Advanced reproductive technologies in buffalo

Bianca Gasparrini, DVM, PhD

Department of Veterinary Medicine and Animal Production, Federico II University of Naples, Italy

Email address: bgasparr@unina.it

Abstract

Artificial insemination (AI) is the most suitable technology to speed up the genetic progress through the paternal lineage. The development of synchronization protocols for fixed timed AI, together with hormonal treatments to control the rate of embryonic mortality, has contributed to a wider diffusion of this technology in the field. The efficiency of AI has greatly increased and sex sorted semen has also been used in AI programs. Due to the limitations of MOET programs, ovum pick-up (OPU) combined with in vitro embryo production (IVEP) is the best alternative for enhancing the maternal contribution to genetic improvement in buffalo. Currently, the IVEP efficiency has greatly increased in terms of both blastocyst yields and pregnancy rates, paving the way for a diffusion in the field. At present, the main limiting factor is the poor number of oocytes recovered, influencing the benefit cost ratios.

Key words: buffalo, artificial insemination, MOET, ovum pick-up, in vitro embryo production

Artificial insemination

Artificial insemination is the best tool to increase the paternal contribution to genetic improvement. In contrast to dairy cattle industry where this technology has been a routine practice since a long time, in buffalo the utilization of AI has been minimal until a decade ago, because of the lack of superior progeny tested bulls and of the low efficiency both on natural or synchronized oestrus [Zicarelli (1997), Zicarelli et al. (1997)]. Undoubtedly, the development of efficient synchronization protocols of estrus and ovulation [Neglia et al (2008), Carvalho et al (2013), Vecchio et al (2013)] contributed to a wider diffusion of the technology, by allowing fixed time artificial insemination (FTAI) and hence, overcoming one of the major limiting factors, that is the difficulty of estrus detection, typical of the species. The most utilized protocol for FTAI is the Ovsynch-TAI Program (Neglia et al., 2003), with AI performed on day 10 at 16-24 hours after the last GnRH. In order to increase the AI efficiency during the non-breeding season, important to ensure a relatively constant milk production throughout the year, eCG can be administered at removal of the intravaginal P4 device in a TAI protocol, as shown by improved ovulation rate, corpus luteum (CL) growth/function and pregnancy rate [Carvalho et al (2013)].
Another critical factor affecting the AI efficiency is the reproductive seasonality of the species, resulting in high incidence of embryonic mortality during months characterized by high daily-light length [Campanile et al (2005, 2007)]. Embryonic loss during the unfavourable season is in part related to impaired luteal function, leading to reduced progesterone level [Campanile et al (2005, 2007)], and in part to decreased oocyte competence [Di Francesco et al (2012)]. Indeed, several studies confirmed the importance

of CL development, vascularization and function during the first weeks after AI on the pregnancy outcome [Russo et al (2010), Vecchio et al (2012)]. Furthermore, the embryo size reached on day 25 after AI is important for subsequent pregnancy outcome [Neglia et al (2012)]. Indeed, a retarded embryonic development, associated to reduced P4 levels, is reflected in altered proteomic profile of the embryonic chorioamnion and uterine caruncles [Balestreri et al (2013)] and abnormal transcriptome embryo profile [Strazzullo et al (2014)]. In order to counteract early embryonic mortality, hormonal treatments to suppress luteolytic mechanisms have been given either on the day of AI or on day 5 after AI [Neglia et al (2008), Campanile et al (2007), Pandey et al (2015, 2016)]. However, in buffalo the major cause of embryonic loss is LEM, where P4 decline occurs later, after day 10 (Russo et al., 2010) interfering with embryo attachment, via disregulation of MUC-1 expression in the uterine epithelium. It follows that, in order to reduce LEM, hormonal treatments to increase P4 should be delayed. It was indeed demonstrated that treatment with progesterone, GnRH agonist and hCG on day 25 post-AI was effective to decrease the incidence of LEM, by keeping P4 concentrations similar to those of pregnant animals [Campanile et al. (2008).
In conclusion, the pregnancy rate after AI has increased by two-fold (from 25 to 50%) in approximately 20 years (Zicarelli et al., 1997; Campanile et al., 2011; Neglia et al., 2015). Currently, AI is routinely applied in intensive river buffalo systems and the good results obtained with sexed semen [Campanile et al (2011, 2013)] make us foresee an even wider diffusion in the field.

Embryo technologies

Currently, the increase of the maternal contribution to genetic improvement is achieved by using advanced reproductive technologies, such as multiple ovulation and embryo transfer (MOET), as well as ovum pick-up (OPU) and in vitro embryo production (IVEP). Although MOET was applied in buffalo for the first time more than thirty years ago [Drost (1983], the technology is still not feasible for commercial purposes, due to both a high incidence of non responsive animals and to the low number of embryos collected. Despite several attempts to improve the MOET efficiency, the embryo recovery per donor is still poor (2.5-3.0) compared to cattle (Neglia et al 2010, Misra et al 2003; Carvalho et al 2002). The lower number of primordial follicles in this species (around 20-30 % of those recorded in cattle) only in part accounts for the low response to super stimulation. In fact, it is now clear that different schedules result in low embryo recovery, despite the growth of high number of ovulatory follicles and a relatively high ovulation rate ([Neglia et al (2010), Carvalho et al (2002)]. This suggests that the low number of embryos recovered is likely due to impaired transport of ova into the oviduct, rather than to the reduced follicular reservoir of the species. It is currently under investigation whether the failure of oocyte uptake is due to altered contractility of the fimbria, that may derive from abnormal steroids ratio or to inappropriate cumulus expansion, likely to occur in buffalo because of the fewer layers of cumulus cells [Gasparrini (2002)].
Due to the limitations of MOET programs, the interest has shifted towards OPU and IVEP for large-scale in vitro production of buffalo embryos. Indeed, OPU linked to IVEP is currently the most suitable technology to accelerate genetic progress through the maternal lineage. The first advantage of OPU over MOET is given by the wider number of donors that can be enrolled, such as non-cyclic animals, pregnant cows, subjects with patent oviducts or genital tract infections, and animals that are not responsive to hormonal stimulation, the last representing a high proportion in buffalo. In buffalo OPU has been carried out on deep anestrous animals under ovarian ipotrophic conditions, on donors of different ages, reproductive status, days in milk [Gasparrini (2013)]. Furthermore, due to non invasivity and repeatability of oocyte collection, carried out by transvaginal follicular aspiration, OPU and IVEP allow higher embryo yields on long term basis. Indeed, the feasibility of repeated oocyte retrieval for up to 6 months was demonstrated, as indicated

by high embryo yields (Neglia et al (2011). However, after approximately 60 days of twice/weekly OPU sampling, a slowing down of the follicular turnover occurred, imposing to extend the interval between sessions from 3-4 to 7 days. Furthermore, after 6 months the number of follicles and oocytes decreased and more intriguingly the oocytes collected failed to develop into viable blastocysts, suggesting a premature aging of the donors. In the last 30 years, the IVEP efficiency has greatly increased, leading to high maturation (90%), cleavage (75-80) and blastocyst rates (30-40%). This improvement was mainly achieved by increasing the antioxidant oocyte capacity [(Gasparrini et al (2006)], and by optimizing the in vitro maturation and gamete co-incubation length [Gasparrini et al (2008). Furthermore, the enhanced pregnancy rates recently recorded after ET of cryopreserved buffalo IVP embryos [Saliba et al (2013; Gasparrini (2013) make this technology mature and suitable for commercial purposes. The major limitation encountered in this species is the reduced predetermined follicular reservoir, resulting in the scarce number of oocytes recovered [Gasparrini (2002)] and hence in the higher production costs per embryo. Hormonal treatments to increase the oocyte availability, such as FSH [(Boni et al (1994)] and rBST [Sá Filho et al (2009)] priming have been so far limitedly effective, although other schedules are currently under investigation. It follows that, as a great individual variability in follicular and oocyte recovery is observed [(Neglia et al. (2003, 2011), Gasparrini et al (2014)], and the follicular recruitment is predetermined within each individual, the best approach to make the technology competitive is to select donors on the basis of the antral follicular count [Gasparrini et al (2014)]. Furthermore, the positive correlation between the intrafollicular anti-Mullerian hormone (AMH) concentration and the antral follicular count suggests a potential use of AMH as a reliable marker to select donors to enroll in IVEP programs [Liang et al (2016)]. Furhermore, despite similar follicular and oocyte population throughout the year, buffalo oocyte developmental competence is strongly affected by season, with a two-fold increase of the blastocysts yields obtained during short day months [(Di Francesco et al (2012)]. It results that in order to improve the benefit/costs ratio oocyte collection should be planned during autumn at our latitudes**.**

Final remarks and recommendations

The utilization of AI in buffalo has greatly increased over the years in intensive breeding systems, with improved efficiency. The use of sexed semen in AI programs will further contribute to the diffusion of the technology in the field. With regard to embryo production technologies, OPU associated to IVEP has reached a competitive efficiency in terms of both embryo yields and pregnancy rates, suggesting a potential commercial exploitation. The possibility to use sexed semen for IVF will undoubtedly boosts the interest in this technology. The major constraint is the low number of oocytes recovered, causing high production costs and hence studies are still needed to increase oocyte availability and improve embryo quality.

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