

Review: Development, adoption, and impact of assisted reproduction in domestic buffaloes



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ABSTRACT

The domestic buffalo (*Bubalus bubalis*), also known as water buffalo, comprises two sub-species the River buffalo (*B. bubalis* ssp. *bubalis*; 50 chromosomes) and the Swamp buffalo (ssp. *carabanensis*; 48 chromosomes). Domestic buffaloes are a globally significant livestock species. In South Asia, the River buffalo is a primary source of milk and meat and has a very important role in food security. The River buffalo also supports high-value, differentiated food production in Europe and the Americas. The Swamp buffalo is an important draft animal and a source of food in Southeast Asia and East Asia. The growing importance of buffaloes requires that they undergo an accelerated rate of genetic gain for efficiency of production, product quality, and sustainability. This will involve the increased use of assisted reproduction. The initial application of reproductive technology in buffaloes had variable success as it relied on the adoption of procedures developed for cattle. This included artificial insemination (AI), sperm cryopreservation, and embryo technologies such as cloning and *in vitro* embryo production (IVEP). Reproductive technology has been progressively refined in buffaloes, and today, the success of AI and IVEP is comparable to cattle. Ovarian follicular superstimulation (superovulation) combined with *in vivo* embryo production results in low embryo recovery in buffaloes and has limited practical application. The contribution of elite female buffaloes to future genetic improvement will therefore rely mainly on oocyte pickup and IVEP. This will include IVEP from females before puberty to reduce generation intervals. This review provides for the first time a clear chronology on the development, adoption, and impact, of assisted reproduction in domestic buffaloes.

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Implications

Assisted reproduction has a very important role in the continuous genetic improvement of domestic buffaloes in different environments and production systems globally. In different parts of the world, buffaloes are important either for food security or high-value, differentiated food. Reproductive technology needs to be practical and efficient and must help deliver the goals of both small and large production systems. Artificial insemination and the production of embryos in the laboratory have been identified as the two current assisted reproductive technologies with the greatest application to facilitate genetic improvement in buffaloes.

Introduction

Assisted reproduction can be considered an enabling technology that allows the livestock industries to achieve faster rates of genetic gain by making greater use of individual males and females with commercially important traits (Ponsart et al., 2014; Granleese et al., 2015; Kasinathan et al., 2015). Assisted reproduction can also be used to manipulate reproduction in females. This includes synchronization of the time of breeding, influencing the age at first breeding, the interval between the calving and the first breeding, and breeding during seasonal anestrus. While an enabling technology, assisted reproduction itself is built on fundamental discovery science that creates new knowledge on reproductive biology. For example, oocyte pickup and *in vitro* fertilization were made possible because of basic studies on ovarian folliculogenesis, oocyte maturation, and early embryonic development. It is common for

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Table 1Chronology of assisted reproduction in domestic buffaloes (*Bubalus bubalis*). Reports that could be considered relevant but are in difficult to source publications are not included.

Year	Assisted breeding technology	References
1939/1943 1956	Artificial insemination Semen cryopreservation	Bhattacharya, 1962, 1968, and 1974; Lonergan, 2018; Vale et al., 2022 Bhattacharya and Srivastava, 1955; Roy et al., 1956; Lonergan, 2018; Kumar et al., 2022; Vale et al., 2022
1964 1979 1983	Pregnancy with frozen-thawed sperm Estrus synchronization <i>In vivo</i> embryo recovery and embryo transfer, and ovarian follicular superstimulation	Basirov, 1964; Kumar et al., 2022 Kamonpatana et al., 1979; de Rensis and López-Gatius, 2007 Drost et al., 1983; Karaivanov et al., 1987; Chantarpateep et al., 1988; Misra et al., 1990
1989/91/92	<i>In vitro</i> fertilization (IVF)	Singh et al., 1989; Totey et al., 1992; Suzuki et al., 1992; Madan et al., 1994; Gasparrini et al., 2001
1994 1998	Oocyte pickup (OPU) OPU + IVF	Boni et al., 1994; Konrad et al., 2017 Galli et al., 1998
2002/2003	Synchronization and fixed-time artificial insemination	Berber et al., 2002; Baruselli et al., 2003; Neglia et al., 2003b; Gutiérrez-Añez et al., 2022; Jeyakumar et al., 2022
2005 2007	Sexed sperm Cloning	Presicce et al., 2005; Presicce, 2013 and 2022 Shi et al., 2007; Selokar et al., 2018
2017/18	Juvenile <i>in vitro</i> embryo transfer (JIVET)	Baldassarre et al., 2017; Silva, 2017; Baldassarre and Bordignon, 2018; Baruselli et al., 2018; Baldassarre, 2021

assisted reproduction protocols that are developed in one species to be translated to another species. An example is the adoption in buffaloes of estrus synchronization protocols originally designed for cattle (Berber et al., 2002; Baruselli et al., 2003; Neglia et al., 2003a). Assisted reproduction is progressively refined to be species-specific as new knowledge emerges on the reproductive biology of different species. This has certainly been the case for domestic buffaloes where initial studies on artificial insemination (AI) through to cloning have relied on information gained from work undertaken mainly in cattle, but also in small ruminants including sheep and goats.

Domestic buffaloes (*Bubalus bubalis*), also known as water buffaloes, comprise two sub-species the River buffalo (*B. bubalis* ssp. *bubalis*; 50 chromosomes) and the Swamp buffalo (ssp. *carabanensis*; 48 chromosomes) (Colli et al., 2022). River buffaloes occur predominantly in South Asia, Europe, Middle-East and the Americas while Swamp buffaloes are found mainly in Southeast and East Asia (Borghese et al., 2022). Domestic buffaloes are a globally significant livestock species. In South Asia, the larger River buffalo is a primary source of milk and meat and has a very important role in food security. The River buffalo is also an important source of high-value, differentiated food such as mozzarella cheese in Europe, Middle-East and the Americas. The Swamp buffalo is predominantly a draft animal in Southeast and East Asia (Borghese et al., 2022). Assisted reproduction has a fundamental role in ensuring there is continuing genetic improvement in domestic buffaloes, and for the manipulation of reproduction generally. This review provides for the first time a chronology of the development, adoption, and impact of assisted reproduction in domestic buffaloes, with a particular focus on water buffaloes where most developments in assisted reproduction have occurred for buffaloes. The approach adopted is to present the first reported use of a particular assisted reproduction technology in water buffaloes, followed by discussion on refinements of the technology, adoption, and impact. A series of comprehensive reviews on buffalo production, genetics, and global distribution have recently been published, and these areas are not covered in this review (Chauhan and Selokar, 2022).

Assisted reproduction in domestic buffaloes

The chronology of assisted reproduction in domestic buffaloes is summarized in Table 1.

Artificial insemination and semen cryopreservation

Similar to cattle, the first assisted reproduction technology applied in buffaloes was artificial insemination. The literature

reports that the first buffalo calf born to AI was produced by Bhattacharya and colleagues in 1943 (Bhattacharya, 1962, 1968; Vale et al., 2022). Unfortunately, it is difficult to source literature on methods used for the collection and processing of semen for the first AI in buffaloes. In the absence of this information, it has been assumed that egg yolk-based semen extenders, which were undergoing development in cattle, were also used in buffaloes (Phillips and Lardy, 1940; Layek et al., 2016; Lonergan, 2018; Vale et al., 2022). As with other livestock, fresh extended semen had limited application in buffaloes and widespread adoption of AI only became possible with sperm cryopreservation (Bhattacharya and Srivastava, 1955; Roy et al., 1956; Roy and Ansari, 1973; Lonergan, 2018; Kumar et al., 2022; Ohashi et al., 2022). Buffalo sperm are more susceptible to damage when frozen-thawed and considerable research has been undertaken on refining conditions for optimizing sperm cryopreservation in buffaloes (Osorio-Meléndez, 2013; Shah and Andrabi, 2021; Kumar et al., 2022; Quintero-Moreno et al., 2022; Vale et al., 2022).

Advances in AI with both fresh and frozen sperm are a major reason for the global increase in milk production in buffaloes from about 20 million tons in 1950 to 180 million tons in 2019 (Gowane and Vohra, 2022). A large part of this increase has occurred in India where, as noted earlier, buffalo milk and meat are very important in food security (Gowane and Vohra, 2022). Artificial insemination has also made possible improvements in the quality of buffalo milk in Asia, Europe, and the Americas (Neglia et al., 2020; Khetra et al., 2022). It could be argued that the development and adoption of AI have been a major factor in making buffaloes a globally important livestock species.

Synchronization of estrus and ovulation, and fixed-time artificial insemination

Artificial insemination in buffaloes initially relied on the detection of estrus (Vale et al., 1994; Baruselli, 1994). However, estrous detection is problematic in buffaloes because of the low intensity of estrus and the wide variation in estrous duration (4–64 hours) (Zicarelli, 1997). For AI to be broadly adopted in buffaloes, it was necessary to develop estrus synchronization protocols similar to cattle (Pursley et al., 1995; Bó et al., 2003). Estrus synchronization also synchronizes ovulation and permits the use of fixed-time AI (FTAI) (Bó et al., 2003). The earliest attempt to control the estrous cycle in buffaloes used PGF_{2α} (Kamonpatana et al., 1979). It was later shown that, as with cattle, combinations of GnRH, estradiol, progestogens, and PGF_{2α} can be used to control ovarian follicular waves and the time of ovulation in buffaloes (Baruselli et al., 2007; Neglia et al., 2016; de Carvalho et al., 2016). Equine chorionic

gonadotropin (**eCG**) can be added at the end of a synchronization protocol to facilitate the final stages of follicle/oocyte development, tighten the time of ovulation, and increase fertility mainly in anestrus buffalo (Carvalho et al., 2013). Estrus synchronization protocols and FTAI are now routinely used in the breeding and non-breeding seasons in buffaloes (de Carvalho et al., 2016). Pregnancy rates are, however, lower when estrus synchronization + FTAI are used in the non-breeding season in buffaloes synchronized with the GnRH + PGF_{2α} + GnRH-based protocol Ovsynch (Pursley et al., 1995; Baruselli et al., 1999). This is because of the higher incidence of anestrus (Carvalho et al., 2021) and relatively high embryonic mortality in buffaloes bred during the non-breeding season (Campanile et al., 2016).

The development of estrus synchronization in combination with AI has been a powerful assisted reproduction platform to accelerate the dispersal of improved genetics in buffaloes. As noted above, there have been major advances in both the quantity and quality of milk in buffaloes, particularly in South Asia, Europe, and the Americas.

Embryo transfer and ovarian follicular superstimulation

Embryo transfer from donor females to recipients allows individual female buffaloes with commercially important traits to make a greater contribution to genetic improvement (Baruselli et al., 2020). The first successful embryo transfer in buffaloes involved the non-surgical collection of a single 7-day blastocyst from a donor and non-surgical transfer to a recipient (Drost et al., 1983). The power of embryo transfer is greatly increased by the collection of multiple embryos from elite donor females. This requires ovarian follicular superstimulation, commonly referred to as superovulation. Early attempts at superovulation in buffaloes drew from experiences in cattle with FSH, PMSG and GnRH in combination with PGF_{2α} (Drost et al., 1983; Karaivanov et al., 1987; Chantaraprateep et al., 1988; Misra et al., 1990). The number of embryos recovered in these early studies was low, and this has remained a feature of superovulation in buffaloes. Notwithstanding these challenges, first-lactation buffaloes generated by embryo transfer from elite donors had greater milk production than buffaloes generated by AI (Castanheira et al., 2021).

Ultrasound monitoring during superovulation in buffaloes shows the growth on average of around 10 follicles (>8 mm; Baruselli et al., 2000). The ovulation rate after treatment averages 63% which is similar in cattle (Desaulniers et al., 1995; Shaw et al., 1995; Stock et al., 1996). However, the main difference between buffaloes and cattle is the low embryo recovery rate (embryos and ova/corpus luteum) in buffaloes of 20–40% compared with 60–80% in cattle (Vos et al., 1994; Shaw et al., 1995). The low embryo recovery rate in buffaloes is thought to be due to failure of the fimbriae to capture oocytes and transfer them to the oviducts (Baruselli et al., 2000). It may also be partly due to follicle rupture and formation of luteal tissue without ovulation. The inefficient low embryo recovery in superovulated buffaloes has restricted the widespread adoption of superovulation as an assisted reproduction technology in buffaloes (Baruselli et al., 2020).

In vitro fertilization

The failure of superovulation as a viable assisted reproduction technology to exploit female genetics in buffaloes has directed focus to *in vitro* fertilization (**IVF**) (Marin et al., 2019; Ohashi et al., 2022). Indeed, oocyte pickup (**OPU**) and *in vitro* embryo production (**IVEP**) are seen as important assisted reproduction technologies for producing a relatively large number of embryos in buffaloes (Konrad et al., 2017; Sakaguchi et al., 2019). Hence, this

area is reviewed in some detail given the importance of IVEP for future genetic improvement in buffaloes. The present major constraint of OPU/IVEP in buffaloes is the relatively small number of oocytes that can be recovered from donors. This is due to the small follicular reserve in buffaloes (Danell 1987; Carvalho et al., 2007) and modest response to follicular superstimulation treatment prior to OPU (Petrovas et al., 2020). Another limiting factor is seasonality in buffaloes that is associated with reduced oocyte competence during the non-breeding season (di Francesco et al., 2011; 2012). This restricts practical OPU/IVEP to the breeding season to optimize the use of assisted reproduction in buffaloes. The low efficiency of IVEP in buffaloes during the 1990s was essentially due to the direct transfer of procedures developed in cattle to buffaloes. The subsequent refinement of IVEP procedures has led to relatively high rates of blastocyst development in buffaloes (Gasparrini et al., 2006; 2008).

Oocyte maturation

Buffalo oocytes commonly undergo IVM in TCM199 supplemented with serum and hormones including gonadotrophins and 17β-estradiol (Totey et al., 1993; Chauhan et al., 1998; Samad et al., 1998). Improved oocyte competence can be achieved by enriching the maturation medium with IGF-I, IGF-2, EGF, FGF and insulin (Nandi et al., 2003; Pandey et al., 2009). The length of IVM is a critical factor for *in vitro* embryo production as it affects chromatin anomalies (Dominko and First, 1997), oocyte aging (Hunter and Greve, 1997) and development (Marston and Chang, 1964). Most buffalo oocytes attain metaphase II between 20 and 24 h of culture. Both cleavage rate and blastocyst rate progressively decline when fertilization is carried out at increasing postmaturation times from 18–30 h (Gasparrini et al., 2008). Hence, fertilization should be performed as early as 18 h of IVM and not later than 24 h. Delaying fertilization beyond 24 h is associated with a higher proportion of degenerated oocytes and poorer developmental competence (Neglia et al., 2001). This indicates that buffalo oocytes mature relatively early during IVM.

Buffalo oocytes have a high lipid content and are susceptible to oxidative stress (Boni et al., 1992). The inclusion of antioxidants during IVM has achieved a major improvement in blastocyst yield in buffaloes. In an early study, the provision of a thiol compound such as cysteamine during IVM increased blastocyst production by stimulating glutathione (**GSH**) synthesis by oocytes (Gasparrini et al., 2000; 2003). The enrichment of IVM medium with cystine in the presence of cysteamine also increases the intra-oocyte GSH reserve, resulting in improved fertilization, cleavage, and embryo yield (Gasparrini et al., 2006). Other antioxidants including taurine and melatonin added to IVM medium improve blastocyst development in buffaloes (Manjunatha et al., 2009).

In vitro fertilization

A low cleavage rate was initially the most inefficient step in IVEP in buffaloes (Galli et al., 2001; Neglia et al., 2003a; Gasparrini et al., 2004). The cleavage rate was higher with fresh sperm compared with frozen-thawed sperm (Totey et al., 1992), and this was consistent with the susceptibility of buffalo sperm to cryopreservation damage (Muer et al., 1988). Cryopreservation has improved in buffaloes and fertilization, and cleavage rates are now similar for fresh and frozen sperm (Wilding et al., 2003). However, variation among bulls to cryopreservation damage remains an unresolved constraint in buffalo IVEP (Wilding et al., 2003). The initial screening of bulls is recommended as bulls of high genetic merit may not be suitable for IVEP in genetic improvement programs.

The duration of sperm-oocyte co-incubation can be another limiting factor in IVEP as the high sperm concentration in a small volume of medium is associated with elevated amounts of hydro-

lytic enzymes (Rehman et al., 1994) and reactive oxygen species (Aitken and Fisher, 1994). An optimal co-incubation time in buffaloes appears to be 16 h (Gasparrini et al., 2008). However, this varies among bulls (Rubessa et al., 2009) and requires the preliminary assessment of individual bulls before large-scale use in industry. With progressive refinements, the cleavage rate is at 75–80% which has helped to make IVEP a viable and practical assisted reproduction technology in buffaloes.

Blastocyst/embryo culture

Buffalo embryos were first cultured *in vivo* in an intermediate host such as the ligated oviducts of sheep (Galli et al., 1998). This was followed by cell co-culture systems (Totey et al., 1992; Madan et al., 1994) and then defined media including synthetic oviduct fluid and potassium simplex optimized medium (Caracciolo di Brienza et al., 2001). Major increases in blastocyst yield in buffaloes have resulted from improvements in IVM whereas advances in IVC have had a lesser impact. The refreshment of IVC medium to remove reactive oxygen species, ammonia, and waste products of metabolism does not impact embryo development in buffaloes as occurs for other species (Boccia et al., 2006). Indeed, buffalo embryos are more susceptible to changes in temperature compared with other species and the fluctuation in temperature that occurs during the refreshment of IVC culture can be detrimental in buffaloes. Also, buffalo embryos require relatively high concentrations (1.5 mM) of glucose during early embryonic development (up to day 4) (Suárez et al., 2011) whereas sheep and cattle embryos show increased glucose consumption during late culture when compaction occurs (Thompson et al., 1991; 1996). The supplementation of IVC medium with hyaluronic acid during late culture improves the cryotolerance of buffalo embryos (Boccia et al., 2012).

Buffalo IVF embryos are advanced in development by 12–24 h compared with cattle IVF embryos 12–24 h (Galli et al., 2001). Blastocysts are observed at day 6 of IVC and most are embryos by day 7. Blastocysts that have a slower development and are embryos by day 8 have a lower cryotolerance (Gasparrini et al., 2001) and are associated with a lower pregnancy rate after transfer (Boccia et al., 2013). Buffalo *in vivo* embryos recovered at day 6.5 after estrus are mostly hatched blastocysts (Drost and Elsdén, 1985).

Oocyte pickup

OPU combined with IVEP allows greater use in genetic improvement programs of female buffaloes with high genetic merit (Boni et al., 1994). The application of OPU in buffaloes is somewhat restricted because of the low number of ovarian follicles and viable oocytes recovered (Gasparrini, 2002; Campanile et al., 2010; Gimenes et al., 2015) and a seasonal effect on oocyte quality (Gasparrini, 2018). Also, buffaloes show large individual variation in the number of antral follicles (AFCs) (Baruselli et al., 1997) and this is associated with a large variation in the number of oocytes recovered by OPU (range of 0–30; mean 8.9 ± 5.0 per donor; Fig. 1; Baruselli et al., 2018). The AFC is, however, highly repeatable within individual buffaloes which provides the opportunity to screen potential oocyte donors to optimize the efficiency of IVEP (Ireland et al., 2007; Batista et al., 2014).

Circulating concentrations of Anti-Mullerian Hormone (AMH) provide an endocrine marker of the AFC and can be used to screen potential oocyte donors (Ireland et al., 2008; Monniaux et al., 2012). In cattle, AMH is a marker for the AFC, the response to follicular superstimulation (Rico et al., 2009), and the response to OPU-IVEP (Guerreiro et al., 2014; Gamarra et al., 2015; Vernunft et al., 2015). In a comparison of cattle and buffaloes, the AFC was greater in Gir (*Bos indicus*) compared with both Holstein (*Bos taurus*) and Murrah buffaloes (Baldrigi et al., 2014). In another study,

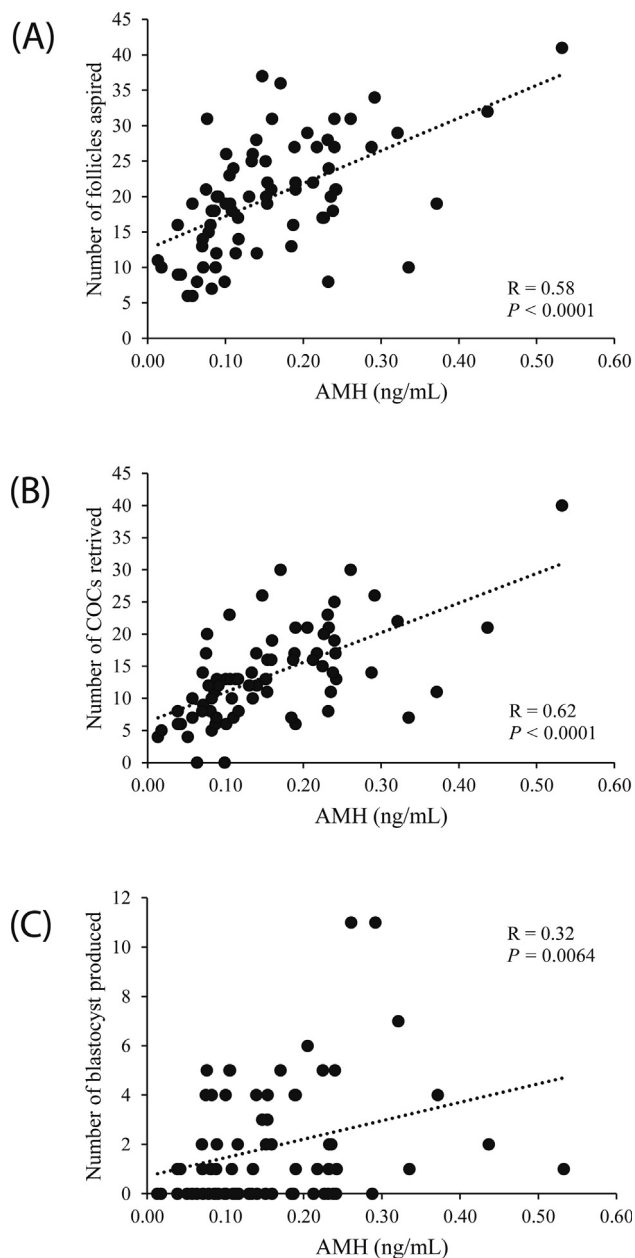


Fig. 1. Correlation between plasma anti-mullerian hormone (AMH) concentrations and number of follicles aspirated (A), total COCs (cumulus-oocyte complexes) retrieved (B) and number of blastocysts produced (C) per buffalo donor ($n = 73$). Blood samples for plasma AMH determination were collected immediately before the ovum pickup (OPU) session (source: Chello, 2020).

there was a positive association between AMH and AFC and AMH and IVEP in buffaloes (Fig. 2; Chello, 2020). This finding showed that AMH can be used as an endocrine marker for AFC and IVEP to optimize the efficiency of this reproductive technology in buffaloes.

Follicular superstimulation with FSH before OPU increases the number of medium and large follicles in buffalo heifers, and primiparous and multiparous cows (de Carvalho et al., 2019). This is associated with a greater number of viable oocytes available for OPU-IVEP which further optimizes the efficiency of this assisted reproduction in buffaloes. At higher latitudes, the time of year influences oocyte quality and the response to IVEP (Gasparrini, 2018). A greater number of embryos are produced by IVEP during short days which coincides with the breeding season at higher

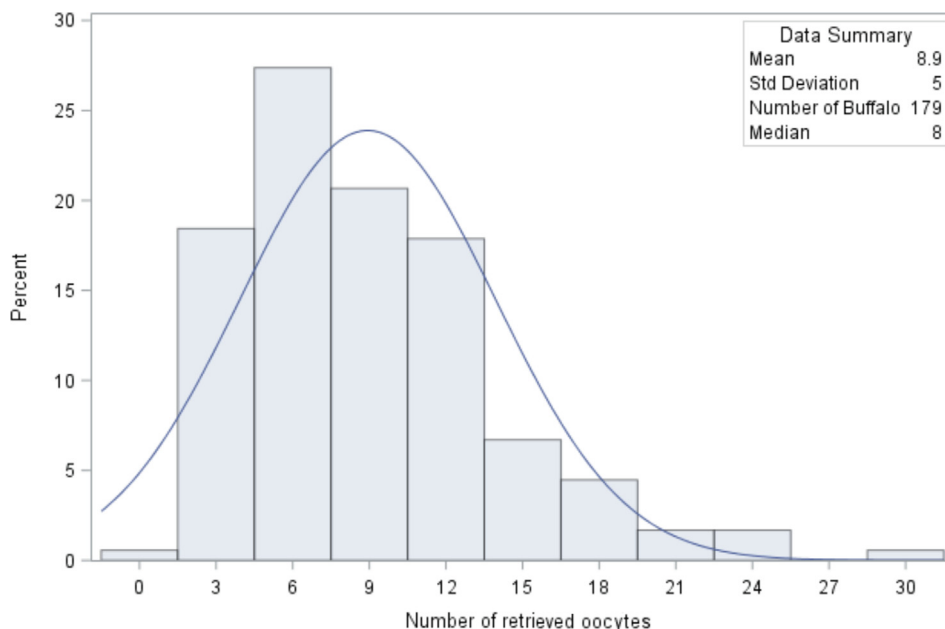


Fig. 2. Distribution of number of oocytes (COC) retrieved per ovum pickup (OPU) session in Murrah buffalo donors (n = 179; Source: Baruselli et al., 2020).

latitudes (di Francesco et al., 2012). There is less evidence of seasonal changes in oocyte quality and IVEP in tropical regions (Macabelli et al., 2012); however, the relationship between time of year and OPU/IVEP requires further research in buffaloes.

Sexed sperm

The history and development of sperm sexing technology, and applications in livestock production including buffaloes, have been thoroughly reviewed (Vishwanath and Moreno, 2018; Presicce, 2022; Yata, 2022). In the first report on the use of sexed sperm in buffaloes, sorted sperm were deposited at the utero-tubal junction in Ovsynch synchronized females which resulted in impressive pregnancies of 43.3% (X-sperm) and 42.8% (Y-sperm) (Presicce et al., 2005). This was equivalent to pregnancies with standard frozen-thawed buffalo sperm and showed the potential for applying sexed semen technology in buffaloes, both *in vivo* (Lu et al., 2010; Presicce, 2022) and *in vitro* (Liang et al., 2008; Presicce, 2022). The *in vivo* application was confirmed by studies that reported similar pregnancy rates with sexed and conventional buffalo sperm (sexed 49.3%; conventional 45.2%) (Campanile et al., 2011; 2013). In the latter studies, higher pregnancy was achieved with sexed sperm deposited in the body of the uterus compared with the horn of the uterus (Campanile et al., 2011). A noteworthy observation has been the difference between buffalo bulls in the fertilizing ability of sperm following sex sorting (Lu et al., 2010). The latter is an area that requires further research to optimize the adoption and impact of sexed sperm in assisted reproduction in buffalo production (Lu et al., 2015).

Cloning

This review only covers cloning in buffaloes that has resulted in the birth of live calves and does not include laboratory experimentation using different cloning methodologies to produce cloned blastocysts (Pandey et al., 2010; Mohapatra et al., 2015). The first live cloned buffalo calves resulted from established somatic cell nuclear transfer using fetal fibroblasts and granulosa cells (Shi et al., 2007). Subsequent reports of cloned calves in buffaloes are summarized in earlier reviews and are not covered in detail here

(Selokar, 2018; Selokar et al., 2018). Cloning has been adopted in India to clone bulls with elite genetics. The cloned bulls are used to obtain semen that is cryopreserved and distributed for use in national buffalo genetic improvement programs (Selokar, 2018; Selokar et al., 2019). This should accelerate the genetic improvement of buffaloes in India and is a good example of the impact-assisted reproduction can have on livestock production resulting in economic and social benefits. Notwithstanding the application in India, technical challenges and inefficiencies have limited the adoption of cloning for genetic improvement in buffaloes.

The emergence of CRISPR-Cas9 technology has provided the opportunity to achieve targeted genetic change in livestock (Komor et al., 2017; Lilloco, 2019; Hansen, 2020; Whitelaw and Lilloco, 2022). CRIPR-Cas9 builds on established somatic cell nuclear transfer and *in vitro* embryo procedures and shows the potential to be a transformative assisted reproduction technology for genetic improvement in livestock (Menchaca et al., 2020; Jabbar et al., 2021; Perisse et al., 2021; Mehra and Kumar, 2021).

Juvenile *in vitro* embryo transfer

Juvenile *in vitro* embryo transfer (JIVET) is an assisted reproduction technology that provides the opportunity to significantly reduce generation intervals and accelerate genetic improvement in buffaloes. It involves the recovery of oocytes from prepubertal buffalo heifers combined with IVF to produce transferable embryos. The ovaries of prepubertal buffalo heifers were reported to have 10 000–15 000 primordial follicles (Danell 1987; Carvalho et al., 2007). Ovarian follicular waves are established in prepubertal heifers, and follicles are responsive to follicular superstimulation treatments (Baldassarre and Bordignon, 2018; Baldassarre, 2021). Hence, it is possible to stimulate follicular growth in prepubertal buffalo heifers and recover oocytes for IVF (Baldassarre and Bordignon, 2018). In calves, oocytes are recovered using laparoscopic ovum pickup (LOPU) (Silva et al., 2017; Baldassarre and Bordignon, 2018; Baruselli et al., 2018).

The first reports of JIVET births in buffaloes were in 2017/2018 (Baldassarre et al., 2017; Silva, 2017; Baldassarre and Bordignon, 2018; Baruselli et al., 2018). In one of our studies, embryo production from buffalo calves (2–4 months of age) was compared with

Table 2

Number of oocytes retrieved and blastocysts (mean \pm SEM) after laparoscopic ovum pickup and *in vitro* embryo production (LOPU-IVP) in buffalo donor calves and after ovum pickup and *in vitro* embryo production (OPU-IVP) in buffalo prepubertal heifers and lactating cows (adapted from [Silva et al., 2017](#)).

Item	Category			P-value
	Calves	Prepubertal heifers	Lactating cows	
Number	8	10	10	
Total oocytes retrieved, n	10.9 \pm 3.3 ^{ab}	15.5 \pm 2.1 ^a	5.8 \pm 1.3 ^b	0.007
Viable oocytes, n	7.63 \pm 2.7	6.20 \pm 1.6	3.20 \pm 0.9	0.11
Viable oocytes rate, %	63.9 ^a	39.3 ^b	54.1 ^a	0.01
Total oocytes cleaved, n	2.75 \pm 0.9	3.10 \pm 0.7	2.10 \pm 0.4	0.52
Cleavage rate, %	30.3 ^{ab}	20.8 ^b	37.6 ^a	0.04
Viable embryos, n	1.00 \pm 0.57 ^b	1.50 \pm 0.34 ^a	1.10 \pm 0.38 ^{ab}	0.02
Embryos rate, %	5.1 ^b	9.3 ^a	15.4 ^a	0.05

^{a,b,c} Values within a row with different superscripts differ significantly at the *P*-value presented.

prepubertal heifers (13–15 months of age) and lactating buffalo cows ([Silva et al., 2017](#); [Baruselli et al., 2018](#)). Calves were treated with a sheep intravaginal progestin device on day 0, and follicular growth was stimulated with FSH (140 mg) in four decreasing doses at 12-h intervals on days 5–6. Oocytes were recovered on day 7 by LOPU in calves and by transvaginal follicular aspiration (OPU) on a random day of the estrous cycle in prepubertal heifers and lactating cows. Both LOPU and OPU were carried out on the same day, and semen from one bull was used for IVF. Calves had a lower blastocyst production rate, but the number of embryos produced was similar between calves and lactating cows ([Table 2](#)). Embryos produced from calves ($n = 8$) resulted in three pregnancies (38%; 3/8) which progressed to the birth of three healthy calves ([Silva et al., 2017](#)). This demonstrated the feasibility of JIVET to significantly reduce generation interval and accelerate genetic progress in buffaloes. However, follicles and oocytes in calves are not exposed to the normal cyclical patterns of gonadotropic stimulation that occur after puberty, and calf oocytes have a lower efficiency of embryo production ([Baldassarre and Bordignon, 2018](#); [Baldassarre, 2021](#)). Further research is required on the *in vivo* follicular stimulation treatments, and the *in vitro* oocyte/embryo procedures, for JIVET to become a practical assisted reproduction technology for broad adoption in buffaloes.

Summary

This review has provided for the first time a clear chronology on the development, adoption, and impact, of assisted reproduction in domestic buffaloes. The global significance of buffaloes as a livestock species is growing, especially throughout Asia where it is particularly important for food security. Buffaloes are also growing in importance for high-value, differentiated food production in Europe and the Americas. The demand for continued genetic improvement in buffaloes will only be met by increased utilization of assisted reproduction. Artificial insemination and sperm cryopreservation are established and practical in buffaloes. Cloning remains restricted notwithstanding the novel application in India to multiple elite bulls for greater semen cryopreservation and distribution in national genetic improvement programs. *In vivo* embryo production is inefficient in buffaloes. Hence, the contribution of elite females to genetic gain will increasingly involve the harvesting of oocytes and IVEP. This will include gene marker-assisted selection and IVEP from females before puberty to reduce generation intervals. CRISPR-Cas9 technology complements genome mapping and IVEP, and the integration of these technologies has the potential to transform the rate of genetic improvement in buffaloes. The global sharing of buffalo germ plasm will be fundamental for buffaloes to achieve their full potential as a globally significant livestock species. The scale-up of assisted reproduction has been relatively recent in buffaloes but it is expected to have the

same cost-effectiveness and impact on production as achieved in cattle ([Baruselli et al., 2018](#)).

Ethics approval

Not applicable.

Data and model availability statement

Data/models were not deposited in an official repository. No new datasets were created.

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Declaration of interest

The authors declare no conflicts of interest.

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Transparency Declaration

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