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OVUM PICK-UP- IN VITRO EMBRYO PRODUCTION - EMBRYO TRANSFER TECHNOLOGY IN THE APPLICATION OF WATER BUFFALO PRODUCTION

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ABSTRACT

The purpose of this study was to explore the feasibility of using ovum pick-up - in vitro embryo production and embryo transfer (OPU-IVEP-ET) in water buffalo production. As the water buffalo superovulatory responses and embryo transfer is inefficient in the past few years, there has been an increasing interest in the in vitro embryo production (IVEP) technologies for speeding up propagation of superior germplasm in buffaloes. OPU combined with IVEP can produce more embryos than MOET and which can improve the reproductive performance of water buffalo. At the same time, if combined with embryo cryopreservation and ET technology can speed up the breeding of water buffalo propagation and accelerate the development of dairy industry. In this study, well-bred frozen-thawed embryos were derived from OPU technology in the Laboratory of Guangxi Buffalo Research Institute. These types of embryos were transferred into the recipients in the Xiangbala Farm in Guilin. Two groups of crossbred water buffaloes were selected as recipients. One group with 11 heads was synchronized by injection PGc (0.6mg) intramuscularly. At the same time, another group of four animals in natural estrus were used. Frozen-thawed embryos were transferred at day 6 after estrous cycle. Results showed that there were 7 buffaloes observed to express estrus in the synchronization group with an estrus rate of 63.64%. Out of 7 buffalo recipients, 5 of them were transferred with embryos with the utilization rate of 71.43%. Pregnancy diagnosis was carried out to recipients at day 45 by B-mode ultrasound. Four recipients in natural estrus group were transferred with embryos. There were 3 recipients in the estrus synchronization group were found pregnant with the pregnancy rate of 60%, one recipient had a case of abortion while the two recipients delivered a healthy calf. On the other hand, the recipients in the natural estrus group were all found not pregnant. As indicated in this experiment, a total of 9 animals were transferred with embryos wherein 3 of the recipients were found pregnant. The pregnancy rate of frozen embryo obtained was 33.33% and two calves were born. Results suggested that OPU-IVEP-ET technology in water buffalo production was feasible and an effective way to speed up the propagation of water buffalo.

Key words: Ovum pick-up; in vitro embryo production; embryo transfer, applications; Water buffalo.

INTRODUCTION

Water buffalo is an important animal in tropical and subtropical regions which can provide high-quality milk, meat, and draught power. Swamp buffalo in China is mainly distributed in southern China covering 18 provinces and autonomous regions. In these areas, 22,745,000 water buffaloes exist (FAO, 2005) accounted to 16.62% of the total number of cattle in China. About one-fifth of the total water buffalo in China (4,197,000) can be found in Guangxi where forage grasses can be found in abundance. Guangxi is a major province of rearing water buffalo and ranking first in China. For a long time in China, the local water buffalo breed mainly showed low milk production performance, with an average annual milk yield of 700-800 kg. Embryo biotechnology in today's world can speed up the propagation of high-yielding dairy cows and can improve rapidly increasing slaughter rates in beef cattle in the most effective technological means. Since 1991, the birth of the first water buffalo derived from in vitro fertilization has made great progress, however, in vitro embryo production efficiency is still low with only 20% of the water buffalo oocytes can develop to blastocyst stage embryos (Nandi et al., 2002). Due to lack of breeding records of slaughtered buffaloes, collecting oocytes from slaughterhouse play a little role in the fast genetic improvement of water buffalo for breeding. Application of OPU-IVF-ET can repeatedly collect oocytes from the ovary of dam that expresses good genetic traits and can fully display the reproductive potential. As buffalo showed poor superovulatory responses to hormonal treatments, applying OPU-IVEP technology in a certain time can produce more embryos. Therefore, OPU-IVEP-ET technology is of great significance to be in practice. The purpose of this study was to apply OPU-IVEP-ET technology in water buffalo production, explore its feasibility and enhance the promotion of this technology for use in genetic improvement in water buffalo.

MATERIALS AND METHODS

Chemical reagents: The reagents used in this experiment for embryo culture were purchased from Sigma Chemical Company while fetal bovine serum was from Gibco Company. Estrus synchronization hormones or PGF2-alpha synthetic analogue (Cloprostenol) were procured from the Shanghai Family Planning Institute.

Instrument: HS2000 Ultrasonic imaging produced by Honda of Japan, with 7.5/5.0MHz vaginal sector scan probe (Aloka) equipped with 60 cm long needle for oocyte collection, vacuum pumps, follicular fluid collection tube, temperature devices, filter cup for embryos, CO_2 training Box (United States Forma), stereomicroscope (Japan), HS101-VB super instrument (Japan), embryo transfer, the gun companies from France IVM Kasuri.

Semen Source: Murrah buffalo semen in straws from Guangxi Livestock Breeder Improvement Station were used in this experiment.

Donor Source: The donors of Murrah and Nili-ravi water buffalo were available from Water buffalo breeding farm in Guangxi Buffalo Research Institute.

In vitro embryo production: The in vitro embryo production method was based and adopted from the procedures of Huang et al., (2004).

Water buffalo embryo frozen and thawed: In vitro fertilization and embryo vitrification was based following the methods of Zhang et al., (2007).

Recipient water buffalo: Water buffalo recipients used were from Xiangbala water buffalo farm. Fifteen Murrah and Nili-Ravi buffaloes with an average body weight of 450 ±46.7 kg and with age that ranged from 3-5 yrs old.

Method: Water buffaloes were classified into two groups: one group with 11 hds synchronized for estrus. In this group, 5 hds with CL and 6 hds with non CL were injected with 0.6 mg Cloprostenol, and then 3 days after injection, these water buffaloes were observed for estrus. The other group with 4 hds of water buffaloes from the naturally occurring estrus, then after day 5 from estrus the status of CL were observed and monitored. B-mode ultrasound machine were used to check the ovarian status of the animals.

Embryo transfer: Water buffalo which have qualified corpus luteum from natural estrus or estrus synchronized were selected for embryo transfer from the day 6 of estrus.

Pregnancy diagnosis: At 35-45 days after embryo transfer, the pregnancy status of recipient water buffaloes was checked by B-mode ultrasound machine.

RESULTS

The CL status in natural estrus water buffaloes: Five days from estrus, the CL status of 4 water buffaloes in natural estrus were checked using the B-mode ultrasound machine. It was found that 1 water buffalo attained class A CL, 2 water buffaloes had class B CL, and 1 water buffalo had class C CL. The ovulation of natural estrus water buffaloes had the better situation over the synchronized water buffaloes.

The estrus and CL status of natural estrus water buffaloes: Eleven water buffaloes were treated with synchronizing agent for synchronization of estrus, then 3-5 days later the experimental buffaloes estrus status were checked. It was found that there were 7 water buffaloes expressed in estrus with the estrus rate of 63.64%. Five of the 7 water buffaloes reached CL qualification (1 class A, 1 class B and 3 classes C) and were utilizable CL getting the rate of 71.43%. Among the 7 estrus observed water buffaloes, 5 had ovulation and had developed CL. These 5 water buffaloes had CL before treatment. The other 2 water buffaloes that had an expressed estrus did not ovulate.

The results of embryo transfer and pregnancy: Among the water buffaloes with natural estrus, 4 had qualified CL. However, 35-45 days after transfer, no one was found to be pregnant as checked by B-mode ultrasound machine. On the other hand, in 5 of synchronized water buffaloes, 3 were found pregnant that accounted to 60% pregnancy rate. One water buffalo had abortion after 1-month and 2 delivered a calf. The present results showed pregnancy rate of 33.33%, and calving rate of 22.22% for the transferred frozen-thawed embryo into 9 recipients.

DISCUSSION

Owing to buffalo production problems with late puberty, silent estrus, long partum estrus and poor superovulatory responses which result to low reproductive performance, application of the embryo-biotechnology can overcome these problems and have a great opportunity towards maximizing production potentials. Ovum-pick-up was first applied by Pieterse (1988) in Netherland. The principle was using ultrasonography as a guiding factor and using ovum pick-up machine penetrate ovary wall and collect oocyte and then finally use in-vitro fertilization and in vitro embryo production to obtain usable embryo, thus, combining embryo frozen-thawed and embryo production that can speed up the breeding technologies. The advantage of ovum pick-up is collecting lots of oocytes from live animal, and can be done repeatly in a certain period of time. Boni et al. (1996) was the one who reported the ovum pick-up techniques applied in water buffalo. On the other hand, Kitiyanant et al. (1995) carried out ovum pick-up in 6 swamp water buffaloes, for 3 consecutive times and the average oocyte recovery; the available oocyte and oocyte number were 12.4, 5.5 and 4, respectively. Galli et al. (1998) conducted ovum pick-up in 2 dry water buffaloes after in vitro maturation and in vitro fertilization, the cleavage oocyte were transferred into oviduct of sheep for incubation, the embryo were collected after day 7 of incubation and frozen embryo using 10% glycerol, and 9 embryos were transferred into 9 recipient water buffaloes. Three animals were found pregnant and had finally delivered 3 calves. In August of 2004, the first case of river water buffalo calf was delivered from swamp water buffalo recipient by ovum pick-up and in vitro embryo production technology. According to the results reported by Zicarelli et al. (1997), compared to superovulation, by ovum pick up could produce more embryos (1.4), and the average in a year can produce 15.7 - 34.6 set of available embryos per water buffalo donor, which is higher than that from superovulation method. Therefore, good advantages in the OPU-IVEP technology can be obtained and beneficial to the industry. In China, Huang et al. (2004) applied the B-mode ultrasound method in ovum pick-up in 42 water buffaloes with the results obtained an average oocyte percent recovery rate of 67.3%, usable oocyte percentage rate of 59.5%. The average collection of oocytes was 5.34±4.25 and usable oocyte of 3.18±2.89. Through OPU-IVEP finally resulted to have 1 case of river water buffalo calf delivered from swamp water buffalo recipient and with this OPU-IVEP technology with frozen embryo and embryo transfer technology with the 9 set of embryos transferred into 9 recipient water buffaloes had at least obtained pregnancy rate of 33.33% with 2 cases of live births with the calving rate of 22.22% which suggested that this technology can be of great importance in water buffalo production.

SUMMARY

From these research results, it was found that the good synchrony between recipient water buffalo and the age of embryo would enhance better result and success in embryo transfer. Mooreover, when estrus synchronization is to be conducted, selection of water buffaloes which has functional corpora lutea in experimental animals is extremely important. The purpose of this study was to apply OPU-IVEP-ET technology in water buffalo production, explore its feasibility and enhance the promotion of this technology for use in genetic improvement in water buffalo.

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REFERENCES

Boni R, S. Roviello and L. Ziearelli (1996). Repeated ovum pick up in Italian Mediterranean buffalo cows. Therio. 46: 899-909.

FAO, 2005, http://www.fao.org.

Galli C, R. Duchi, G. Crotti, and G. Lazzari (1998). Embryo production by ovum pick up in water buffalo. Therio. 49 (1): 400.

Huang, Y. J., G. A. Presicce, B. Gasparrini, B. Z. Yang, X. W. Liang, X. F. Zhang, M. T. Chen, Z.Q. Li and F. X. Huang (2004). Study on ovum pick-up and in vitro fertilization in water buffalo. China Animals Husb. & Vet. Med., 2004, 11:22-24.

Kitiyanant, Y., C. Tocharus, M. Areekijseree and K. Pavasuthipaisit (1995). Swamp buffalo oocytes from transvaginal ultrasound-guided aspiration fertilized and co-cultured in vitro with bovine oviductal epithelial cells. Therio. 43: 250.

Nandi S, H M., Raghu and B. M. Ravindranatha (2002). Production of Buffalo (Bubalus bubalis) Embryos in vitro: Premises and Promises. Reprod. Dom. Anim. 37: 65-74.

Pieterse, M. C., Kappen, K. A., ThAM. Kruip (1988). Aspiration of bovine oocytes during transvaginal ultrasound scanning of the ovaries [J]. Therio., 30: 75l-762.

Zhang, X. F., X. W. Liang, M. T. Chen, C. Y. Pang, F. X. Huang, G. S. Qin, C.H. Liao and S. J. Wei (2007). In vitro fertilization and subsequent derived from abattoir ovaries follicle aspiration embryo development using oocytes or collected by transvaginal in water buffalo. Anim. Husb. and Vet. Med. 39(11):15-17.

Zicarelli L., Boni, Gasparrini B, et al., 1997. News on buffalo reproduction. Proceedings of 5th World Buffalo Congress, Caserta, Italy. 748-753.