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A N I N T E R N A T I O N A L J O U R N A L

Estrus Synchronization and Fixed Time Artificial Insemination (FTAI) in Dairy Buffaloes during Seasonal Anestrus

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ABSTRACT

The aim of this work was to study estrus synchronization and fixed time artificial insemination (FTAI) in dairy buffaloes during season anestrus. One hundred thirty-nine dairy buffaloes in seasonal anestrus were divided in two groups as G1(n=66) and G2(n=73). The protocols for both the groups were the same until day (D)14:D0 administration of 2.0 mg estradiol benzoate and implantation of progesterone device (P_4) for 14 days; D14 removal of P_4 plus 150 mg of cloprostenol and 400 IU of equine chorionic gonadotropin. On D16, G1 received 10 mg of buserelin and G2 100 mg deslorelin acetate. On D17, both the groups were submitted to FTAI. Ultrasonographic examinations of ovaries were performed on D0, D14, D16 and D17. Results showed that pregnancy rates in G1 and G2 were 20 and 41% ($p<0.05$) and the ovulation rates were 16.6 and 37%, respectively ($p<0.05$). The dominant follicle (DF) diameter on D16 was 7.9 mm in G1 and 8.9 mm in G2 ($p>0.05$). Thirty-five percent of the animals in G1 and 54.1% in G2 showed a diameter DF greater than 8.0 mm on D16 ($p>0.05$). Thus, it could be concluded that the protocols synchronized the estrus, leading the concentration of the parturitions in the period of low milk production. Deslorelin was more efficient than buserelin due the higher percentage of DF ovulation and higher pregnancy rates.

Key words: Estrus Synchronization, Seasonal Anestrus, Dairy Buffaloes, Deslorelin, Buserelin, FTAI

INTRODUCTION

The buffalo herd plays an important role in the world's production of animal protein, especially in third world countries. It is estimated that this herd is increasing by 10% per year, reflecting the growing interest worldwide in this species (Vale 1988). The Brazilian population of Bubalus bubalis is estimated as 2.5 million animals and has shown an average annual growth of 12% (Diaz et al. 2001). Hence, it is necessary to improve the quality and quantity in the production and reproduction of these animals.

The reproductive seasonality of the dairy buffalo is the physiological factor that exerts the greatest impact on this economic activity. The highest

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concentration of parturitions in this species is seen from March to June in the Southern and Southeastern Brazil (mainly between parallels 24 and 26), and it determines the concentration of milk production until September, with a significant reduction, or even absence of milk in the summer months (Baruselli et al. 2003). In this region, poor buffalo farmers depend heavily on the milk production from the buffaloes. Thus, the development and implementation of reproductive biotechnologies, such as estrus synchronization associated to FTAI in this species, could be of significant relevance in this region. In places far from the equatorial region, the reduction in hours of light per day has a positive influence on the female buffaloes' reproductive behavior. There is evidence that high environmental temperatures, photoperiod, relative humidity, and high levels of rainfall influence the reproductive endocrine system (Shah et al. 1990; Zicarelli et al. 1990). Due to this seasonality, the concentration of parturition results in a reduction in the milk production.

One of the limiting factors in the application of artificial insemination (AI) in buffaloes is the difficulty in the estrus detection. A small number of females exhibit homosexual behavior, the signs of estrus are discrete, and it occurs mainly at night. Therefore, the use of hormonal protocols associated with FTAI makes reproduction in these animals more advantageous and practical, especially during the seasonal anestrus (Baruselli and Carvalho 2005).

Methods for the detection of estrus have been developed to facilitate the management of reproductive programs and to increase the efficiency of AI protocols. These protocols synchronize follicular growth and ovulation, allowing the performance of FTAI. Many female buffaloes present prolonged periods of anestrus during the unfavorable breeding season (spring/ summer) and do not respond properly to the treatment with only the Ovsynch protocol. Protocols using P_4, estradiol, prostaglandin, GnRH, and eCG improve the pregnancy rates in female buffaloes (Baruselli et al. 2003). The use of deslorelin acetate (a GnRH agonist) has been reported in the dogs (Trigg et al. 2006), cattle (Bergfeld et al. 1996; Ambrose et al. 1998; Ramajahendran et al. 1998; Bartolome et al. 2004; Padula and Macmillan 2005; Silvestre et al. 2009), horses (Mckinnon et al. 1993; Squires et al. 1994; Mumford et al. 1995; Vanderwall et al. 2001; Melo et al. 2005; Raz et al. 2009; Lindholm et al. 2010) and sheep (Schneider and Rehbock 2003).

There are no reports on the use of deslorelin acetate in female buffaloes for estrus synchronization and ovulation. The use of deslorelin acetate in the female buffaloes has only been reported in the protocols used for ovary superovulation, involved in embryo transfer procedures. This agonist induces ovulation in fertile buffaloes and the recovery rate of embryos is reported at about 46% (Carvalho et al. 2002).

This study aimed at the synchronization of estrus in dairy buffaloes during the period of seasonal anestrus using hormonal protocols associated with different inductors of ovulation and FTAI in order to concentrate the parturitions in the period of low milk production in the buffalo herd from Southern Brazil.

MATERIALS AND METHODS

Animals

The experiment was carried out between September/October until January 2010, which corresponded to the yearly period of seasonal anestrus (absence of estrus, ovulation and pregnancy) in *Bubalus bubalis* in Southern Brazil. The breeding season extends from March to June (parturitions and estrus after puerperium period, determining the concentration of milk production until September). None of the buffaloes showed the signs of estrus during the protocol (before, or after buserelin/deslorelin). One hundred thirty-nine pluriparous Murrah female buffaloes showing body condition scores of 3.5 (in a 1-5 scale, Edmondson et al. 1989) from three dairy farms were used. The animals were maintained on pasture (*Brachiaria decumbens*) and mineral supplements were given *ad libitum* in troughs. The daily mechanical milking in the presence of calves was performed in the mornings and the average milk production per animal was 10 liters.

Experimental Design

The animals were divided in two groups (G): G1 (n=66) and G2 (n=73). On D0, the buffaloes (both groups) received 2.0 mg of estradiol benzoate (EB) (Cronibest ®, Biogenesis-Bago, Brazil) IM and were implanted with an intravaginal progesterone device (P_4) (Cronipres monodose ®, Biogenesis-Bago, Brazil), which remained implanted for 14 days (Sing 2003). On D14, 150 g of D-cloprostenol (Croniben ®, Biogenesis-Bago, Brazil) and 400 IU of equine chorionic gonadotropin - eCG (Folligon ®, Intervet Schering-Plough Animal Health, Brazil) were administered to both the groups (IM) and the P_4 was removed from every animal. On D16, the G1 animals received 10 mg of buserelin acetate IM (Conceptal ®, Intervet Schering-Plough Animal Health, Brazil) and the G2 animals received 100 mg of deslorelin acetate IM. The FTAI occurred on D17 as presented in Figures 1 and 2.

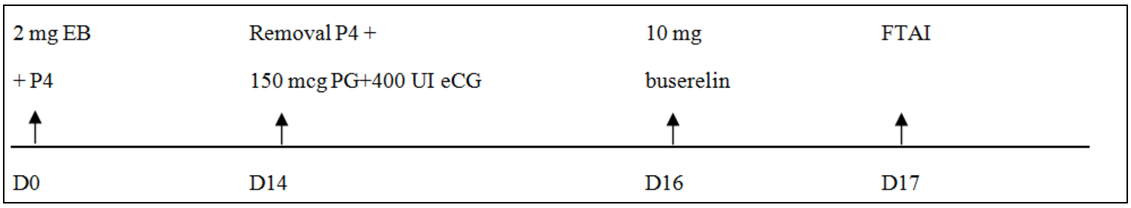

Figure 1 - Hormonal protocol in group 1 of buffaloes aiming at the FTAI in seasonal anestrus.

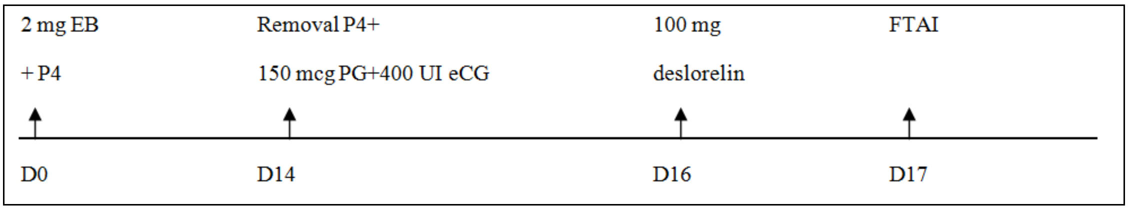

Figure 2 - Hormonal protocol in group 2 of buffaloes aiming at the FTAI in seasonal anestrus.
EB=estradiol benzoate; P4= progesterone; PG= prostaglandin F2 α; eCG=equine chorionic gonadotropin.

Ultrasonography

Ultrasonographic examinations (U.S.) (5 MHz linear transducer - Aloka model 500) of the ovaries were performed in all the animals to assess the ovarian activity before the start of the hormonal protocols. The examinations were performed on D0, D14, D16, and D17. The examination on D16 was performed to verify the presence of DF presenting diameters greater than 8.0 mm (Fig. 3) and on D17 (day of FTAI) to verify the occurrence of ovulation. Thus, the ultrasonography results on D17 showing the absence of DF, previously detected on D16, was considered as an indicator of ovulation (Nasser et al. 1993). Synchronization of ovulation was considered present when occurred within 24 h after the application of buserelin, or deslorelin acetate. Pregnancy diagnosis was performed 55 days after AI via ultrasonography.

Statistical Analyses

Ovulation rate, pregnancy rate after FTAI, the percentage of animals with follicles greater than 8 mm in diameter, and the percentage of pregnancy with follicles greater than 8 mm in diameter were analyzed using the Chi-Square test. The diameter of the DF was analyzed with the Student's t test. In both the tests, differences with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The P_4 protocol, applied in this study was based on a study on buffaloes during an unfavorable reproductive season (Sing 2003). In that study, the author suggested that the treatment with P_4 was more effective when the implants remained for 10 to 14 days compared to eight days only. The use of 400 IU of eCG, at the time of the P_4 removal, increased the ovulation and conception rates in the buffaloes without CL at the time of the P_4 implantation (Baruselli et al. 2002). In the present study (Table 1), the pregnancy rate in 37% of the animals submitted to hormonal protocols for synchronization of estrus and induction of ovulation followed by FTAI was observed. This rate would not be possible without the administration of hormonal protocols because the animals were in seasonal anestrus, outside of the reproductive season, thus demonstrating the importance of present study. The 37% pregnancy rate resulted in the concentration of parturitions and subsequently in improved milk production in the period when milk availability was beneficial for the small local producers. G2 (deslorelin acetate) showed better rates than G1 (buserelin acetate), not only for the ovulation but also for the pregnancy ($p<0.05$).

Table 1 - Ovulation (D17) and pregnancy rates after the FTAI in Murrah breed dairy buffaloes during seasonal anestrus (%).(n=139).

	G1(buserelin)	G2 (deslorelin)	Total
Ovulation rate	11/66 (16.6)^a	27/73 (37.0)^b	
Pregnancy rate	13/66 (20.0)^a	30/73 (41.0)^b	
Total pregnancy rate			37.0

Different letters in the same row indicate significant difference ($p<0.05$).

There are currently no reports on the effects of deslorelin in inducing the ovulation in estrus synchronization and FTAI protocols in female buffaloes. Thus, the present work is first report on this. The results from this study showed smaller percentage rates than the rates reported by Presicce et al. (2005). However, these authors applied the P_4 protocol added by the administration of eCG or the Ovsynch protocol in the animals that were cycling (40.9%). Other studies demonstrated that when the Ovsynch protocol was used in female buffaloes during the unfavorable reproductive season (seasonal anestrus), the conception rates were quite variable: 28.2% (Baruselli et al. 2002); 6.9% (Baruselli et al. 2003); 34% (Campanile et al. 2005); 30% (Warriach et al. 2008). In the present study, deslorelin acetate was used to induce the ovulation (GnRH agonist), which resulted in conception rates (39.6%) higher than the rates reported in other studies using the Ovsynch protocol, indicating that deslorelin acetate was more efficient in female buffaloes under these conditions.

The pre-ovulatory follicle (Fig. 3) was the largest follicle at pro-estrus and estrus (El-Wishy and Ghoneim 1995) and presented constant growth (Marion et al. 1968). In this study, the DF presented an average diameter of 7.9 mm in G1 (buserelin) and 8.9 mm in G2 (deslorelin) (Table 2). These results were similar to an average diameter of 9.0 mm for the DF reported in the literature (Berber et al. 2002). Rastegarnia et al. (2004) observed the DF average diameter as 12.7 mm in the experiments using synchronized ovulation and comparing different doses of gonadorelin in female buffaloes in the season breeding. This DF average value was higher than the values observed in the present study, which was performed outside the favorable reproductive season.

It has reported that the DF in female buffaloes could ovulate when the diameter was around 5-8 mm (Baruselli et al. 1997), which corroborated the results observed in this study with the DF diameters reaching averages between 7.9 and 8.9 mm. These results were also similar to results previously reported (Campanile et al. 2007; Campanile et al. 2008) of follicles with diameters from 4.2 to 13.0 mm, when ovulation was induced with hCG ,or GnRH agonist. On D16, 48.5% of the animals presented DF in both the groups, indicating that the protocols used for estrus synchronization showed satisfactory efficiency rates. These results were promising compared to previously reported results about synchronized estrus in cyclic and acyclic pluriparous female buffaloes during a favorable reproductive season and using the Ovsynch protocol, or P_4 added by the administration of eCG (Presicce et al. 2005).

Table 2 - Parameters of the DF diameter (mm), percentage of animals with follicle greater than 8 mm in diameter, and pregnancy rate with follicles greater than 8 mm in diameter observed on D16 in Murrah breed dairy buffaloes during the seasonal anestrus (%).(n=139).

Parameter (D16)	G1 (buserelin)	G2 (deslorelin)
Animals (n)	66	73
DF (diameter x ± s)	7.9 ± 0,7^a	8.9 ± 0.4^a
DF (larger diameter, x ± s)	15.0	20.0
DF (smaller diameter, x ± s)	4.0	2.0
Ovaries with follicles > 8 mm (%)	35.0^a (7/20)	54.1^a (26/48)
Pregnancy with follicles > 8 mm (%)	50.0^a	63.1^a

Figure 3 - Dominant follicle showing 11.0 mm in diameter, detected by ultrasound examination on D16 in animals submitted to the protocol using deslorelin.

CONCLUSION

The hormonal protocols used in this study showed satisfactory efficiency in order to estrus synchronization and pregnancy rate. The application of the both the protocols allowed the concentration of parturitions during the period of low, or absent production of milk, which represented an important business improvement for the small local milk producers. The protocol using deslorelin acetate was more effective than the protocol using buserelin acetate; the deslorelin protocol provided greater percentage of DF ovulation and higher pregnancy rates.

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