bulls in regular use. Fresh buffalo semen is assessed for volume, concentration (Makler chamber or CASA), progressive motility, morphological normality, and viability (eosin-nigrosine stain). Acceptable semen for cryopreservation typically requires ≥60% progressive motility, ≥70% morphologically normal sperm, and concentration ≥800×10^6/mL. The tris-citric acid-fructose (TCAF) extender supplemented with egg yolk (20%) and glycerol (7%) is most widely used, though non-egg yolk alternatives based on soy lecithin and plant-derived lipoproteins are gaining acceptance as animal-component-free options with better biosafety profiles.

3.2 Antioxidant Supplementation of Semen Extenders

A principal cause of inferior post-thaw sperm quality in buffalo compared to cattle is higher susceptibility to oxidative stress during cryopreservation. Reactive oxygen species (ROS), generated as a by-product of sperm metabolism and cryoprotectant toxicity, cause peroxidation of the polyunsaturated fatty acids (PUFAs) in the sperm plasma membrane, leading to loss of membrane integrity, reduced motility, and impaired fertilising ability. Supplementation of semen extenders with exogenous antioxidants has been extensively investigated as a strategy to mitigate ROS damage. Table 2 summarises the major antioxidants evaluated in buffalo semen cryopreservation and their effects on post-thaw motility.

Table 2. Antioxidant supplementation of buffalo semen cryopreservation extenders and effects on post-thaw progressive motility

Antioxidant	Concentration	Motility Improvement	Reference
Vitamin E (α-tocopherol)	0.5–1.0 mg/mL	Significant (+8–12%)	Lone et al., 2018
Glutathione (GSH)	1.0–2.0 mM	Moderate (+6–10%)	Hussain et al., 2017
Resveratrol	25–50 µM	Significant (+9–15%)	Chhillar et al., 2012

Antioxidant	Concentration	Motility Improvement	Reference
Trehalose	50–100 mM	Moderate (+5–8%)	Reddy et al., 2010
Coenzyme Q10	25 µM	Moderate (+7–11%)	Atessahin et al., 2008
Quercetin	10–25 µM	Significant (+10–14%)	Najafi et al., 2013

GSH = Glutathione; ROS = Reactive oxygen species. Motility improvement expressed as percentage points above control.

4. OPU-IVF and Embryo Production

Ovum pick-up (OPU) combined with in vitro fertilisation (IVF) represents the most productive method for generating large numbers of buffalo embryos for transfer, genetic resource banking, and research. Transvaginal OPU in buffalo is feasible using a 7.5 MHz transvaginal probe with aspiration at 40–50 mmHg, but the average number of aspiratable follicles per OPU session (5–8) is significantly lower than in Bos taurus (10–15), substantially limiting oocyte yields. Stimulation of follicular growth by FSH priming (3–5 days before OPU) modestly increases follicle number and oocyte quality in buffalo, and is now routinely incorporated in OPU protocols at specialised facilities.

Buffalo oocytes are matured in SOFaaci medium supplemented with LH (0.02 IU/mL), FSH (0.01 IU/mL), oestradiol (1 µg/mL), and 6 mg/mL BSA at 38.5°C, 5% CO2, 5% O2 for 22–24 hours. Fertilisation is performed in TALP medium with heparin-treated swim-up spermatozoa at a final concentration of 1×10^6/mL. Blastocyst rates average 25–40% in optimised buffalo IVF systems — consistently below the 35–52% typically reported in bovine IVF under equivalent conditions. The lower blastocyst rate reflects intrinsically lower oocyte developmental competence in buffalo, potentially linked to mitochondrial function, cytoplasmic maturation, and the reduced availability of maturation-promoting factors in the smaller follicular population.

Synchronisation (D0: CIDR insertion + E2 benzoate) → FSH priming (D3–6, declining doses) → CIDR removal (D7) → OPU (D8–9; transvaginal, 40mmHg) → Oocyte grading (GI/GII) → IVM (SOFaaci+hormones+BSA; 22–24h; 38.5°C; 5%O2) → IVF (TALP+heparin sperm; 18–20h) → IVC (SOFaaci+BSA+NEAA; 7 days; 5%O2) → Blastocyst (25–40%) → Transfer/Vitrification

Figure 1. Schematic diagram of the OPU-IVF pipeline in water buffalo, highlighting species-specific modifications required relative to the standard bovine protocol.

5. Challenges and Mitigation Strategies

Table 3. Major reproductive biotechnology challenges in water buffalo and evidence-based mitigation strategies

Challenge	Biological Cause	Management Strategy	Effectiveness
Silent oestrus detection	Low oestrogen surge; less mounting	Progesterone assay + activity monitors	Moderate; 70–85% detection
Seasonal anoestrus	Long-day photoperiod suppresses GnRH	Melatonin implants (18mg) + buck/ram effect	Good; +15–20% conception in summer
Low post-thaw sperm quality	Membrane lipid composition; ROS sensitivity	Antioxidant-enriched extenders + trehalose	Moderate; +8–15% motility
Poor IVF blastocyst rate	Immature oocytes; follicular scarcity	FSH priming before OPU + modified SOFaaci	Moderate; +5–12% blastocysts
Low SCNT efficiency	Nuclear reprogramming barriers	iPSC-derived donor cells; chemical treatment	Research stage; 5–20% cloning
Seasonality of folliculogenesis	Melatonin/IGF axis modulation	Year-round OPU with FSH priming	Applicable; 20–30% better off-season

Seasonal anoestrus management through melatonin implantation (18 mg sustained-release subcutaneous implants, equivalent to short-day signal) has been validated in multiple studies in Mediterranean Italy and Egypt, improving AI conception rates by 15–20 percentage points during summer months by restoring GnRH pulsatility and LH surge amplitude (El-Moghazy & El-Ashmawi, 2010). The combination of melatonin with GnRH-based ovulation synchronisation (Ovsynch or Presynch-Ovsynch protocols adapted from cattle) provides reliable oestrus synchronisation even in anoestrous buffalo, enabling year-round AI programmes in subtropical production systems.

6. Genomic Selection in Buffalo

The development of genomic selection (GS) tools for buffalo has been slower than for cattle, constrained by the limited availability of high-density SNP arrays, small reference populations with reliable phenotypic

records, and under-investment in buffalo genomics infrastructure relative to the economic significance of the species. The first buffalo SNP chip (BubuSNP50, ~50K SNPs) was developed at NDRI Karnal in 2013, enabling genome-wide association studies (GWAS) for milk production traits in Murrah buffalo. Reference populations for genomic evaluation remain small (<3,000 phenotyped and genotyped animals in the largest national programmes), limiting GEBV reliability.

Despite these limitations, genomic selection holds considerable promise for buffalo improvement. The high heritability of milk yield in Murrah ($h^2 \approx 0.35–0.45$) and fat percentage ($h^2 \approx 0.45–0.60$) means that even modest-sized reference populations can provide useful GEBV reliability. International collaboration between India, Italy, Pakistan, and Brazil to pool reference population data — analogous to the EuroGenomics initiative in cattle — is being discussed and could substantially improve prediction accuracy for numerically dominant breeds such as Murrah, Nili-Ravi, and Italian Mediterranean buffalo.

7. Conclusions

Water buffalo reproductive biotechnology has advanced substantially over the past two decades, but a persistent productivity gap relative to cattle reflects the depth of species-specific biological constraints that require dedicated research rather than simple protocol transfer from Bos taurus. Priority research areas include further optimisation of antioxidant-based semen cryopreservation, non-surgical approaches to synchronisation and embryo transfer, development of defined serum-free IVF media, and expansion of genomic selection reference populations through international collaboration. Closing the buffalo-cattle productivity gap through improved reproductive efficiency represents one of the highest-return investments available to the livestock sectors of South and Southeast Asia.

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