Effect of Frozen Semen from Italian Mediterranean Buffalo on Some Reproductive Parameters and Conception Rate Performed to Different Species Water Buffalo in Southern China

Guangsheng QIN ， **Bingzhuang YANG, Zhengzhun TAN** ， **Hui LI, Jian HUANG** ， **Xianwei LIANG***

Key Laboratory of Buffalo Genetics, Breeding and Reproduction technology, Ministry of Agriculture and Guangxi, Buffalo Research Institute, Chinese Academy of Agricultural Sciences, 24-1 Yongwu Road, Nanning 530001, P.R. China.
*Corresponding email: liangbri@126.com

ABSTRACT

The aim of the present study was to determine the effect of insemination of Italian Mediterranean buffalo by artificial insemination in China. The frozen semen of Italian Mediterranean buffalo imported to China was used to inseminate the existing Murrah, Nili-Ravi and local buffalo from Guangxi Buffalo Research Institute. During 3 years, 65 Murrah buffaloes, 48 Nili-Ravi buffaloes and 52 local buffaloes their ages range between 2.5 and 9 years old with natural estrus were selected and inseminated with the Italian Mediterranean buffalo frozen semen in the uterine horn by the rectum deep grasp. 40 days after mating, the early pregnancy diagnosis of these inseminated animals was checked by B Mode ultrasound. The results showed that the conception rate in cow of Murrah, Nili-Ravi and local buffalo were 45.31%, 52.08%, 48.08%, respectively. And the average conception rate was 47.88%. Although there was no significant difference (P>0.05) among the different buffalo species. The results suggested that it was practicable that the introduction of Mediterranean water buffalo frozen semen to carry out hybrid combinations, to improve the existing water buffalo species and to improve their production performance.

Keywords: Mediterranean Water Buffalo ；Frozen Semen; Artificial Insemination; Conception rate

INTRODUCTION

Italian Mediterranean buffalo is a genetic type used as milk production, which is known as one of the best river buffalo with good milk performance in the world. The average milk yield in lactation can reach to 2168 kg, even 4000-5000 kg from some of the high productive buffalos. In Italy, buffalo milk is processed into fresh cheese that is contributed in both local and international markets. Guangxi Buffalo Research Institute is the only institute in China which focuses on buffalo studies. In addition, it has two types of river buffalo (Murrah and Nili-Ravi buffalo). China imported the frozen semen of Italian Mediterranean buffalo in July of 2007 at the first time and it was used to inseminate the existing Murrah, Nili-Ravi and local buffalo for research. This program was supported by the fund of International technical cooperation projects and "948" Program of Ministry of Agriculture of China. The aim of importing Italian Mediterranean buffalo frozen semen, in one hand, was to breed a new buffalo species by selecting and cultivating inseminated animals with the frozen semen. In another hand, the aim was to be selected the best cross-combination of mating and to improved production performance of offspring. The present study has been designed to determine the effect of artificial insemination with frozen semen of Italian Mediterranean buffalo in China, as well as to provide a basis for future scientific research and clinical applications.

MATERIALS AND METHODS

The study was conducted at Guangxi Buffalo Research Institute, China. A total of 165 water Buffalos ages range between 2.5 and 9 years were assigned for this study, Murrah buffaloes, Nili-Ravi buffaloes and local buffaloes was 65、48 and 52 respectively. All animals with good health and have no reproductive disease history. The body weight ranged from 350 to 650 kg. The straw frozen semen of Italian Mediterranean buffalo imported from Cooperativa Fecondazione Artificiale (CoFA). The effective sperm count of straw frozen semen was above 107/ml and the spermatozoa motilities of thawing semen were above 0.4.

Estrus detection

Estrus detection was performed to all animals visually observed by trained technician everyday. Cows also exposed to vasectomized bulls to observe spontaneous estrus. When a cow displayed spontaneous, overtly evident estrous-behavior, follicular development was checked by B-mode everyday to make sure the location of dominant follicle. Ovulation was also observed every 4 to 6 hours per day. Cows were artificially inseminated until ovulation was verified based on estrous behavior and mucus discharge.

Method of semen thawing

The frozen semen of Italian Mediterranean buffalo first was taken it out from a nitrogen canister, and stayed in the air for a short 5 to 8 seconds, then put it on a water bath at 37.5 -39℃ for 10-15s. The motility and modality of semen was checked by microscope, post-thaw motility of semen was above 0.40.

Method of artificially insemination

Buffalo cows were artificially inseminated with frozen semen approximately 5×10^6 sperm cell of Italian Mediterranean buffalo after confirming the estrus 12- 24h.

Pregnancy diagnosis

Pregnancy diagnosis was checked on day 40 post insemination by both rectal palpation and ultrasonography.

Data collection and statistical analyses

Incidence of estrus duration, estrous behavior signs, ovulation time, follicular development and conception rate were recorded. Take different buffalo species as a parameter, the number of inseminated cows and pregnant ones as results. All data were analyzed using a chi-squared test by SAS 8.0. Difference was not significant (P > 0.05).

RESULTS

Effect of frozen semen from Mediterranean buffalo bull on conception rate under different buffalo species

As can be seen from table 1, 65 Murrah buffaloes, 48 Nili-Ravi buffaloes and 52 local buffaloes with natural estrus were selected and inseminated with the Italian Mediterranean buffalo frozen semen in the uterine horn by the rectum deep grasp during 3 years. The conception rate in artificial insemination of Murrah, Nili-Ravi and local buffalo was 45.31%, 52.08%, 48.08%, respectively. According to table 1, there is no significant difference on conception rate between species that artificial inseminated with Italian Mediterranean buffalo frozen semen (P > 0.05) .Moreover, the average conception rate was 47.88%.

Effect of frozen semen from Mediterranean buffalo bull on conception rate under different buffalo ages

In order to know whether there are different in pregnancy of AI between heifer buffalos and multiparous buffalos.The results of heifer buffalos and multiparous buffalos pregnancy was 54.55% and 44.44% respectively in the Table 2.The pregnancy rate per AI was higher in heifer buffalos than multiparous buffalos, however, the difference is not significantly (P > 0.05) .

Effect of ovulation time on conception rate

Comparison pregnancy rate with AI before ovulation time and after ovulation time, it was

found that their pregnancy rate was 43.08%, 52.56% and 28.57% when AI was done in 6-8 hours before ovulation, 1-3 hours after ovulation and 4-6 hours after ovulation. There was an apparently effect of ovulation time on conception rate presented in table 3, the best time for mating was in 1-3 hours after ovulation and the conception rate of it was significantly higher than that in 4-6 hours after ovulation（ $P < 0.05$ ）. But no difference was found in conception rate between 6-8 hours before ovulation and 1-3 hours after ovulation（ $P > 0.05$ ）.

DISCUSSIONS

According to the results, the average pregnancy rate per AI was 47.88% which is slightly lower than the result reported by Moioli (Moioli et al., 1998)（ 56% ）, and in agreement with Zicarelli (Zicarelli et al., 1997) （ 42.5%-51.1% ）. This means it is practicable for the existing buffalos in China to adapt to breeding system by submitting AI with imported Italian Mediterranean buffalo frozen semen.

From these results we also conclude that the conception rate of Nili-Ravi buffalo was the highest one among all inseminated cows (52.08%). However, the difference of conception rate between species was not significant（ $P > 0.05$ ）, which is similar to the results reported by Cai (1997). Cai reported that buffalos aged from 3 to 8 had the highest pregnancy rate (66.5%), buffalos aged from 9 to 12 and above 13 had a pregnancy rate of 45.6%, 35.3% respectively. These studies show that the pregnancy rate of AI conducted on heifer buffalos was higher than multiparous buffalos. The main reason, which could be the uninfected uterus and tractus genitalis of heifer animals, resulted to be beneficial to spermatiation and embryo nidation. Moreover, the multiparous buffalos often suffer a variety of reproductive diseases. Also the less activated ovary of old aged buffalos may lead to delayed ovulation, which can affect the conception rate in return.

The effect of ovulation time on AI is given in Table 3, which indicated that ovulation time has a great influence on the pregnancy rate. But estrous detection is difficult in buffalo because of the scarce behavior signs. It is also difficult to recognize buffalo cows in heat because of their asynchronism. In addition, due to variable duration of estrus (4–64 h) (Ohashi, 1994; Baruselli, 2001) and the difficulty encountered in predicting the time of ovulation, artificial insemination (AI) in buffaloes is limited. Therefore it is important to know the regular pattern of ovulation and the best time for artificial insemination in buffalos. In this study, 125 estrous cycles of 85 buffalos were monitored by B-mode (HS-101V, Japan), the average time from the start of estrous to ovulation was 40.7 hours (19-96h), which is similar to the results of 40.8 h (Zhou, 2004; Qisheng, 2004) and 47 h (Li, 2007). The results (Wu, 2007) also showed the best time for AI to almost all buffalos is at the 40 hours after estrous. Artificial insemination submitted at 36 to 40 hours after estrous had a conception rate of 69.86%. On the contrary, artificial insemination performed at 24 hours before estrous or 60 hours after estrous had a lower conception rate (25.71% and 32% respectively). The difference was significant (P<0.05).

In conclusion, artificial insemination performed to the existing Murrah, Nili-Ravi and local buffalo in China with the imported Italian Mediterranean buffalo frozen semen improves the conception rate. In our opinion it is practicable to carry out hybrid combination research and improve the existing buffalo species as well as their production performance by introducing Italian Mediterranean buffalo frozen semen.

ACKNOWLEDGEMENT

REFERENCES

Baruselli P.S. 2001.Control of follicular development applied to reproduction Biotechnologies in buffalo. *In: Proc I Congresso Nazionale sull'alleva   ento del bufalo:* 128–46.

Cai, Q.Z. 1997. Effect of breed, season, age and parity on pregnancy of AI buffalo. Chinese Journal of   Veterinary Science 1：98-99.

Li, Z.Q. 2007. Resarch on artificial insemination of buffalo in breeding season [J]. Guangxi Journal of Animal Husbandry & Veterinary Medicine 23(3): 116-117.

Moioli B.M.，F. Napolitano，S. Puppo，V.L. Barile，G.M. Terzano，A. Borghese，A. Malfatti，A. Catalano and A.M. Pilla. 1998. Pattern of estrous，time of LH release and ovulation and effects of time of artificial insemination in Mediterranean buffalo cows. Anim. Sci. 66：87-91.

Ohashi O.M. 1994. Estrous detection in buffalo cow. Buffalo J.   (Suppl. 2):61–64.

Wu, S.，J.R. Bin，Y.X. Zhang   ，Y.M. Wei，Z. Y. Wu. 2007. Research estrous characteristics and timely insemination of Guangxi buffalo. China Herbivores 27(6)：34-35.

Zhou, Q.S. 2004. Experience of artificial insemination. Guangxi Journal of Animal Husbandry & Veterinary Medicine 20（2）：61-64.

Zicarelli, L.，C. De Filippo，M. Francillo，C. Pacelli and E. Villa. 1997. Influence of insemination technique and ovulation time on fertility percentage in synchronized buffaloes. Proc. Fifth world buffalo congress，Caserta，Italy，13-16：732-73

Table 1.   Comparison of buffalo artificial fertilization effect on different breeds.

Breed	Numbers of AI	Numbers of pregnancy	pregnancy rate(%)
Murrah buffalo	65	29	45.31
Nili-Ravi buffalo	48	25	52.08
Local buffalo	52	25	48.08
Total	165	79	47.88

Table 2.   Effect of buffalo pregnancy rate on different ages.

Age	Numbers of AI	Numbers of pregnancy	pregnancy rate（%）
Heifer buffalos （2.5-4years）	44	24	54.55
multiparous buffalos (5-9years)	72	32	44.44

Table 3. Effect of artificial insemination to buffalo conception rate time on ovulation time.

ovulation time	Numbers of AI	Numbers of pregnancy	pregnancy rate（%）
6-8 hours before ovulation	65	28	43.08
1-3 hours after ovulation	78	41	52.56
4-6 hours after ovulation	21	6	28.57