EVALUATION OF A STRATEGY TO INCORPORATE REPRODUCTIVE BIOTECHNOLOGIES INTO BUFFALO FARMING WITHOUT AFFECTING REPRODUCTIVE PARAMETERS OF THE HERD : A COLOMBIAN CASE

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ABTRACT

Buffalo breeders need to improve buffalo production to satisfy the market needs, reproduction is one of the parameters to be improved specially if it is combined with reproductive biotechnologies. The aim of this paper is to show a Colombian Experience in which artificial insemination is introduced to the current management of a buffalo herd without affecting the reproductive parameters. It has been obtained a decrease of 26 days in the day open of the farm, pregnancy rates of the natural heat and fixed time artificial insemination are not statistically different and are similar to those reported in the literature. The feasibility of using AI in buffalo herds without affecting reproductive parameters is reported here and also allowing to get the benefits of artificial insemination to improve production.

Keywords: *Bubalus bubalis*, buffaloes, articial insemination, reproduction, FTAI, strategies

INTRODUCTION

Improving the production of animal protein is mandatory for social and technical reasons, since it is important to consider the strengths and weaknesses of each system. Buffalo production being introduced and increasing in various countries; it is seen as the animal of choice in coming years for many countries across the globe, due to its inherent qualities of adaptability to harsh environments, resistance against diseases, milk with high quality A2 casein, production based on minimal rations and in lands in which cattle does not perform well, low cost of meat and milk production as well as of work, and the possibility of transforming their products into food of high value for human nutrition.

Buffaloes contribute about 13% to the world milk production with 3.5% of annual growth rate, compared with over 2.1% for cow milk production (INPA, 2013). The buffalo population in Colombia has an annual growth rate of (16%) during the last five years, and the demand for reproductively more efficient animals has also increased (Berdugo, 2012). In Colombia, from the beginning of the production of buffalo, the producers began to use

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the same biotechnologies as in cattle but due to low pregnancy rates of buffaloes the use of artificial insemination (AI) or embryo transfer (ET) needs to be improved. Experience in India shows that the average milk production of buffaloes reached 4.6 kg/day/buffalo in 2009 while in 1993 it was only 3.4 kg/day/buffalo. This success is attributed to the extensive AI programs used in the country with the active participation of buffalo owners.

The first AI in Colombia was reported in 1976, but it was only in 2002 that sufficiently good and reproducible results were obtained. Artificial insemination has been diffused especially at fixed time, however, exact data on the use of AI in our country. AI is practiced very little in Europe and East Asian countries with a coverage of only 5% buffaloes in Italy, 3.7% in Azerbaijan, 0.3% in Egypt, and 0.1% in Romania although in Bulgaria, 80% buffaloes in large cooperative state farms are subjected to AI . (Singh and Balhara, 2016).

One of the main problems of AI in buffaloes is heat detection (Barile, 2005). It is accepted worldwide that the use of timed AI is the ideal way to start the application of the technology and afterwards, natural heat with the aid of a teaser bull can be used (Zicarelli *et al*., 1997). It is very important to consider that the reproductive parameters and the biological aspects for buffalo reproduction in the herd must be the best in order to be successful in establishing AI programs.

Many reports exist of the application of AI in Colombia (Angulo *et al*., 2004; Saldarriaga, *et al*., 2016) but the technique is not widely used as an impact over production is required, especially because of the farmers` fears to alter reproductive parameters of the herd. Baruselli *et al*. (2014) proposed a model that combines de use of AI and natural mating without affecting reproductive parameters and to use AI in daily farming, with the aid of progeny testing programs to test the breeding value of bulls; this approach would favor an increase in productivity and the development of the industry.

The aim of this study is to present the results of the application of a model developed to use reproductive biotechnologies without affecting the reproductive parameters of the herd, and provide information related to the reproductive performance in a buffalo farm in Colombia.

MATERIALS AND METHODS

The study was performed in the Fortaleza Farm, Monteria, Colombia, South America, (8`75``N, 75`88``W) during the reproductive season 2015 to 2016. Sixty-six healthy, pluriparous females without anatomical abnormalities, were included in this study, and the age, parity and average intercalving period were recorded. All animals were kept in the same conditions, grazing *Brachiaria pastures*. It was selected at random in one of the following groups: fixed timed artificial insemination (FTAI) and natural heat. The females in the FTAI group (n=46) have 72±18 days after calving and were synchronized using OVSYNCH (Baruselli, 2014) protocol, natural heat (NH) group were mixed with three vasectomized bulls 15 days after calving. NH females were inseminated 6 hours at the end of the oestrus. After the first insemination either FTAI or NH, the animals continued in the program until the next natural heat and insemination, No more than 2 inseminations were performed. Twenty-eight days after the last insemination, the buffaloes that did not present estrus were evaluated for pregnancy using an ELISA KIT (IDEXX visual Pregnancy Test Cat No 99-41369) and 45 days later, the pregnancies were confirmed by ultrasound. Frozen semen fulfilled the minimum standards to get pregnancies from 10 Murrah bulls were used this program.

Data were analyzed using the Mann-Whitney Test to identify statistical differences between the groups; statistical differences were considered with a $P<0.05$.

RESULTS

In this study 66 animals were included: 41 animals in the FTAI and 25 in the NH Group. The median age for the FTAI was 7.3 years (4.3 to 16.2 years) and 10 years for the NH Group (4.3 to 18.7 years, $P=0.18$). In addition, the parity for the FTAI group was 4 calvings (2 to 11 calvings) and 2 calvings in the NH Group (2 to 11 calvings, $P=0.01$). The mean intercalving period was 425 days (364 to 675 days) and 427 days (367 to 586 days) with no differences between the groups ($P=0.95$). No differences in pregnancy rates of the bulls were detected.

Pregnancy rate was 41.5% (17/41) and 36.6% (8/22) for the FTAI and NH Group, respectively; no statistical differences between groups were observed ($P=0.79$). After 45 days, 31.7% (13/41) and 36.6% (8/22) continued to be pregnant. The pregnancy rate for the second insemination was 41.6% (5/12) and 75% (3/4) for FTAI and NH, respectively.

The time of appearance of oestrus after FTAI or NH insemination was 22 days, with a range of 11 to 38 days, and 10 to 29 days, respectively, with no statistical differences ($P=0.63$). The first post-partum oestrus was observed after 55 days (range 27 to 76 days).

At the end of the study, 81.5% (22/27) of females summited to FTAI and 61.1% (11/18) to NH were pregnant.

DISCUSSION

In this study, we present information about the routine application in a herd of a combined scheme of natural mating and FTAI as a means to introduce AI into buffalo herds without affecting the reproductive parameters. In this experiment we demonstrate a reduction from 425 days to 399 days in the intercalving period. In order to improve reproduction of the herds, Rossi and coworkers propose the use of ultrasonography to identify not pregnant animals on day 20 after FTAI, and to re-synchronize them, twice and report that 95% of the animals became pregnant after the third insemination (Rossi *et al.*, 2014).

There was no significant effect on pregnancy outcome of body condition score, days of milk production, milk yield, bull, age (parity), or year of FTAI. In the study of Rossi *et al*. (2014), the intercalving interval was 487 days, whereas using FTAI plus sonography it was 416 days. In the present study, the duration of intercalving was 399 days for the FTAI and 382 for the NH Group compared to 425 for the herd. This difference in intercalving intervals could be explained by the proposed model for the application of the reproductive technologies as a consequence of the greater control of reproduction in the present study.

Certainly, it is necessary to remember that reproduction technology has had a major impact on cattle improvement during the last century, especially the development and introduction of AI (Harris, 1998). AI made the dissemination of superior genetic material possible, and progeny testing (Robertson and Randel, 1950) was introduced as a complement for reliable and stable genetic progress in many countries over 60 years ago.

Sparse information exists regarding the bull effect in AI programs in buffaloes. Certainly, researchers and farmers agree the programs need to use of aids for heat detection, to identify the best moment to inseminate the female, surgical prepared males are the best aid to do that. It has been reported (since 1997) that females with vasectomized bulls demonstrate a significantly higher reproductive efficiency than groups without them. There was also a higher incidence of spontaneous estrus (92 versus 69%, P<0.01), spontaneous estrus of high intensity (62.2% versus 31.1%, P<0.01*)*, and higher incidence of functional estrous cycles following spontaneous (65.8% versus 57.1%) or induced (77.0% versus 59.5%, P<0.05*)* estrus. Exposure to vasectomised bulls also increased the incidence of consecutive functional estrous cycles (90.5% versus 68.1%, P<0.01), and the pregnancy rate in cows inseminated at spontaneous (42.5% versus 18.9%, P<0.01) or induced (51.1% versus 33.3%; since 1997<0.05*)* estrus (Zicarrelli *et al.*, 1997).

The results obtained here agree with those of other authors related to oestrus length (Baruselli *et al.*, 1997_b), (Drost, 2007) report a duration of 21 days with a range of 18 to 24 days.

A recent review by Singh and Bakara (2016), presents the results of different protocols of FTAI with an average pregnancy rate from 28.0% to 66.6% with an average of 45.64% that is not very different from the results obtained here. It must be noticed that the lower results 28.0 and 28.2 correspond to the FTAI protocol used here. It might be possible to increase pregnancy rates up to 66.6% using other synchronization protocols with progestagens and a more intensive management of the follicular wave.

Many researchers have been evaluating different strategies to improve follicle developed in cattle submitted to FTAI to increase fertility in cattle, which could be applied to buffaloes. These strategies include the reduction in the circulating concentration of progesterone during the protocols, stimulation of endogenous gonadotropin release, and exogenous gonadotropin treatments (Menenghetti *et al.*, 2009; Vasconcelos *et al.*, 2009). In buffaloes, in spite of having examined many strategies, the only advance has been the use of specific synchronization protocols associated with the season (Gimenes *et al.*, 2011) as well as additionally allowing to schedule the time for procedures in the case of FTAI or using vasectomized bulls in NH programs. The results presented here show that it is entirely feasible to apply FTAI and NH in buffalo herds without affecting reproductive parameters and taking advantage of the benefits for improving production of the AI.

There is little published information related the use of AI to increase productivity in buffalo herds. In Colombia, the buffalo population has been increasing by 16% each year during the last 5 years, making the development of improvement programs mandatory, with the use of AI. In Colombia, AI needs to be used more frequently since no more than 2% of the females are inseminated, Borghese reported that AI is practiced very little in Europe and East Asian countries such as Iran and Egypt, efforts to increase the number of animals born using AI must be supported (Borghese, 2010).

It is important to know the genetic parameters and genetic correlations between traits associated with breeding and production of milk and meat because of their economic relevance. Agudelo-Gomez reports that the trait that could have a greater response to the selection process is weight at 18 months. Nevertheless, milk yield at 270 days was the trait with the greatest genetic progress during the evaluation period, probably due to the empirical selection conducted for this trait (Agudelo-Gomez *et al.*, 2007). All these parameters could be established faster with the use of AI.

Colombian breeders need genetically qualified bulls to increase productivity. It has been calculated that the needs are about 5000 genetically qualified bulls. Lohuis in (1995) reported an annual increase in milk production of 1 to 1.5% using AI but if proven bulls are used the gain is of 5%. The United States Deparment of Agriculture (USDA) improvement program indicates that it is possible to increase by 665 lb of milk every 5 years Dikinson *et al.*, 1974). Certainly we must keep in mind that with AI it is possible to concentrate desirable genes for the population. It is possible to select not only for quantity, it is possible to choose for quality. It is also possible to select for other parameters with high heretability such as fat, protein (Pai *et al*., 1971), other parameters such us meat quality, somatic cell score, making calving easy, and longevity could be introduced to the selection and will add more value to the use of AI.

In conclusion, the feasibility of using AI in buffalo herds without affecting reproductive parameters is reported here. Future work will demonstrate the heritability of the parameters that have been reported and that are not the same as in cattle (Rosati and Van Vleck, 2002), and will show the results of the effect of AI in the productivity of the farms.

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