Animal 17 (2023) 100764

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

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

Pietro S. Baruselli [a,*], Nelcio A.T. de Carvalho [b], Bianca Gasparrini [c], Giuseppe Campanile [c], Michael J. D'Occhio [d]

[a] Department of Animal Reproduction, Faculty of Veterinary Medicine and Animal Science, University of Sao Paulo, Sao Paulo, Brazil
[b] Research and Development Unit of Registro, Diversified Animal Science Research Center/Institute of Animal Science, Registro, São Paulo-SP, Brazil
[c] Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy
[d] School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Sydney, Australia

ARTICLE INFO

Article history:
Received 1 November 2022
Revised 26 February 2023
Accepted 27 February 2023

Keywords:
Artificial insemination
Biotechnology
Embryo transfer
Genetic
Water buffalo

ABSTRACT

The domestic buffalo (*Bubalus bubalis*), also known as water buffalo, comprises two sub-species the River buffalo (B. bubalis ssp. *b*ubalis; 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.

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

* Corresponding author.
E-mail address: barusell@usp.br (P.S. Baruselli).

https://doi.org/10.1016/j.animal.2023.100764
1751-7311/

Table 1
Chronology 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	Artificial insemination	Bhattacharya, 1962, 1968, and 1974; Lonergan, 2018; Vale et al., 2022
1956	Semen cryopreservation	Bhattacharya and Srivastava, 1955; Roy et al., 1956; Lonergan, 2018; Kumar et al., 2022; Vale et al., 2022
1964	Pregnancy with frozen-thawed sperm	Basirov, 1964; Kumar et al., 2022
1979	Estrus synchronization	Kamonpatana et al., 1979; de Rensis and López-Gatius, 2007
1983	*In vivo* embryo recovery and embryo transfer, and ovarian follicular superstimulation	Drost et al., 1983; Karaivanov et al., 1987; Chantaraprateeep et al., 1988; Misra et al., 1990
1989/91/92	*In vitro* fertilization (IVF)	Singh et al., 1989; Totey et al., 1992; Suzuki et al., 1992; Madan et al., 1994; Gasparrini et al., 2001
1994	Oocyte pickup (OPU)	Boni et al., 1994; Konrad et al., 2017
1998	OPU + IVF	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	Sexed sperm	Presicce et al., 2005; Presicce, 2013 and 2022
2007	Cloning	Shi et al., 2007; Selokar et al., 2018
2017/18	Juvenile *in vitro* 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. *b*ubalis; 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 $PGF_{2\alpha}$ (Kamonpatana et al., 1979). It was later shown that, as with cattle, combinations of GnRH, estradiol, progestogens, and $PGF_{2\alpha}$ 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 anestrous 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 + $PGF_{2\alpha}$ + GnRH-based protocol Ovsynch (Pursley et al., 1995; Baruselli et al., 1999). This is because of the higher incidence of anestrous (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 supertimulation, commonly referred to as superovulation. Early attempts at superovulation in buffaloes drew from experiences in cattle with FSH, PMSG and GnRH in combination with $PGF_{2\alpha}$ (Drost et al., 1983; Karaivanov et al., 1987; Chantaraprateeep 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-l, 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 Elsden, 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 (Baldrighi et al., 2014). In another study,

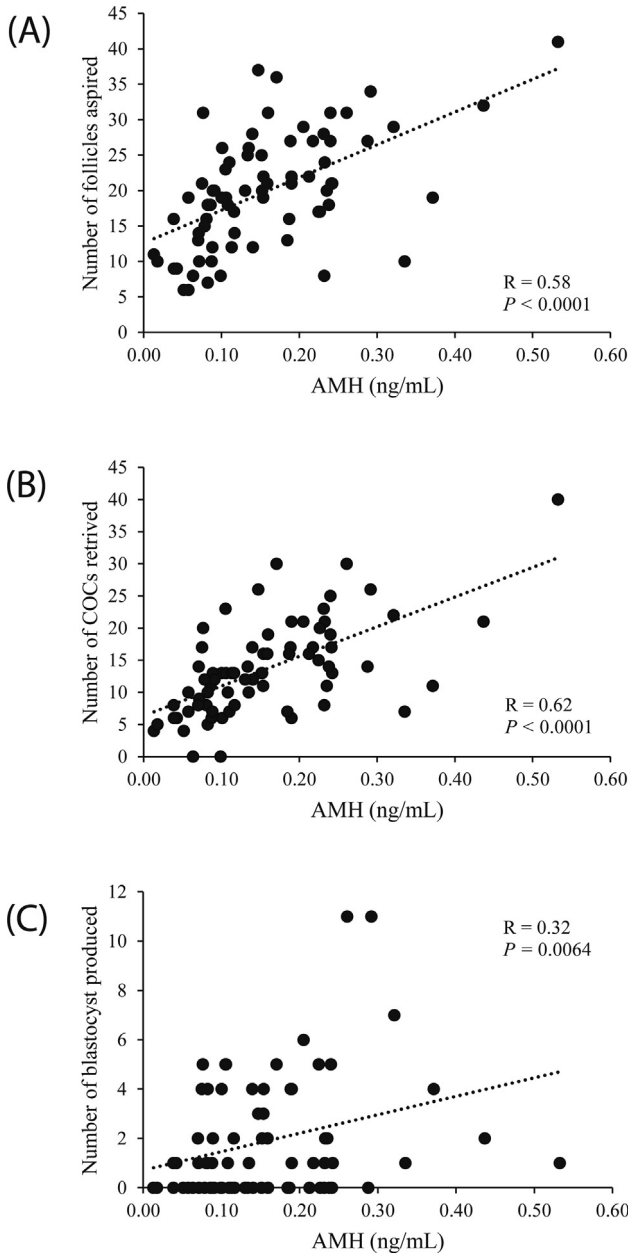

Fig. 1. Correlation between plasma anti-mullerian hormone (AMH) concentrations and number of follicles aspirated (A), total COCs (cumulus-oocyte complexes) retrieved (B) and number of blastocysts produced (C) per buffalo donor (n = 73). Blood samples for plasma AMH determination were collected immediately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

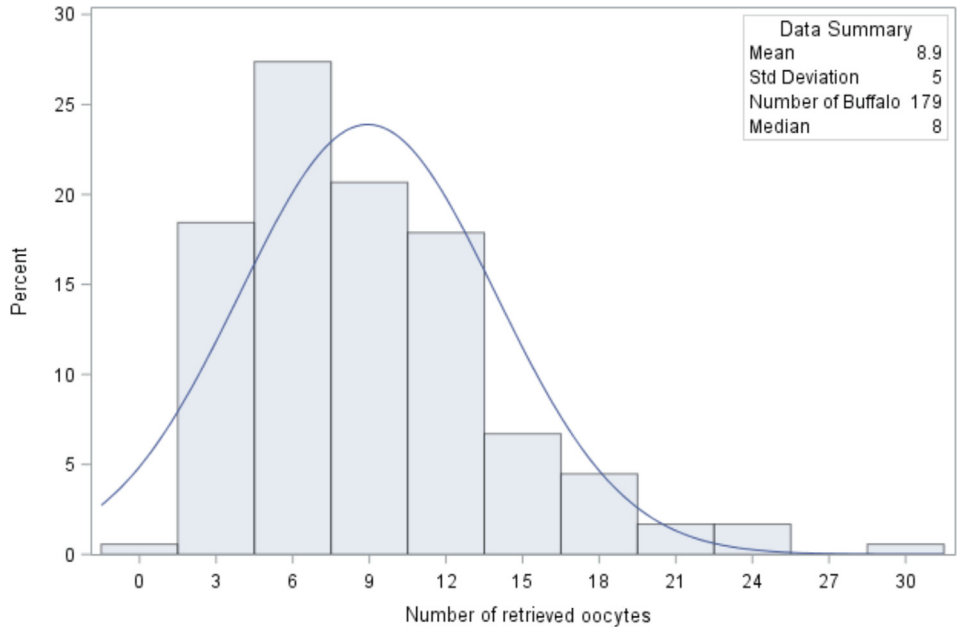

Fig. 2. Distribution of number of oocytes (COC) retrieved per ovum pickup (OPU) session in Murrah buffalo donors (n = 179; Source: Baruselli et al., 2020).

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; Lillico, 2019; Hansen, 2020; Whitelaw and Lillico, 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 supertimulation 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 ± 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).

	Category			
Item	Calves	Prepubertal heifers	Lactating cows	*P*-value
Number	8	10	10	
Total oocytes retrieved, n	10.9 ± 3.3[ab]	15.5 ± 2.1[a]	5.8 ± 1.3[b]	0.007
Viable oocytes, n	7.63 ± 2.7	6.20 ± 1.6	3.20 ± 0.9	0.11
Viable oocytes rate, %	63.9[a]	39.3[b]	54.1[a]	0.01
Total oocytes cleaved, n	2.75 ± 0.9	3.10 ± 0.7	2.10 ± 0.4	0.52
Cleavage rate, %	30.3[ab]	20.8[b]	37.6[a]	0.04
Viable embryos, n	1.00 ± 0.57[b]	1.50 ± 0.34[a]	1.10 ± 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.

Author ORICDs

Pietro S. Baruselli: https://orcid.org/0000-0002-6773-4450.
Nelcio A.T. Carvalho: https://orcid.org/0000-0003-2867-3648.
Bianca Gasparini: https://orcid.org/0000-0002-5086-7767.
Giuseppe Campanile: https://orcid.org/0000-0002-3242-7274.
Michael J. D'Occhio: https://orcid.org/0000-0001-8952-3091.

Author contributions

Pietro S. Baruselli: Conceptualization, Writing – original draft, Writing – review and editing, Formal analysis;
Nelcio A.T. Carvalho: Conceptualization, Writing – original draft;
Bianca Gasparini: Conceptualization, Writing – original draft;
Giuseppe Campanile: Conceptualization, Writing – original draft;
Michael D'Occhio: Conceptualization, Writing – original draft, Writing – review and editing, Formal analysis.

Declaration of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors are grateful for the opportunity to build an effective research relationship between universities to study topics related to the biotechnology of reproduction in buffaloes.

Financial support statement

The author (Baruselli, P.S.) would like to acknowledge the support from CNPq (Process 306759/2016-0 and 426227/2016-6), FAPESP (Process 2017/50.339-5) and CAPES of Brazil.

Transparency Declaration

This article is part of a supplement entitled Proceedings from the 11th International Ruminant Reproduction Symposium supported by the British Society of Animal Science.

References

Aitken, J., Fisher, H., 1994. Reactive oxygen species generation and human spermatozoa: The balance of benefit and risk. BioEssays 16, 259–267.

Baldassarre, H., 2021. Laparoscopic ovum pick-up followed by in vitro embryo production and transfer in assisted breeding programs for ruminants. Animals 11, 1–12.

Baldassarre, H., Bordignon, V., 2018. Laparoscopic ovum pick-up for in vitro embryo production from dairy bovine and buffalo calves. Animal Reproduction 15, 191–196.

Baldassarre, H., Littkemann, M., Currin, L., et al., 2017. Efficient shortening of generation interval by applying laparoscopic ovum pick-up and in vitro embryo production technologies in Mediterranean buffalo of 2–6 months of age. Proceedings of the 9th Symposium of the International Buffalo Federation, 20–25 November 2017, Campeche, Mexico, pp 10.

Baldrighi, J., Sá Filho, M., Batista, E., Lopes, R., Visintin, J., Baruselli, P., Assumpção, M., 2014. Anti-Mullerian Hormone Concentration and Antral Ovarian Follicle Population in Murrah Heifers Compared to Holstein and Gyr Kept Under the Same Management. Reproduction in Domestic Animals 49, 1015–1020.

Baruselli, P., 1994. Basic requiriments for artificial insemination and embryo transfer in buffaloes. Buffalo Journal 1, 53–60.

Baruselli, P.S., Antonio, N., Carvalho, T., Gimenes, L.U., Crepaldi, G., 2007. Fixed-time artificial insemination in buffalo. Italian Journal of Animal Science 6, 107–118.

Baruselli, P.S., de Carvalho, J.G.S., Elliff, F.M., da Silva, J.C.B., Chello, D., de Carvalho, N. A.T., 2020. Embryo transfer in buffalo (Bubalus bubalis). Theriogenology 150, 221–228.

Baruselli, P., Madureira, E., Barnabé, V., Barnabé, R., Berber, R. de A., Amaral, R., 2000. Timed insemination using synchronisation of ovulation in buffalo. In: Proceedings of the 14th International Congress on Animal Reproduction, 2–6 July 2000, Stockholm, Sweden, pp.14–18.

Baruselli, P., Madureira, E., Visintin, J., Barnabe, V., Barnabe, R., Amaral, R., 1999. Inseminação artificial em tempo fixo com sincronização da ovulação em bubalinos. Revista Brasileira de Reprodução Animal 23, 360–362.

Baruselli, P.S., Mucciolo, R.G., Visintin, J.A., Viana, W.G., Arruda, R.P., Madureira, E.H., Oliveira, C.A., Molero-Filho, J.R., 1997. Ovarian follicular dynamics during the estrous cycle in buffalo (Bubalus bubalis). Theriogenology 47, 1531–1547.

Baruselli, P.S., Madureira, E.H., Barnabe, V.H., Barnabe, R.C., de Berber, R.C., A,, 2003. Evaluation of synchronization of ovulation for fixed timed insemination in buffalo (Bubalus bubalis). Brazilian Journal of Veterinary Research and Animal Science 40, 431–442.

Baruselli, P.S., Soares, J.G., Bayeux, B.M., Silva, J.C.B., Mingoti, R.D., Carvalho, N.A.T., 2018. Assisted reproductive technologies (ART) in water buffaloes. Animal Reproduction 15, 971–983.

Basirov, E.B., 1964. The biology of reproduction and artificial insemination of buffaloes. In: Proceedings of the 5th International Congress on Animal Reproduction and Artificial Insemination, 6–13 September 1964, Trento, Italy, Vol. 4, p 10.

Batista, E., Macedo, G., Sala, R., Ortolan, M., Sá Filho, M., del Valle, T., Jesus, E., Lopes, R., Rennó, F., Baruselli, P., 2014. Plasma Antimullerian Hormone as a Predictor of Ovarian Antral Follicular Population in *Bos indicus* (Nelore) and *Bos taurus* (Holstein) Heifers. Reproduction in Domestic Animals 49, 448–452.

Berber, R.C. de A., Madureira, E.H., Baruselli, P.S., 2002. Comparison of two Ovsynch protocols (GnRH versus LH) for fixed timed insemination in buffalo (Bubalus bubalis). Theriogenology 57, 1421–1430.

Bhattacharya, P., 1974. Reproduction. In: Cockrill, W.R. (Ed.), The husbandry and health of domestic buffalo. Australian Freedom from Hunger Campaign, FAO, Rome, Italy, pp. 105–158.

Bhattacharya, P., Srivastava, P., 1955. Studies in deep freezing of buffalo semen. In: Proceeding of the 42nd Indian Science Congress, no date, Baroda, India, pp. 348.

Bhattacharya, P., 1962. Artificial insemination in water buffalo. In: The Semen of Animals and Artificial Insemination (ed Maule, J.P.), pp 184–204. Commonwealth Agricultural Bureaux, Cambridge, UK.

Bhattacharya, P., 1968. Buffaloes. In: Johnson PE (Ed.), The artificial insemination of farm animals, 4th Revised Edition. Rutgers University Press, New Brunswick, NJ, USA.

Bó, G.A., Baruselli, P.S., Martínez, M.F., 2003. Pattern and manipulation of follicular development in Bos indicus cattle. Animal Reproduction Science 78, 307–326.

Boccia, L., de Rosa, A., Attanasio, L., Neglia, G., Vecchio, D., Campanile, G., Zicarelli, L., Gasparrini, B., 2013. Developmental speed affects the cryotolerance of in vitro produced buffalo (Bubalus bubalis) embryos. Italian Journal of Animal Science 12, 492–496.

Boccia, L., Monaco, E., Attanasio, L., de Rosa, D., Gasparrini, B., 2006. Influenza del cambio di coltura (doppio vs singolo) sullo sviluppo di embrioni bufalini in vitro. In: Proceedings of the 12nd Giornate Scientifiche, no date, Napoli, Italy, pp. 171.

Boccia, L., Rubessa, M., de Blasi, M., di Francesco, S., Albero, G., Neglia, G., Gasparrini, B., 2012. Hyaluronic acid improve cryotolerance of buffalo (Bubalus bubalis) in vitro derived embryos. Reproduction, Fertility and Development 24, 138.

Boni, R., di Palo, R., Barbieri, V., Zicarelli, L., 1994. Ovum pick-up in deep anestrus buffaloes. In: Proceedings of the 4th World Buffalo Congress, 27–30 July 1994, São Paulo, Brazil, pp. 480–482.

Boni, R., Santella, L., Dale, B., Roviello, S., di Palo, R., Barbieri, V., 1992. An ultrastructural study of maturation in buffalo oocytes. Acta Medica Veterinaria 38, 153–161.

Borghese, A., Chiariotti, A., Barile, V.L., 2022. Buffalo in the World: Situation and Perspectives. In: Chauhan, M.S., Selokar, N. (Eds.), Biotechnological Applications in Buffalo Research. Springer, Singapore, pp. 3–31.

Campanile, G., Baruselli, P.S., Neglia, G., Vecchio, D., Gasparrini, B., Gimenes, L.U., Zicarelli, L., D'Occhio, M.J., 2010. Ovarian function in the buffalo and implications for embryo development and assisted reproduction. Animal Reproduction Science 121, 1–11.

Campanile, G., Gasparrini, B., Vecchio, D., Neglia, G., Senatore, E.M., Bella, A., Presicce, G.A., Zicarelli, L., 2011. Pregnancy rates following AI with sexed semen in Mediterranean Italian buffalo heifers (Bubalus bubalis). Theriogenology 76, 500–506.

Campanile, G., Vecchio, D., Neglia, G., Bella, A., Prandi, A., Senatore, E.M., Gasparrini, B., Presicce, G.A., 2013. Effect of season, late embryonic mortality and progesterone production on pregnancy rates in pluriparous buffaloes (Bubalus bubalis) after artificial insemination with sexed semen. Theriogenology 79, 653–659.

Campanile, G., Neglia, G., D'Occhio, M.J., 2016. Embryonic and fetal mortality in river buffalo (Bubalus bubalis). Theriogenology 86, 207–213.

Caracciolo di Brienza, V., Neglia, G., Masola, N., Gasparrini, B., di Palo, R., Campanile, G., 2001. In vitro embryo production in chemically defined media. In: Proceedings of the 1st Congresso Nazionale sull'Allevamento del Bufalo, 3–5 October 2001, Eboli, Italy, pp. 341–344.

Carvalho, N.A.T., Soares, J.G., Porto Filho, R.M., Gimenes, L.U., Souza, D.C., Nichi, M., Sales, J.S., Baruselli, P.S., 2013. Equine chorionic gonadotropin improves the efficacy of a timed artificial insemination protocol in buffalo during the nonbreeding season. Theriogenology 79, 423–428.

Carvalho, N.A.T., Carvalho, J.G.S., Souza, D.C., Madureira, E.H., Sá Filho, M.F., Sales, J. N.S., Ghuman, S.S., D'Occhio, M.J., Baruselli, P.S., 2021. Lack of effect of melatonin on ovarian function and response to estrous synchronization and fixed-time AI during the nonbreeding season in lactating dairy buffalo (Bubalus bubalis). Animal Reproduction Science 231, 106796.

Carvalho, F.C.A., Oba, E., Leal, L.S., 2007. Morphologic and quantitative evaluation of preantral follicles of buffalo (Bubalus bubalis) in different reproductive phases. Italian Journal of Animal Science 6, 691–693.

Castanheira, R., Ligori, V., Bernardes, O., Baruselli, P., Zadra, L., Carvalho, N., 2021. Biotecnologias reprodutivas aliadas à avaliação genética promovem aumento da produção de leite em búfalas. In: Proceedings of the 14th Congresso Brasileiro de Melhoramento Animal, 18 a 19 October 2021, Santa Catarina, Brazil, pp. 1.

Chantaraprateeep, P., Lohachit, G., Kobayashi, M., Techakumphu, M., Virakul, P., Kunavongkrit, A., Prateep, P., 1988. Ovarian responses to gonadotrophin stimulation in swamp buffalo (Bubalus bubalis). Buffalo Bulletin 7, 82–86.

Chauhan, M.S., Selokar, N., 2022. Biotechnological Applications in Buffalo Research. Springer, Singapore.

Chauhan, M.S., Singla, S.K., Palta, P., Manik, R.S., Madan, M.L., 1998. In vitro maturation and fertilization, and subsequent development of buffalo (Bubalus bubalis) embryos: effects of oocyte quality and type of serum. Reproduction, Fertility and Development 10, 173.

Chello, D., 2020. Factors influencing donor embryo production and fertility of recipients in buffalo. Master thesis, Sao Paulo University, Sao Paulo, Brazil.

Colli, L., Williams, J.L., Ajmone-Marsan, P., 2022. Water Buffalo Genomic Diversity. In: Biotechnological Applications in Buffalo Research (ed. Chauhan, M.S., Selokar, N.), pp. 33–55. Springer, Singapore, Singapore.

Danell, B., 1987. Oestrus behavior, ovary morphology and cyclical variation in follicular system and endocrine pattern in water buffalo heifers. PhD thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

de Carvalho, J.G.S., de Carvalho, N.A.T., Bayeux, B.M., Watanabe, Y.F., Watanabe, O.Y., Mingoti, R.D., Baruselli, P.S., 2019. Superstimulation prior to the ovum pick-up improves the in vitro embryo production in nulliparous, primiparous and multiparous buffalo (Bubalus bubalis) donors. Theriogenology 138, 164–168.

de Carvalho, N.A.T., Soares, J.G., Baruselli, P.S., 2016. Strategies to overcome seasonal anestrus in water buffalo. Theriogenology 86, 200–206.

de Rensis, F., López-Gatius, F., 2007. Protocols for synchronizing estrus and ovulation in buffalo (Bubalus bubalis): A review. Theriogenology 67, 209–216.

Desaulniers, D.M., Lussier, J.G., Goff, A.K., Bousquet, D., Guilbault, L.A., 1995. Follicular development and reproductive endocrinology during and after superovulation in heifers and mature cows displaying contrasting superovulatory responses. Theriogenology 44, 479–497.

di Francesco, S., Boccia, L., Campanile, G., di Palo, R., Vecchio, D., Neglia, G., Zicarelli, L., Gasparrini, B., 2011. The effect of season on oocyte quality and developmental competence in Italian Mediterranean buffaloes (Bubalus bubalis). Animal Reproduction Science 123, 48–53.

di Francesco, S., Novoa, M.V.S., Vecchio, D., Neglia, G., Boccia, L., Campanile, G., Zicarelli, L., Gasparrini, B., 2012. Ovum pick-up and in vitro embryo production (OPU-IVEP) in Mediterranean Italian buffalo performed in different seasons. Theriogenology 77, 148–154.

Dominko, T., First, N.L., 1997. Timing of meiotic progression in bovine oocytes and its effect on early embryo development. Molecular Reproduction and Development 47, 456–467.

Drost, M., Elsden, R., 1985. Blastocyst development in water buffalo (Bubalus bubalis). Theriogenology 23, 191.
Drost, M., Wright, J.M., Cripe, W.S., Richter, A.R., 1983. Embryo transfer in water buffalo (Bubalusbubalis). Theriogenology 20, 579–584.
Galli, C., Duchi, R., Crotti, G., Lazzari, G., 1998. Embryo production by ovum pick up in water Buffalo. Theriogenology 49, 400.
Galli, C., Crotti, G., Notari, C., Turini, P., Duchi, R., Lazzari, G., 2001. Embryo production by ovum pick up from live donors. Theriogenology 55, 1341–1357.
Gamarra, G., Ponsart, C., Lacaze, S., le Guienne, B., Humblot, P., Deloche, M.-C., Monniaux, D., Ponter, A.A., 2015. Dietary propylene glycol and in vitro embryo production after ovum pick-up in heifers with different anti-Müllerian hormone profiles. Reproduction, Fertility and Development 27, 1249.
Gasparrini, B., 2002. In vitro embryo production in buffalo species: state of the art. Theriogenology 57, 237–256.
Gasparrini, B., 2018. Effects of reproductive season on embryo development in the buffalo. Reproduction, Fertility and Development 31, 68.
Gasparrini, B., Neglia, G., di Palo, R., Campanile, G., Zicarelli, L., 2000. Effect of cysteamine during in vitro maturation on buffalo embryo development. Theriogenology 54, 1537–1542.
Gasparrini, B., Neglia, G., Caracciolo di Brienza, V., Campanile, G., di Palo, R., Zicarelli, L., 2001. Preliminary analysis of vitrified in vitro produced embryos. Theriogenology 55, 307.
Gasparrini, B., Sayoud, H., Neglia, G., de Matos, D.G., Donnay, I., Zicarelli, L., 2003. Glutathione synthesis during in vitro maturation of buffalo (Bubalus bubalis) oocytes: effects of cysteamine on embryo development. Theriogenology 60, 943–952.
Gasparrini, B., Boccia, L., de Rosa, A., Vecchio, D., di Palo, R., Zicarelli, L., 2004. In vitro fertilization of buffalo (Bubalus bubalis) oocytes: effects of media and sperm motility inducing agents. Reproduction, Fertility and Development 16, 255.
Gasparrini, B., Boccia, L., Marchandise, J., di Palo, R., George, F., Donnay, I., Zicarelli, L., 2006. Enrichment of in vitro maturation medium for buffalo (Bubalus bubalis) oocytes with thiol compounds: Effects of cystine on glutathione synthesis and embryo development. Theriogenology 65, 275–287.
Gasparrini, B., de Rosa, A., Attanasio, L., Boccia, L., di Palo, R., Campanile, G., Zicarelli, L., 2008. Influence of the duration of in vitro maturation and gamete co-incubation on the efficiency of in vitro embryo development in Italian Mediterranean buffalo (Bubalus bubalis). Animal Reproduction Science 105, 354–364.
Gimenes, L.U., Ferraz, M.L., Fantinato-Neto, P., Chiaratti, M.R., Mesquita, L.G., SáFilho, M.F., Meirelles, F.V., Trinca, L.A., Rennó, F.P., Watanabe, Y.F., Baruselli, P. S., 2015. The interval between the emergence of pharmacologically synchronized ovarian follicular waves and ovum pickup does not significantly affect in vitro embryo production in Bos indicus, Bos taurus, and Bubalus bubalis. Theriogenology 83, 385–393.
Gowane, G.R., Vohra, V., 2022. Advances in Buffalo Breeding: A Journey from Classical Breeding to Genomic Selection. In: Chauhan, M.S., Selokar, N. (Eds.), Biotechnological Applications in Buffalo Research. Springer, Singapore, pp. 57–77.
Granleese, T., Clark, S.A., Swan, A.A., van der Werf, J.H.J., 2015. Increased genetic gains in sheep, beef and dairy breeding programs from using female reproductive technologies combined with optimal contribution selection and genomic breeding values. Genetics Selection Evolution 47, 70.
Guerreiro, B.M., Batista, E.O.S., Vieira, L.M., Sá Filho, M.F., Rodrigues, C.A., Castro Netto, A., Silveira, C.R.A., Bayeux, B.M., Dias, E.A.R., Monteiro, F.M., Accorsi, M., Lopes, R.N.V.R., Baruselli, P.S., 2014. Plasma anti-mullerian hormone: an endocrine marker for in vitro embryo production from Bos taurus and Bos indicus donors. Domestic Animal Endocrinology 49, 96–104.
Gutiérrez-Añez, J.C., Camacho de Gutiérrez, A., Nava-Trujillo, H., 2022. Application of fixed-time artificial insemination in water buffaloes. In: Chauhan, M.S., Selokar, N. (Eds.), Biotechnological Applications in Buffalo Research. Springer, Singapore, pp. 295–318.
Hansen, P.J., 2020. Prospects for gene introgression or gene editing as a strategy for reduction of the impact of heat stress on production and reproduction in cattle. Theriogenology 154, 190–202.
Hunter, R., Greve, T., 1997. Could Artificial Insemination of Cattle be More Fruitful? Penalties Associated with Ageing Eggs. Reproduction in Domestic Animals 32, 137–141.
Ireland, J.L.H., Scheetz, D., Jimenez-Krassel, F., Themmen, A.P.N., Ward, F., Lonergan, P., Smith, G.W., Perez, G.I., Evans, A.C.O., Ireland, J.J., 2008. Antral Follicle Count Reliably Predicts Number of Morphologically Healthy Oocytes and Follicles in Ovaries of Young Adult Cattle. Biology of Reproduction 79, 1219–1225.
Ireland, J.J., Ward, F., Jimenez-Krassel, F., Ireland, J.L.H., Smith, G.W., Lonergan, P., Evans, A.C.O., 2007. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. Human Reproduction 22, 1687–1695.
Jabbar, A., Zulfiqar, F., Mahnoor, M., Mushtaq, N., Zaman, M.H., Din, A.S.U., Khan, M. A., Ahmad, H.I., 2021. Advances and Perspectives in the Application of CRISPR-Cas9 in Livestock. Molecular Biotechnology 63, 757–767.
Jeyakumar, S., Balasubramanian, S., Vedamurthy, G.V., Lavanya, M., Chethan, H.S., Kumaresan, A., Ramesha, K.P., 2022. Advances in Estrous Synchronization and Timed Breeding Programs for Fertility Enhancement in Cattle and Buffaloes. In: Current Concepts in Bovine Reproduction (ed. Kumaresan, A., Srivastava, A.K.), pp. 119–167. Springer, Singapore.
Kamonpatana, M., Kunawongkrit, A., Luvira, Y., 1979. Effect of PGF-2alfa on serum progesterone levels in the swamp buffalo (Bubalus bubalis). Journal of Reproduction and Fertility 56, 445–449.
Karaivanov, C., Ylahovl, K., Petrov, M., Kacheva, D., Stojanova, N., Alexiev, A., Polihronov, O., Danev, A., 1987. Studies on preimplantation development of Buffalo embryos. Theriogenology 28, 747–753.
Kasinathan, P., Wei, H., Xiang, T., Molina, J.A., Metzger, J., Broek, D., Kasinathan, S., Faber, D.C., Allan, M.F., 2015. Acceleration of genetic gain in cattle by reduction of generation interval. Scientific Reports 5, 8674.
Khetra, Y., Meena, G.S., Arora, S., 2022. Buffalo Milk and Its Products: Composition, Nutrition and Benefits. In: Chauhan, M.S., Selokar, N. (Eds.), Biotechnological Applications in Buffalo Research. Springer, Singapore, pp. 121–143.
Komor, A.C., Badran, A.H., Liu, D.R., 2017. CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. Cell 168, 20–36.
Konrad, J., Clérico, G., Garrido, M.J., Taminelli, G., Yuponi, M., Yuponi, R., Crudeli, G., Sansinena, M., 2017. Ovum pick-up interval in buffalo (Bubalus bubalis) managed under wetland conditions in Argentina: Effect on follicular population, oocyte recovery, and in vitro embryo development. Animal Reproduction Science 183, 39–45.
Kumar, P., Kumar, D., Jerome, A., 2022. Buffalo Semen Cryopreservation: An Update. In: Current Concepts in Bovine Reproduction. In: Current Concepts in Bovine Reproduction, Kumaresan A., Srivastava, A., Editors, pp. 243–262. Springer Nature, Singapore.
Layek, S.S., Mohanty, T.K., Kumaresan, A., Parks, J.E., 2016. Cryopreservation of bull semen: Evolution from egg yolk based to soybean based extenders. Animal Reproduction Science 172, 1–9.
Liang, X.W., Lu, Y.Q., Chen, M.T., Zhang, X.F., Lu, S.S., Zhang, M., Pang, C.Y., Huang, F. X., Lu, K.H., 2008. In vitro embryo production in buffalo (Bubalus bubalis) using sexed sperm and oocytes from ovum pick up. Theriogenology 69, 822–826.
Lillico, S., 2019. Agricultural applications of genome editing in farmed animals. Transgenic Research 28, 57–60.
Lonergan, P., 2018. Review: Historical and futuristic developments in bovine semen technology. Animal 12, s4–s18.
Lu, Y., Zhang, M., Lu, S., Xu, D., Huang, W., Meng, B., Xu, H., Lu, K., 2010. Sex-preselected buffalo (Bubalus bubalis) calves derived from artificial insemination with sexed sperm. Animal Reproduction Science 119, 169–171.
Lu, Y., Liao, Y., Zhang, M., Yang, B., Liang, X., Yang, X., Lu, S., Wu, Z., Xu, H., Liang, Y., Lu, K., 2015. A field study on artificial insemination of swamp and crossbred buffaloes with sexed semen from river buffaloes. Theriogenology 84, 862–867.
Macabelli, C., Gimenes, L., Carvalho, N., Soares, J., Ferraz, M., Watanabe, Y., et al., 2012. Molecular evaluation of developmental competence of buffalo oocytes collected in vivo during winter and summer. Animal Reproduction 9, 674.
Madan, M.L., Singla, S.K., Chauhan, M.B., Manik, R.S., 1994. In vitro production and transfer of embryos in buffaloes. Theriogenology 41, 139–143.
Manjunatha, B., Devaraj, M., Gupta, P., Ravindra, J., Nandi, S., 2009. Effect of Taurine and Melatonin in the Culture Medium on Buffalo *In Vitro* Embryo Development. Reproduction in Domestic Animals 44, 12–16.
Marin, D.F.D., de Souza, E.B., de Brito, V.C., Nascimento, C.V., Ramos, A.S., Filho, S.T. R., da Costa, N.N., Cordeiro, M. da S., Santos, S. do S.D., Ohashi, O.M., 2019. In vitro embryo production in buffaloes: From the laboratory to the farm. Animal Reproduction 16, 260–266.
Marston, J.H., Chang, M.C., 1964. The fertilizable life of ova and their morphology following delayed insemination in mature and immature mice. Journal of Experimental Zoology 155, 237–251.
Mehra, V.K., Kumar, S., 2021. The Application of CRISPR/Cas9 Technology for Farm Animals: A Review. Agricultural Reviews 43, 54–61.
Menchaca, A., dos Santos-Neto, P.C., Mulet, A.P., Crispo, M., 2020. CRISPR in livestock: From editing to printing. Theriogenology 150, 247–254.
Misra, A.K., Joshi, B.V., Agrawala, P.L., Kasiraj, R., Sivaian, S., Rangareddi, N.S., Siddiqui, M.U., 1990. Multiple ovulation and embryo transfer in Indian buffalo. Theriogenology 33, 1131–1141.
Mohapatra, S.K., Sandhu, A., Neerukattu, V.S., Singh, K.P., Selokar, N.L., Singla, S.K., Chauhan, M.S., Manik, R.S., Palta, P., 2015. Buffalo embryos produced by handmade cloning from oocytes selected using brilliant cresyl blue staining have better developmental competence and quality and are closer to embryos produced by in vitro fertilization in terms of their epigenetic status and gene expression pattern. Cellular Reprogramming 17, 141–150.
Monniaux, D., Drouilhet, L., Rico, C., Estienne, A., Jarrier, P., Touzé, J.-L., Sapa, J., Phocas, F., Dupont, J., Dalbiès-Tran, R., Fabre, S., 2012. Regulation of anti-Müllerian hormone production in domestic animals. Reproduction, Fertility and Development 25, 1.
Muer, S.K., Roy, S.B., Mohan, G., Dhobl, R.L., 1988. Cryogenic changes in seminal protein of cattle and buffalo. Theriogenology 30, 1005–1010.
Nandi, S., Ravindranatha, B.M., Gupta, P.S.P., Raghu, H.M., Sarma, P.V., 2003. Developmental competence and post-thaw survivability of buffalo embryos produced in vitro: effect of growth factors in oocyte maturation medium and of embryo culture system. Theriogenology 60, 1621–1631.
Neglia, G., Marino, M., di Palo, R., Wilding, M., Caracciolo di Brienza, V., Dale, B., Gasparrini, B., Zicarelli, L., 2001. A comparison of in vitro maturation in buffalo (Bubalus Bubalis) and bovine oocytes using confocal microscopy. In: Proceedings Annual Conference of the International Embryo Transfer Society, Omaha, Nebraska, USA, January 13–16, 2001. Theriogenology 55, 488.
Neglia, G., Gasparrini, B., Caracciolo Di Brienza, V., di Palo, R., Campanile, G., Presicce, G.A., Zicarelli, L., 2003a. Bovine and buffalo in vitro embryo production using oocytes derived from abattoir ovaries or collected by transvaginal follicle aspiration. Theriogenology 59, 1123–1130.
Neglia, G., Gasparrini, B., di Palo, R., de Rosa, C., Zicarelli, L., Campanile, G., 2003b. Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean Buffalo cows. Theriogenology 60, 125–133.

Neglia, G., Gasparrini, B., Salzano, A., Vecchio, D., de Carlo, E., Cimmino, R., Balestrieri, A., D'Occhio, M.J., Campanile, G., 2016. Relationship between the ovarian follicular response at the start of an Ovsynch–TAI program and pregnancy outcome in the Mediterranean river buffalo. Theriogenology 86, 2328–2333.
Neglia, G., de Nicola, D., Esposito, L., Salzano, A., D'Occhio, M.J., Fatone, G., 2020. Reproductive management in buffalo by artificial insemination. Theriogenology 150, 166–172.
Ohashi, O.M., Kumar, S., Rolim Filho, S.T., Ribeiro, H.F.L., Freitas, V.J. de F., Vale, W.G., 2022. Advances in Embryo Production in Buffaloes: In Vivo Versus In Vitro Procedures. In Biotechnological Applications in Buffalo Research (ed. Chauhan, M.S., Selokar, N.), pp. 279–294. Springer, Singapore.
Osorio-Meléndez, C., 2013. Computerized evaluation of the functional integrity of the plasma membrane, motility and sperm morphology in cryopreserved buffalo semen. Master thesis, University of Zulia, Maracaibo, Venezuela.
Pandey, A., Gupta, N., Gupta, S.C., 2009. Improvement of in vitro oocyte maturation with lectin supplementation and expression analysis of Cx43, GDF-9, FGF-4 and Fibronectin mRNA transcripts in Buffalo (Bubalus bubalis). Journal of Assisted Reproduction and Genetics 26, 365–371.
Pandey, A., Gupta, S.C., Singh, N., Rana, J.S., Gupta, N., 2010. Efficiency of scnt buffalo (bubalus bubalis) embryos in different culture medium and analysis of mRNA expression of insulin-like growth factors during embryogenesis. Reproduction in Domestic Animals 45, 786–795.
Perisse, I.V., Fan, Z., Singina, G.N., White, K.L., Polejaeva, I.A., 2021. Improvements in Gene Editing Technology Boost Its Applications in Livestock. Frontiers in Genetics 11, 614688.
Petrovas, G., Kosior, M.A., Presicce, G.A., Russo, M., Zullo, G., Albero, G., Alkan, S., Gasparrini, B., 2020. FSH Stimulation with Short Withdrawal Improves Oocyte Competence in Italian Mediterranean Buffalo (Bubalus bubalis). Animals 10, 1997.
Phillips, P.H., Lardy, H.A., 1940. A Yolk-Buffer Pabulum for the Preservation of Bull Semen. Journal of Dairy Science 23, 399–404.
Ponsart, C., le Bourhis, D., Knijn, H., Fritz, S., Guyader-Joly, C., Otter, T., Lacaze, S., Charreaux, F., Schibler, L., Dupassieux, D., Mullaart, E., 2014. Reproductive technologies and genomic selection in dairy cattle. Reproduction, Fertility and Development 26, 12–21.
Presicce, G.A., 2013. Sexed semen and AI in the Mediterranean buffaloes (Bubalus bubalis). Buffalo Bulletin 32, 181–187.
Presicce, G.A., 2022. Semen Sexing in the Buffalo (Bubalus bubalis). In: Chauhan, M. S., Selokar, N. (Eds.), Biotechnological Applications in Buffalo Research. Springer, Singapore, pp. 319–332.
Presicce, G.A., Verberckmoes, S., Senatore, E.M., Klinc, P., Rath, D., 2005. Short Communication First Established Pregnancies in Mediterranean Italian Buffaloes (Bubalus bubalis) Following Deposition of Sexed Spermatozoa near the Utero Tubal Junction. Reproduction Domestic Animal 40, 73–75.
Pursley, J.R., Mee, M.O., Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 44, 915–923.
Quintero-Moreno, A., Trujillo, H.N., Osorio-Meléndez, C., 2022. Advances in Semen Quality Assessments in AI Programs in Buffalo. In: Chauhan, M.S., Selokar, N. (Eds.), Biotechnological Applications in Buffalo Research. Springer, Singapore, pp. 377–388.
Rehman, N., Collins, A.R., Suh, T.K., Wright, R.W., 1994. Effect of sperm exposure time on in vitro fertilization and embryo development of bovine oocytes matured in vitro. Theriogenology 41, 1447–1452.
Rico, C., Fabre, S., Medigue, C., Clemente, N.D., Clement, F., Bontoux, M., Touze, J.-L., Dupont, M., Briant, E., Remy, B., Beckers, J.-F., Monniaux, D., 2009. Anti-Mullerian Hormone Is an Endocrine Marker of Ovarian Gonadotropin-Responsive Follicles and Can Help to Predict Superovulatory Responses in the Cow. Biology of Reproduction 80, 50–59.
Roy, D., Ansari, M., 1973. Studies on freezability of buffalo spermatozoa using two freezing techniques. Indian Journal of Animal Science 43, 1031–1033.
Roy, D., Srivastava, R., Pandey, M., 1956. Deep freezing of buffalo semen diluted and preserved in glycine-egg yolk medium. Indian Journal of Dairy Science 9, 61–62.
Rubessa, M., di Fenza, M., Mariotti, E., di Francesco, S., de Dilectis, C., di Palo, R., Zicarelli, L., Gasparrini, B., 2009. Kinetics of sperm penetration is correlated with in vitro fertility of buffalo (Bubalus Bubalis) bulls. Reproduction, Fertility and Development 21, 206–207.
Sakaguchi, K., Maylem, E.R.S., Tilwani, R.C., Yanagawa, Y., Katagiri, S., Atabay, E.C., Atabay, E.P., Nagano, M., 2019. Effects of follicle-stimulating hormone followed by gonadotropin-releasing hormone on embryo production by ovum pick-up and in vitro fertilization in the river buffalo (Bubalus bubalis). Animal Science Journal 90, 690–695.
Samad, H.A., Khan, I.Q., Rehman, N.U., Ahmad, N., 1998. The Recovery, In Vitro Maturation and Fertilization of Nili-Ravi Buffalo Follicular Oocytes. Asian-Australasian Journal of Animal Sciences 11, 491–497.
Selokar, N.L., 2018. Cloning of breeding buffalo bulls in India: Initiatives & challenges. Indian Journal of Medical Research 148, 120–124.
Selokar, N.L., Saini, M., Palta, P., Chauhan, M.S., Manik, R.S., Singla, S.K., 2018. Cloning of Buffalo, a Highly Valued Livestock Species of South and Southeast Asia: Any Achievements? Cellular Reprogramming 20, 89–98.
Selokar, N.L., Sharma, P., Saini, M., Sheoran, S., Rajendran, R., Kumar, D., Sharma, R.K., Motiani, R.K., Kumar, P., Jerome, A., Khanna, S., Yadav, P.S., 2019. Successful cloning of a superior buffalo bull. Scientific Reports 9, 11366.
Shah, S.A.H., Andrabi, S.M.H., 2021. A systematic review and meta-analysis of spermatozoa cryopreservation, *in vitro* and *in vivo* fertility practices in water buffalo. Veterinary Research Communications 45, 47–74. https://doi.org/10.1007/s11259-021-09789-0.
Shaw, D.G., Kidson, A., van Schalkwyk, J.O., Bartels, P., Loskutoff, N.M., Bezuidenhout, C., Rogers, P., Barry, D.M., Lishman, A.W., 1995. In-vitro production of african buffalo (Syncerus caffer) embryos derived from follicular oocytes and epididymal sperm. Theriogenology 1, 322.
Shi, D., Lu, F., Wei, Y., Cui, K., Yang, S., Wei, J., Liu, Q., 2007. Buffalos (Bubalus bubalis) cloned by nuclear transfer of somatic cells. Biology of Reproduction 77, 285–291.
Silva, J.C.B., 2017. RRCMBBMRO-ROBANJBP 2017. In vitro embryo production in buffalo: comparison between calves, prepubertal Heifers and lactating cows. Animal Reproduction 14, 766.
Silva, J.C.B., Rezendre, R.G., Colli, M.H.A., Bayeux, B.M., Mingoti, R.D., Ojeda-Rojas, O. A., Basso, A.C., Naves, J.R., Baruselli, P.S., 2017. In vitro embryo production in buffalo: comparison between calves, prepubertal Heifers and lactating cows. Animal Reproduction 14, 766.
Singh, G., Totey, S.M., Talwar, G.P., 1989. In vitro fertilization of Buffalo (Bubalus bubalis) oocyte matured in vitro. Theriogenology 31, 255.
Stock, A.E., Ellington, J.E., Fortune, J.E., 1996. A dominant follicle does not affect follicular recruitment by superovulatory doses of FSH in cattle but can inhibit ovulation. Theriogenology 45, 1091–1102.
Suárez, M., di Francesco, S., Rubessa, N., Boccia, L., Longobardi, V., de Blasi, M., Gasparrini, B., 2011. Effect of reducing glucose concentration during in vitro embryo culture in buffalo (Bubalus bubalis). Reproduction, Fertility and Development 23, 168.
Suzuki, T., Singla, S.K., Sujata, J., Madan, M.L., 1992. In vitro fertilization of water buffalo follicular oocytes and their ability to cleave in vitro. Theriogenology 38, 1187–1194.
Thompson, J.G., Partridge, R.J., Houghton, F.D., Cox, C.I., Leese, H.J., 1996. Oxygen uptake and carbohydrate metabolism by in vitro derived bovine embryos. Reproduction 106, 299–306.
Thompson, J., Simpson, A., Pugh, P., Wright, R., Tervit, H., 1991. Glucose utilization by sheep embryos derived in vivo and in vitro. Reproduction, Fertility and Development 3, 571.
Totey, S.M., Pawshe, C.H., Singh, G.P., 1993. In vitro maturation and fertilization of buffalo oocytes : Effects of media, hormones and sera. Theriogenology 39, 1153–1171.
Totey, S., Singh, G., Taneja, M., Pawshe, C., Talwar, G., 1992. In vitro maturation, fertilization and development of follicular oocytes from buffalo (Bubalus Bubalis). Journal Reproduction Fertility 95, 597–607.
Vale, W.G., Castro, S.R.S., Almeida-Silva, A.O., Gutiérrez-Añez, J.C., Singh, P., 2022. Advances in Cryopreservation of Buffalo Semen. In: Biotechnological Applications in Buffalo Research (ed. Chauhan, M.S., Selokar, N.), pp. 333–376. Springer, Singapore.
Vale, W., Sousa, J., Ribeiro, H.F.L., et al., 1994. Preparation of a 'Teaser' buffalo bull for use in artificial programs. Buffalo Journal, Bangkok 10, 75–79.
Vernunft, A., Schwerhoff, M., Viergutz, T., Diederich, M., Kuwer, A., 2015. Anti-Muellerian hormone levels in plasma of Holstein-Friesian heifers as a predictive parameter for ovum pick-up and embryo production outcomes. Journal of Reproduction and Development 61, 74–79.
Vishwanath, R., Moreno, J.F., 2018. Review: Semen sexing - Current state of the art with emphasis on bovine species. Animal 12, s85–s96.
Vos, P.L.A., van der Schans, A., de Wit, A.A.C., Bevers, M.M., Willemse, A.H., Dieleman, S.J., 1994. Effects of neutralization of pregnant mares' serum gonadotrophin (PMSG) shortly before or at the preovulatory LH surge in PMSG-superovulated heifers on follicular function and development. Journal of reproduction and fertility 100, 387–393.
Whitelaw, B., Lillico, S., 2022. Increasing livestock farming sustainability using genome editing technology. The Biochemist 44, 9–12.
Wilding, M., Gasparrini, B., Neglia, G., Dale, B., Zicarelli, L., 2003. Mitochondrial activity and fertilization potential of fresh and cryopreserved buffalo sperm. Theriogenology 59, 59–466.
Yata, V.K., 2022. Sperm Sexing and its Role in Livestock Production. Springer Nature, Singapore.
Zicarelli, L., 1997. Reproductive seasonality in buffalo. Bubalus bubalis 4, 29–52.