EFFICACY OF DIFFERENT PROTOCOLS OF OVULATION SYNCHRONIZATION AND RESYNCHRONIZATION IN ARGENTINIAN BUFFALO HERDS

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ABSTRACT

During the last years buffalo reproductive researchers have been tried to develop and apply fixed timed artificial insemination protocols to increase pregnancy rates to maximize improvement. The objective of this paper is to evaluate the pregnancy rates of different protocols of synchronization (Ovsynch and progesterone implants) and resynchronization of ovulation in a meat water buffalo herd during reproductive season of 2016. 194 mature Mediterranean females, were randomly assigned to four different protocols, ultrasound evaluation were used to determine cyclicity (presence of corpus luteum or follicles ≥7 mm de diameter). 18 days after insemination all females were resynchronized and at day 25 ultrasound were performed, those nonpregnant females were inseminated 28 days after first insemination. All inseminations were performed by the same technician and only one bull was used. InfoStat software was used, statistical significance was considered when p value was lower than α of the 5%. No statistical significance were found within the protocols. Early embryonic dead 50 days after IA was 3.4%. Pregnancy rate was 47.9% and 53.5% for insemination and reinsemination respectively and the final pregnancy rate after the adjust for early pregnancy loss 73.2%, during the 28 days of the breeding period. The results obtained allow breeders to choose the best pharmacological option to use FTAI in their herds based on their own needs to improve production without affecting pregnancy rates.

Keywords: *Bubalus bubalis*, buffaloes, artificial insemination, pregnancy, FTAI, embryonic mortality

INTRODUCTION

The products derived of the buffalo herds are an emergent business that could contribute to strength and diversify the country economy, based on the quality and diversity: meat, milk

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and draught. Annually the buffaloes contribute to 1.2% of the meat and 12.4% of the milk produced around the world, with an increased rate of 12.4% in meat and 41.6% of milk (FAO, 2016). The first buffalo were introduced to Argentina in 1907 and buffalo meat industry began in Corrientes in 1972 with 1.200 head. In 2004, Crudeli *et al.* (2004) reported 65000 heads with an annual increase of 13% that has been maintained to date, the growing Argentinean buffalo industry needs to increase production using available reproductive biotechnologies specially in the North East (NEA) part of the country in which there are low and wet lands that are good for buffaloes (Baruselli *et al.*, 1997).

Artificial insemination (AI) is an important tool for improving the genetic progress in herds and has been used in buffalos (*Bubalus bubalis*) with relatively low success. One cause is the low homosexual behavior and discrete manifestation of estrus in buffalo (Singh and Singh, 1985). Other causes are estrus length (Kamonpatana *et al.*, 1979; Soares *et al.*, 1985), therefore it is very difficult to predict the time of ovulation (Geary *et al.*, 1998; Dhillon *et al.*, 1994) to define the best moment for the insemination that make necessary to evaluate the application of cattle derived protocols in the specie.

Hormonal therapies to induce estrus and ovulation in buffalo cows are important strategies to regulate reproduction in herds and to take advantage of the benefits of AI. Many efforts have been made to apply protocols designed in cattle to synchronize the ovulation and perform fixed timed artificial insemination. All of them combine the use of hormones to lyse the corpus luteum (CL), facilitate follicular development and manage the time of ovulation (Agrawal *et al.*, 1978; Singh and Singh, 1985). Lowering progesterone levels (P4) induces follicular development, estrus and ovulation (Markandeya and Bharkad, 2002). It has been reported that luteal regression could be induced by administration of prostaglandins (PGF2 alfa) after day 5 of the cycle, a similar effect that has been informed in cattle (Seguin *et al.*, 1977; Singh and Singh, 1985). Pregnancy rates (PR) after insemination using one single injection of PGF was 45 to 50% (Hattab *et al.*, 2000; Snel-Oliveira *et al.*, 2010) and it is very close to the results obtained by natural heat detection and insemination (Peter *et al.*, 1987; Taponen *et al.*, 1999).

Ovulation was induced with progestagens alone or combined with estradiol benzoate (EB), and prostaglandin injection at the time of implant removal induces estrus in 80 to 93% of the animals 40 to 96 h later (Singh and Singh, 1985). It has been reported that gonadotropin releasing factor (GnRH) induce ovulation 33±8 h after administration in 60 to 86% of the cases (Odde, 1990). The Ovsynch protocol combines the use of GnRH with PGF to lyse the CL and induce to ovulate the new dominant follicle generated (Odde, 1990; Thatcher *et al.*, 1993; Snel-Oliveira *et al.*, 2010). Other researchers have added the use of intravaginal device (DIV) for P4 release to the Ovsynch protocol (Thatcher *et al.*, 1993; Snel-Oliveira *et al.*, 2010), DIV, PGF and equine chorionic gonadotropin (eCG) (Twagiramungu *et al.*, 1994; Snel-Oliveira *et al.*, 2010), norgestomet and eCG (Usmani *et al.*, 2001), DIV y PGF combination (Dhaliwal *et al.*, 1987), or BE, DIV y PGF (Vale *et al.*, 1986).

There is a possibility of combining at the same time strategies for the reproductive management and to increase production of the herd. It can be used during the breeding season the benefits of synchronization protocols plus the benefits of fixed timed artificial insemination, without affecting the reproductive herd parameters.

Each country needs to evaluate the effects of the different developed protocols to adjust them to the management of the herds. The objective of this work was to evaluate the effect over pregnancy rate of different protocols for synchronization and resynchronization of ovulation in a fixed timed artificial insemination (FTAI) program using different protocols in beet type Argentinian buffalo herds.

MATERIALS AND METHODS

This research was conducted in an Argentinean buffalo farm, located in Corrientes Province, at 27º 20´ 33¨ de latitude south y 58º 08´ 27¨ de western longitude. 194 parous Mediterranean females, with body sore of 3.48±0.11 (scale 1 to 5), without genital anatomical abnormalities and ovarian cyclicity evaluated by corpus luteum presence and/or follicles of ≥7 mm of diameter), determined by ultrasound (Pie Medical S-100 with a sectorial probe of 5.0 to 7.5 MHz (Maastricht, The Nederland), all animals were maintained in grazing supplemented with minerals and water ad libitum were randomly assigned to one of the following protocols (TRT). Day 0, were considered the day of insemination, all injections were intramuscular, Figure 1.

The TRT1 (Ovsynch, n=50), Day 10, 8 µg of GnRH, day 3 150 µg de PGF2α, day 1, 8 µg de GnRH were administered, FTAI were performed 64 h after prostaglandin injection. 18 days after insemination, 8 µg of GnRH were administered and on day 25 pregnancy detection were performed by ultrasound. Non pregnant females were reinjected with 150 µg de PGF2α, on day 8 µg de GnRH were administered, FTAI were performed 64 h after the last prostaglandin injection.

The TRT2 (DIV-BE, n=80), day 10 an intravaginal dispositive for progesterone release (DIV) were inserted and 2 mg of estradiol benzoate (BE) were injected, on day -3, DIV was removed and 150 µg de PGF2α were administered, day 2, 1 mg de BE. FTAI were performed 56 h after DIV removal. 18 days after insemination a second use DIV was inserted plus 1 mg of BE, day 25 pregnancy detection were performed by ultrasound and DIV removal, non-pregnant females were reinjected with 150 µg de PGF2α, on day 26, 1 mg of BE were administered and FTAI were performed 56 h after DIV removal.

The TRT3 (DIV-GnRH, n=44) day 10 an intravaginal dispositive for progesterone release (DIV) were inserted plus 8 µg de GnRH, on day 3, DIV was removed and 150 µg de PGF2α, day 1, 8 µg de GnRH were administered and FTAI were performed 56 h after DIV removal. 18 days after insemination a second use DIV was inserted plus 8 µg de GnRH, day 25 pregnancy detection were performed by ultrasound and DIV removal, non-pregnant females were reinjected with 150 µg de PGF2α, on day 27 8 µg de GnRH were administered and FTAI were performed 56 h after DIV removal.

The TRT4 (DIV-GnRH+BE, n=20) day 10 an intravaginal dispositive for progesterone release (DIV) were inserted plus 8 µg de GnRH, on day 3, DIV was removed and 150 µg de PGF2α on day 2 1 mg of BE was administered. FTAI were performed 56 h after DIV removal. 18 days after insemination a second use DIV was inserted plus 8 µg of GnRH, on day 25 DIV removal and pregnancy detection by ultrasound were performed, non-pregnant females were reinjected with 150 µg de PGF2α, on day 26, 1 mg of BE were administered and FTAI were performed 56 h after DIV removal.

All resynchronized females were evaluated

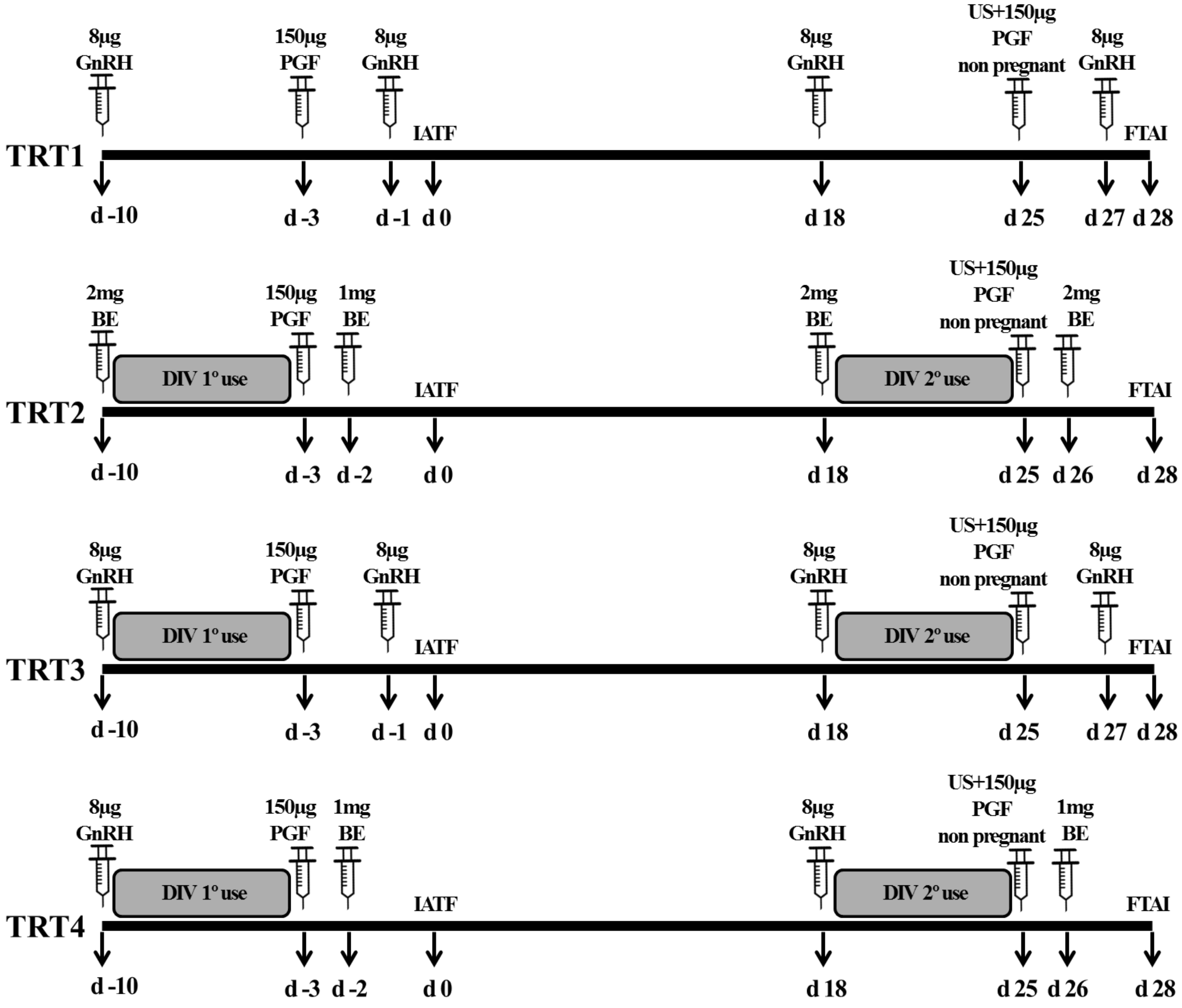

Figure 1. Experimental design used to study the follicular dynamics of four different treatments to synchronize and resynchronize estrus and ovulation in fixed timed IA in beef buffaloes. TRT1 (Ovsynch), TRT2 (DIV+BE), TRT3 (DIV+GnRH), y TRT4 (DIV+GnRH+BE).

25 days after the last insemination. From those pregnant females early embryonic mortality was ultrasound evaluated 50 days after insemination Figure 1, different protocols used.

All data were recorded in an special format designed for the experiment and pregnancy rates were evaluated in the different treatments using variance analysis with InfoStat software (Version), statistical significance were considered when p value was lower than α del 5% (Di Renzo *et al.*, 2018).

RESULTS

Pregnancy rates for TRT1, TRT2, TRT3, TRT4 was 42%, 52.5%, 50% and 40% respectively with no statistical significant differences (P≥0,05) (Table 1). Early embryonic loss and fetal mortality (EM) was observed in treatments TRT1 (5.4%) and TRT2 (5.1%) with no statistical differences (p≥0.05), for treatments TRT3 y TRT4 no losses were observed. The overall pregnancy rate at the first insemination was 47.9%, and at reinsemination was 53.5%, the combinatory pregnancy rate at day 50 after AI, adjusting for the pregnancy losses was 73.2% at the beginning of breeding season.

DISCUSSION

The results of this work show no differences in pregnancy rates after the use of different synchronization protocols. There are many reports that obtain similar results, in different parts of the world. Odde (1990) evaluate the use of GnRH or luteinizing hormone (LH) on day 9 in an Ovsynch protocol and obtained 56.5% y 64.2% respectively. Other researchers (Carvalho *et al.*, 2017), compared GnRH with BE and obtained 47.5% and 42.7% respectively. Vale (1988), compared the Ovsynch protocol with the use of presynchronization with prostaglandin (day 0 y 13 + GnRH day 15 and FTAI after 16 h) and found non significative differences in pregnancy rates (56.0% y 47.5%, respectively).

In Thailand (Tuchadaporn *et al.*, 2010)

Table 1. Pregnancy rates obtained with different FTAI protocols, early pregnancy diagnosis and resynchronization in buffalo.

Pregnancy rates	TRT1	TRT2	TRT3	TRT4	Total
First IA	42% 21/50	52.5% 42/80	50% 22/44	40% 8/20	47.9% 93/194
Resynchronization	55.2% 16/29	44.7% 17/38	59.1% 13/22	66.7% 8/12	53.5% 54/101
Losses	5.4% 2/37	5.1% 3/59	0% 0/35	0% 0/16	3.4% 5/147
Total pregnancy	70% 35/50	70% 56/80	79.5% 35/44	80% 16/20	73.2% 142/194

and Colombia (Bolívar-Vergara *et al.*, 2016), other researchers got lower pregnancy rates, but essentially the same results, no different pregnancy rates in spite of the use of different protocols used. They used Murrah females and the Ovsynch protocol and compared double insemination (12 and 24 h after GnRH) or insemination at estrus detection and obtained 33.3% vs. 30.7%, respectively without significantly statistical differences.

In Pakistán (Warriach *et al.*, 2008), researchers added the use of progestogens to the Ovsynch protocol and found that the inclusion of progestogens were beneficial to improve PR (30.4% vs. 55.5%). Also in Pakistán, Arshad *et al.* (2017) evaluate the effect over PR and EM of the resynchronization comparing two protocols classical Ovsynch and the use P4 and did not find differences, but the cumulative pregnancy rate of Ovsynch were better 81% compared with controls 59%, showing the beneficial effects of resynchronization. EM 45 days after AI were lower 18% in treated animals compared to controls 42%.

Italian researchers (Campanile *et al.*, 2013) have been reported high levels of EM 47.7%. Others (Usmani *et al.*, 1985) using Ovsynch protocol associated EM to the increasing length of the day that naturally corresponds with a decrease in the reproductive activity of the animals. They obtained a PR of 63% at day 26 post AI, at day 40 decline to 34%, it represents 45% of EM, of them only 8% was associated with infectious causes, 49% to decreasing P4 levels 0.6 ng/ml y 1.1 ng/ml at day 10 and 20 respectively and the other 43% in spite of having sustained P4 levels have EM this observation needs to be explained.

Regarding EM in this work we obtained lower rates 3.4% than others Italian researchers (Zicarelli, 1994), others in the equatorial zone, report 20% (Zicarelli, 1997) the most probable explanation of this difference is the season in which all protocols were used.

Discussing the practical application of the results, it can be observed the high pregnancy rate obtained after the two inseminations allow us to propose to the breeders, taking advantage of the fact that the buffalo reinitiate their ovarian activity very early in the postpartum period, to introduce these insemination protocols as part of the reproductive program of the herd with the certainty that the reproductive parameters of the herd don't have alterations, the very low proportion of non-pregnant females could be covered by the males. Other aspect that should be considered is the management of animals, in the case of TRT1, animals should be grouped less often than in the other protocols, which in the conditions of the Argentina buffalo farms is beneficial, and the other issue is that each producer in their country must evaluate the economic feasibility of each protocol.

CONCLUSION

The use of synchronization and resynchronization protocols are efficient tools to establish FTAI programs in buffalo farms and obtained pregnancy rates comparable to the ones reported in natural reproduction, each breeder has the opportunity to choose the most fitted protocols for his needs. Argentinean breeders have the opportunity to take the advantages of AI to increase the productivity and to satisfy commercial demands.

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