Theriogenology xxx (2015) 1–6

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

A field study on artificial insemination of swamp and crossbred buffaloes with sexed semen from river buffaloes

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ARTICLE INFO

Article history:
Received 31 December 2014
Received in revised form 6 May 2015
Accepted 9 May 2015

Keywords:
Buffalo
Sexed semen
Artificial insemination

ABSTRACT

Sex preselection by flow sorting of X- and Y-sperm has been proven to be an efficient and economically feasible strategy for use in Holstein dairy cow breeding, and previous reports have demonstrated the feasibility of altering the sex ratio in buffalo species by using sexed semen in either artificial insemination or IVF. However, because buffalo reproductive physiology and farm management are different from Holsteins, factors involved in artificial insemination by sexed semen need to be further addressed before being applied in buffalo breeding at village-level husbandry. In this study, a total of 4521 swamp or crossbred (F1 or F2) buffaloes with natural estrus were inseminated with X-sorted sperm from river buffaloes, resulting in a 48.5% (2194 of 4521) pregnancy rate and 87.6% (1895 of 2163) sex accuracy in the derived calves. The pregnancy rate obtained with sexed semen from Murrah bulls was higher than that of Nili-Ravi, 52.5% (895 of 1706) versus 46.1% (1299 of 2815; $P < 0.01$), respectively. Also, significant variations were seen in pregnancy rates from inseminations performed in different seasons ($P < 0.01$) and by different technicians ($P < 0.01$). In contrast to Holsteins, no difference was seen in the pregnancy rate between heifers and parous buffalo cows, and buffalo cows with different genetic backgrounds (swamp type, crossbred F1 and F2) showed similar fertility after insemination with sexed semen. The findings in the present study under field conditions pave the way for application of sexing technology to buffalo breeding under village-level husbandry and diverse genetic backgrounds.

1. Introduction

Buffaloes have been domesticated for use as farm animal for a long time in Asia and are well adapted to the environment of high humidity and high temperature in tropical and subtropical countries. It is the most important noncattle dairy animal and provides over 13% of the total milk production across the globe, whereas in India and Pakistan, the majority of the dairy products are derived from dairy buffaloes instead of other well-known dairy breeds [1]. There is a huge population of buffaloes in China and the Southeast Asian countries, but most of these are swamp buffaloes which have much lower milk production than the river types of South Asia or the Mediterranean countries. To develop a sustainable alternative to meet the increasing demand for dairy products in terms of both quantity and quality in these

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http://dx.doi.org/10.1016/j.theriogenology.2015.05.022

regions, there is an urgent need to improve the productive performance of swamp buffaloes [2], and the introduction of gene from river type buffalo through crossbreeding has proven to be an efficient strategy for improving milk productivity of native swamp buffaloes [3].

In animals such as dairy cows where productivity depends on the sexual phenotype, preselecting the sex of offspring is of significant importance. Flow sorting of X- and Y-sperm combined with artificial insemination (AI) is an effective strategy for skewing the sex ratio in mammals and rapidly extending the animal population by increasing the percentage of females [4]; this strategy has been proven to be economically feasible through commercial application in Holstein cows [5]. Furthermore, buffalo sperm can be sorted into X- and Y-enriched populations on the basis of difference in their DNA contents [6], and sexed buffaloes have been produced by AI or IVF [7,8]. A comparable pregnancy rate was obtained by AI using sexed and unsexed semen in buffalo species after spontaneous or synchronized estrus [8,9]. Thus, introduction of sexed semen from river buffalo to the swamp buffalo could be a practical strategy for rapidly expanding the dairy buffalo herds and improving the productivity of buffaloes in East and Southeast Asia. However, given the limited data in documented reports, the unique characteristics of buffalo reproductive physiology, and the diversity in buffalo management in Asian countries where most buffaloes are household raised with small herd size, the factors affecting pregnancy from insemination with sexed semen need to be further addressed before commercial applications of this technology at this level of implementation.

In the current field study, sexed semen from river buffalo was randomly inseminated into the swamp or crossbred buffaloes in Guangxi, China. The effects of age, parity, season, and genetic background on pregnancy were evaluated under field conditions, and the outcome of this study is of significant value for implementing the use of sexing technology in buffalo breeding.

2. Materials and methods

2.1. Animals

The bulls used for semen collection were river-type buffaloes, 21 Murrah and 23 Nili-Ravi, raised at the Guangxi Livestock and Poultry Breeding Station, China. All bulls were of proven fertility, and semen was routinely collected twice a week using an artificial vagina for regular or sexed semen production. A total of 4521 female buffaloes were used in this study, which included 4324 cows and 197 heifers, of which 4038 were swamp type and 272 F1 and 68 F2 crossbreds. All the females were household raised in herds, sizes ranging from one to five heads. Experiments were conducted and animals used following the established standards and policies specified by the Animal Care and Use Committee of Guangxi University.

2.2. Sexed semen production

Semen samples collected from the bulls were subjected to quality control by computer-assisted sperm analysis (Hamilton Thorne Research), and only samples with greater than 75% morphologically normal sperm and greater than 65% progressive motility were used for flow sorting. The buffalo sperm was sorted into X- and Y-sperm–enriched populations on the basis of difference in DNA content using a MoFlo SX flow cytometer [6]. Briefly, sperm was stained with 40 μg/mL of Hoechst 33342, incubated in modified Tyrode's solution at 35 °C for 45 minutes, filtered through cell strainer to remove the dead cell clumps, and then sorted into X- or Y-chromosome–bearing sperm–enriched populations using the flow cytometer operated at 40 psi, with 197-mM Tris-base as the sheath fluid. Sperm were sorted into 50-mL tubes containing 2 mL of Tris extender supplemented with 20% (v:v) egg yolk, and the sorted sperm was centrifuged at 800 × *g* for 20 minutes and finally frozen using Tris extender supplemented with 20% egg yolk (v:v) and 6% (v:v) glycerol, with each 0.25-mL straw containing a total of $\sim 2 \times 10^6$ sperm. The sorting accuracy was determined by reanalysis of the sperm nuclei, and only the lots with greater than 90% purity and greater than 30% motility were used.

2.3. Artificial insemination

The frozen sexed semen was randomly assigned to 23 breeding stations, each operated by one technician, located in five counties in Guangxi, China. The buffalo cows or heifers were inseminated after spontaneous estrus. Estrus with overt signs was first observed by the owners of the animals and then confirmed by technicians through transrectal palpation to detect the presence of the dominant follicle before insemination. One dose of sexed semen was thawed and deposited in the deep uterine horn ipsilateral to the side with follicle impending ovulation.

2.4. Data collection and analysis

Data on the pregnancies were collected by diagnosis 2 to 3 months after insemination through transrectal palpation, and those that failed to develop to term were considered as embryo loss or abortion. The effects of parity, season, genetic background, and AI technician were analyzed. Some of the household buffaloes without detailed records were excluded from the analysis of any factor that could not be clearly identified. Statistical analyses were performed by the chi-square test using SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA), and a P value less than 0.01 was considered significant.

3. Results

In the current field study, a total of 4521 female buffaloes were inseminated with X-sorted sperm from river buffaloes, and 2194 were confirmed pregnant 2 to 3 months after insemination, which represents a 48.5% pregnancy rate (Table 1). Of the total conceptions with sexed semen, 31 (1.4%) failed to develop to term, whereas 2163 (98.6%) gave birth to healthy calves, of which 1895 (87.6%) were females and 268 (12.4%) males.

Sperm samples used in flow sorting were collected from 21 Murrah and 23 Nili-Ravi bulls, two pure-breed