

Advanced reproductive technology in the water buffalo

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Abstract

Embryo transfer techniques in water buffalo were derived from those in cattle. However, the success rate is much lower in buffaloes, due to their inherent lower fertility and poor superovulatory response. The buffalo ovary has a smaller population of recruitable follicles at any given time than the ovary of the cow (89% fewer at birth). In addition, estrus detection is problematic. Progress in the field of embryo transfer in water buffalo has been slow, and is primarily due to a poor response to superovulation. The average yield of transferable embryos is less than one per superovulated donor. In vitro embryo production could considerably improve the efficacy and logistics of embryo production. The technique of Ovum Pick Up is superior to superovulation; it can yield more transferable embryos per donor on a monthly basis (2.0 versus 0.6). The feasibility of intergeneric embryo transfer between buffalo and cattle has been investigated. No pregnancy resulted after transfer of 13 buffalo embryos to synchronized Holstein heifers. Preliminary successes with nucleus transfer of *Bubalus bubalis* fetal and adult somatic nuclei into enucleated bovine oocytes and subsequent development to the blastocyst stage have been reported.

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1. Introduction

Reproductive technology in water buffalo (*Bubalus bubalis*) has not developed to the extent it has in cattle (*Bos taurus*, *Bos indicus*). Although AI is practiced commercially, it is hampered by difficulties in estrus detection. More advanced procedures such as embryo transfer and IVF remain in the experimental stages due to poor yield of embryos. There are some preliminary successes with the esoteric approaches of intergeneric embryo transfer and nucleus transfer. This condensed review will touch on the current status of the above approaches.

2. Embryo transfer

Estrus detection, AI, and estrous cycle control are basic methods in assisted reproductive technology in

livestock. They are prerequisites to success with advanced procedures such as embryo transfer, transfer of frozen embryos and in vitro-derived embryos, including cloned embryos. Embryo transfer is a factorial process that consists of a series of carefully integrated sequential steps including donor selection, donor treatment, recipient selection, insemination of the donor, embryo recovery, embryo handling and evaluation, embryo transfer, and recipient care.

Although bovine embryo transfer technology has been successfully applied in the water buffalo, progress has been slow and results have been modest to poor. The first successful transfer in buffalo was performed in the United States of America in 1983 [1]. Subsequent successful transfers have been reported in Bulgaria [2], India [3] and Thailand [4]. Live buffalo calves have also been born after the transfer of in vivo-derived embryos frozen by conventional freezing methods [5]. Generally the numbers of animals involved have been small.

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It is of interest to look at the differences between the two genera, *Bubalus* and *Bos*, which contribute to lower success rates in buffalo. First among the inherent differences is the lower level of fertility in buffalo. The latter is largely due to lack of selection of breeding stock. Problems of delayed puberty and prolonged calving intervals can be reduced with adequate nutrition, parasite control, and disease management as has been demonstrated in the excellent (large) milk buffalo herds in Italy and Bulgaria. Seasonality of the breeding season may further restrict the success rate of embryo recovery.

3. Superovulation

Superovulation is even less predictable in buffalo than in cattle [1,3,5–9]; an optimal treatment regimen with FSH remains elusive. The buffalo ovary has a smaller population of recruitable follicles at any given time than the ovary of the cow, at birth an average of 12,000 primary follicles in the buffalo [10] versus an average of 133,000 in the cow [11].

Estrus detection in buffaloes is problematic because overt signs are few. All available techniques should be employed, including frequent observation, use of a non-entry teaser animal [12,13] transrectal palpation, and ultrasonography. Accurate determination of the onset of estrus (estrus = Day 0) is critical for precise determination of the stage of development of the embryo, as the onset of estrus is used as a reference point for the age of the embryo.

The Ovsynch protocol effectively synchronized ovulation in Murrah buffaloes and resulted in conception rates (to two fixed-time inseminations) that were comparable to those achieved with a single insemination after detection of estrus [14]. This study indicates the opportunity for the use of Ovsynch, with confirmation of ovulation by transrectal palpation or ultrasonography, to synchronized embryo recipients.

Embryo development is more rapid in buffalo than in cattle [15,16]. The zona pellucida serves as a distinguishing feature in the identification of embryos in the effluent recovery medium. After hatching, when this characteristic landmark has been shed, the blastocyst is difficult to recognize, particularly because the blastocoele is frequently collapsed. Hatching generally occurs 6.5–7.0 days after the beginning of estrus in buffalo compared to 8.5–10.0 days in cattle. Consequently, nonsurgical recovery of buffalo embryos must be done between 5 and 6 days after the onset of estrus (versus 7 days in the bovine) when they are in the morula and blastocyst stages. The negative implication

of this is that on Day 5 the corpus luteum has not yet reached full development and is both small and relatively soft, hence difficult to identify by palpation per rectum. The latter is in addition to the fact that the corpus luteum of the buffalo is already smaller per se and more deeply embedded (hence more difficult to palpate) than that of the cow. This difficulty extends to the proper evaluation of synchronized recipients on Day 5, in order for the embryo to be transferred to the horn ipsilateral to the corpus luteum. The use of ultrasonography has become a valuable, if not indispensable, adjunct.

4. In vitro embryo production

The birth of a live calf was reported as a result of the transfer of fresh in vitro-derived buffalo embryos to buffalo recipients in 1991 [17]. Subsequently, in 2004, six live calves were born after the transfer of 95 in vitro-derived vitrified buffalo embryos to 55 recipients (1–3 per recipient) [18]. Although numbers were small, no signs of abnormal offspring syndrome calves were observed.

That IVF could considerably increase embryo production (relative to the inconsistent response to superovulation) has led to an increasing interest in large-scale embryo production and the opportunity to enhance the maternal contribution to genetic improvement. Oocytes recovered from abattoir-derived ovaries generally have little genetic value. The mean number of oocytes collected per ovary and used for IVF was approximately 0.43–0.70 in India, and 2.4–3.3 in Italy [19]. This number increased to 2.25 when oocytes were collected by transvaginal ultrasound guided puncture, often referred to as Ovum Pick Up (OPU) [20,21]. These differences may depend on genetic, nutritional, environmental, and stressful conditions. However, this variation substantially affects the potential of IVF. The buffalo ovary is smaller than that of cattle [22]. With the OPU method, the recovery rate by aspiration of 2–8 mm follicles is much lower, from 0.7 [23] to 2.4 per ovary [24], compared with cattle in which 8–12 good quality oocytes are obtained on average from abattoir-derived ovaries [25]. The technique of OPU is superior to superovulation because it can yield more transferable embryos per donor on a monthly basis (2.0 versus 0.6, respectively). Therefore this technology has the greater potential to improve the genetic potential of the water buffalo via the maternal lineage [26].

Overall, high oocyte maturation, fertilization, and cleavage rates, but low rates of blastocyst yield, and calving after the transfer of in vitro produced buffalo

embryos have been obtained. The efficiency of IVF in buffalo is much lower than that in cattle. Several problems need to be resolved before IVF technology can be used commercially in producing buffalo breeding stock [27].

5. Intergeneric embryo transfer

The feasibility of reciprocal embryo transfer between water buffalo and domestic cattle has been investigated [28]. Eight mature water buffalo females were superovulated and hand-bred naturally to a male water buffalo. Sixteen buffalo embryos were recovered nonsurgically with 13 flushes 5.5, 6.0, 7.0, and 7.5 days after they were first bred by the bull. Three morulas were recovered on Day 5.5, six blastocysts on Day 6.0, one hatched blastocyst on Day 7, and six hatched blastocysts on Day 7.5. Compared to cattle embryos, the diameter of the embryo was generally the same, and the color, particularly that of the inner cell mass, darker. The stage of development was 24–36 h in advance of that of bovine embryos collected on corresponding days [29]. No pregnancies resulted after transfer of 13 buffalo embryos to synchronized Holstein heifers.

Intergeneric embryo transfers have been reported between sheep and goats [30–32] but have otherwise largely been limited to descriptions of attempts in rodents [33,34]. Selection of a recipient that is capable of hybridizing with the donor has been suggested [35] which would maximize the chances for an interspecies embryo transfer. There are no reports of hybridization (by natural service or AI) between *Bubalus bubalis* and *Bos taurus*, whose respective chromosome numbers are $2n = 48$ (swamp buffalo) or $2n = 50$ (river buffalo) and $2n = 60$ in cattle.

The feasibility of producing hybrid embryos of domestic cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*) was investigated by exposing cattle oocytes to buffalo sperm, and buffalo oocytes to cattle sperm [36]. The cleavage rate in buffalo oocytes exposed to cattle sperm was low (40.8%), with only 8.8% of these hybrid embryos reaching the blastocyst stage. Cattle oocytes exposed to buffalo sperm showed 86.3% cleavage, whereas 25.9% of these hybrid embryos attained the blastocyst stage [36].

A different approach to establish intergeneric pregnancies was explored by using adult fibroblasts as donor cells in nucleus transfer (NT) between buffaloes and cattle. Bubaline and bovine oocytes, matured in vitro for 22 h, were enucleated by micromanipulation. Subsequently, an ear fibroblast was injected into the enucleated oocyte (cytoplasm).

When bubaline adult fibroblasts were used as donor cells, there were no differences in the cleavage rates (66.2% versus 64.0%) between bovine and bubaline recipient oocytes, but more embryos derived from bovine cytoplasts developed to blastocysts than from bubaline cytoplasts (13.3% versus 3.0%). When bovine adult fibroblasts were used as donor nuclei, both cleavage rate (45.3%) and blastocyst yield (4.5%) of NT embryos derived from bubaline cytoplasts were lower than those of NT embryos derived from bovine cytoplasts (65.5% and 11.9%). The authors concluded that embryos constructed by intergeneric NT of adult fibroblasts between buffalo and cattle developed to blastocysts, but bovine cytoplasts may facilitate embryonic development more effectively than bubaline cytoplasts, irrespective of donor cell genus [37]. Additional success has been reported with the intergeneric nucleus transfer of *Bubalus bubalis* fetal and adult somatic cell nuclei into *Bos indicus* cytoplasts and subsequent development to the blastocyst stage [38].

6. Conclusions

Despite the inherent infertility of the water buffalo (*Bubalus bubalis*) slow progress has been made in the application of reproductive technology in this genus. The domestic cow (*Bos taurus*) serves as a good model, but idiosyncrasies of estrous behavior, ovarian characteristics, and embryo development in the water buffalo must be acknowledged. Although AI is practiced commercially, embryo transfer, in vitro embryo production, and nucleus transfer remain in the realm of experimentation. A recent report from China affirmed that even sexed semen by flow cytometric sorting of X- and Y-bearing sperm of buffalo is feasible [39].

Illustrations

Go to the Visual Guide to Theriogenology (<http://drostproject.vetmed.ufl.edu>), select the Bubaline Guide and/or the Bovine Guide, then select Reproductive Technology.

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