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Use of Human Chorionic Gonadotropin (hCG) for fixed-time artificial insemination in buffalo (B*ubalus bubalis*)

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Abstract

The aim of this study was to evaluate the efficacy of the Ovsynch protocol used for fixed-time artificial insemination in buffalo when replacing the last injection of GnRH with hCG in order to increase plasma progesterone (P4) concentrations and therefore increase conception rates. Two-hundred twelve buffaloes were randomly assigned into 2 treatment groups: G-GnRH (Day 0, GnRH; Day 7, $PGF_{2\alpha}$; Day 9, GnRH; n = 94) and G-hCG (Day 0, GnRH; Day 7, $PGF_{2\alpha}$; Day 9, hCG; n = 118). All buffaloes were inseminated on Day 10, 16 h after the last hormonal injection. On Day 22, 80 buffaloes were randomly selected for blood collections in order to measure plasma P4 concentration. Pregnancy diagnosis was performed on Day 40 by ultrasonography. Conception rates of G-GnRH and G-hCG animals were 46.8% (44/94) and 50.8% (60/118; $P > 0.05$), respectively. Plasma P4 concentration was lower in G-GnRH (2.94 ± 1.51 ng/ml) than in G-hCG (4.02 ± 2.34 ng/ml; $P < 0.05$) animals. The plasma P4 of pregnant buffaloes was higher in G-hCG than in G-GnRH (4.66 ± 1.73 ng/ml vs. 3.54 ± 1.05 ng/ml; $P < 0.05$). This effect was not observed in non-pregnant animals (G-GnRH = 3.18 ± 2.78 ng/ml vs. G-hCG = 2.19 ± 1.69 ng/ml, respectively; $P > 0.05$). Pregnant G-GnRH and G-hCG buffaloes had higher plasma P4 concentrations than the non-pregnant buffalo of both groups (3.54 ± 1.05 vs. 2.19 ± 1.69 ng/ml and 4.66 ± 1.73 vs. 3.18 ± 2.78 ng/ml, respectively; $P < 0.05$). In summary, when using the Ovsynch protocol for FTAI in buffalo, the replacement of the last GnRH administration with hCG increased the plasma P4 concentration. However, no positive effect on the conception rate was observed.

Keywords: hCG; conception rate; progesterone; corpus luteum; buffaloes.

Introduction

In the last few years, many fixed-time artificial insemination (FTAI) protocols have been developed. However, there are several factors, from the correct administration of the treatments to the artificial insemination procedure, which could influence the results when employing such biotechnology. Previous researches indicate that 85 to 90% of the bovine oocytes are fertilized after natural breeding (Kidder *et al*., 1954; Bearden *et al*., 1956); however, around 30% of those embryos die after Day 8 to 16 (Diskin and Sreenan, 1980) or even 25 days after conception (Lamming *et al*., 1989). This high embryonic mortality leads to decreased conception rates and increased service periods and birth intervals, which can cause significant economic losses in a cattle production system.

High environmental temperatures (Badinga *et al*., 1985; Putney *et al*., 1989; Wolfenson *et al*., 2000), bacterial endometritis (Bouters, 1985), nutritional problems (Broadbent *et al*., 1991), high milk production, and excessive dry matter consumption (Parr *et al*., 1993a, b; Vasconcelos, 1998) leading to increased liver metabolism of progesterone and luteal insufficiency (Remsen and Roussel, 1982; Stubbings and Walton, 1986) are some of the factors that are known to cause early embryonic death. It was also verified that low plasma P4 concentrations are related to reduced pregnancy rates (Lukaszewska and Hansen, 1980).

Previous studies indicate that bovine females showing high plasma P4 concentrations after ovulation had higher conception rates (Nishigai *et al*., 1998, 2000) and generated embryos with an improved capacity of synthesizing and secreting Interferon-Tau (bIFN-τ; Mann *et al*., 1999). These studies suggest that the increase in P4 concentrations may have a positive effect on the embryonic development through the improvement on the maternal pregnancy recognition (Geisert *et al*., 1992; Mann and Lamming 1995, 2001). The bIFN-τ is secreted through the uterine lumen, which interacts with the endometrium and blocks the synthesis of $PGF_{2\alpha}$ (Binelli *et al*., 2001).

Campanile *et al*. (2005) found higher plasma P4 concentration in pregnant buffaloes than in buffaloes which suffered embryonic mortality until Day 10 after AI, whilst P4 in non-pregnant buffaloes was intermediate. Pregnant buffaloes had also higher plasma P4 concentration on Day 20 than both non-pregnant buffaloes and buffaloes that showed embryonic mortality. Plasma progesterone concentration significantly decreased only in non-pregnant buffaloes between Days 10 and 20. In a further trial, pregnant buffaloes had higher P4 concentration in milk whey than both animals showing embryonic mortality and non-pregnant buffaloes on Day 20 (Campanile *et al*., 2007). The

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authors hypothesized that embryonic mortality in buffalo species is primarily due to a reduced secretion of P4 by corpus luteum.

Strategies that aim to increase P4 concentrations as well as to inhibit the synthesis of $PGF_{2\alpha}$ during the critical period of maternal recognition of pregnancy (i.e., 15 to 17 days after the artificial insemination) may be fundamental to the conceptus development (Binelli *et al*., 2001). In this circumstance, human chorionic gonadotropin (hCG), a hormone with biological activity similar to the luteinizing hormone (LH; Donaldson *et al*., 1964), appears to be an alternative. Human chorionic gonadotropin is a hormone with peculiar traits with regards to its pharmacokinetics. It remains in the circulatory system for a long period, and its dissociation from the LH receptors occurs in a gradual way. Therefore, its administration may promote the ovulation and subsequent differentiation of granulosa cells to luteal cells (Schmitt *et al*., 1996a). The corpus luteum formed after administration of hCG has a higher steroidogenic capacity (Chenault *et al*., 1990; Schmitt *et al*., 1996b) as well as a higher capacity of secreting P4 (Schmitt *et al*., 1996a). Based on these studies, the hypothesis of the present experiment was that by replacing the last injection of GnRH of the Ovsynch protocol with hCG, an increase in P4 plasma concentrations would be observed resulting in a consequent improvement in conception rates in buffaloes.

Materials and Methods

Animals

A total of 212 Murrah, Mediterranean, and crossbred (Murrah x Mediterranean) buffalo cows, raised in the regions of Vale do Ribeira and São Carlos (São Paulo State, Brazil), were used from May to September. Animals raised at São Carlos were kept on pasture with mineral salt and water ad libitum and received a protein, mineral and energy supplementation. Females raised at Vale do Ribeira received the same treatment except for the supplementation. All animals showed body condition scores (BCS) equal or higher then 3.0 (1 to 5 scale; 1 = thin, and 5 = fat). Eighty of these animals were randomly selected for blood collections in order to evaluate the plasma P4 concentration.

Hormonal treatment

Animals of G-GnRH (n = 94, control) received 25 μg GnRH i.m. (Lecirelina; Gestran-plus®; Arsa, Argentina) on Day 0 and 150 μg $PGF_{2\alpha}$ analogue, i.m. (d-Cloprostenol; Prolise®; Arsa, Argentina) 7 days later (Day 7). Forty-eight hours later (Day 9), animals received 25 μg GnRH, i.m. Buffaloes of the G-hCG (n = 118) group received 25 μg GnRH (Lecirelina) and 150 μg of $PGF_{2\alpha}$ (d-Cloprostenol) on Day 0 and on Day 7, respectively. On Day 9, animals were treated with human chorionic gonadotropin (hCG; 1,500 IU, i.m.; Vetecor®; Calier, Espanha). Animals of both groups were inseminated approximately 16 hours after the last hormonal treatment (Day 10). The semen was distributed equally between groups. The pregnancy diagnosis was performed 30 days after the FTAI by transrectal ultrasonography (Pie Medical 480 with a 5.0-MHz linear-array probe). Hormonal treatments are depicted in Fig. 1.

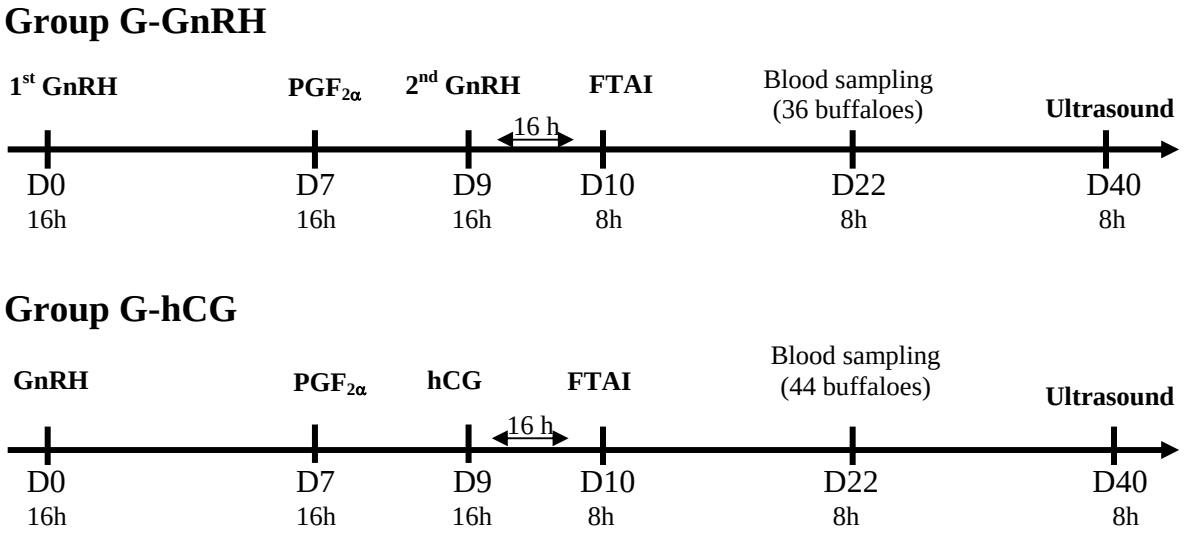

Figure 1. Schematic representation of synchronization treatments for FTAI, blood collections and ultrasonographic examinations of buffaloes (*Bubalus bubalis*) treated with GnRH/$PGF_{2\alpha}$/GnRH (G-GnRH) and GnRH/$PGF_{2\alpha}$/hCG (G-hCG).

Blood collections and hormonal evaluation

Blood samples were taken from the jugular vein using heparinized Vacutainer® tubes (Becton & Dickinson, USA). Collections were performed 12 days after the artificial insemination (Day 22) from 80 randomly selected cows (36 from G-GnRH and 44 from G-hCG; Fig.1). Blood samples were kept at 2 to 8ºC for a maximum period of 4 hours, centrifuged (3000*g* for 10 minutes), and the plasma was stored at -20ºC until hormone analysis.

The plasma P4 concentrations were measured using solid-phase radioimmunoassay kits (Coat-a-Count®; Diagnostic Products Corporation, USA) and using 100 µl of sample in duplicate, according to the manufacturer's recommendations. The 80 samples were processed in one assay, with 3.3 and 6.5 % coefficients of variation for low and high values, respectively. The minimum detectable concentration was 0.03 ng/ml. All the hormone assay procedures were performed at the Laboratory de Hormonal Dosages of the Department of Animal Reproduction of the College of Veterinary Medicine and Animal Science of the University of São Paulo (São Paulo, SP, Brazil).

Statistical analysis

Conception rates were analyzed using the Chi-Square test (χ^2). The plasma P4 concentrations were analyzed using the Student's *t*-test to compare the groups. For all the statistical analyses, the minimum significance level was $P < 0.05$. Results are presented as means ± SEM.

Results

No differences were found ($P > 0.05$) in conception rates between G-GnRH (46.8%; 44/94) and G-hCG (50.8%; 60/118) animals. As expected, no differences ($P > 0.05$) were found in conception rates between animals selected for blood collections (n = 80) either. Buffaloes treated with GnRH/$PGF_{2\alpha}$/GnRH (G-GnRH) had a similar conception rate to buffalo treated with GnRH/$PGF_{2\alpha}$/hCG (G-hCG). However, the mean plasma P4 concentration was lower ($P < 0.05$) in buffaloes from the G-GnRH group when compared to animals from the G-hCG group (Table 1).

When comparing only the pregnant animals of both groups, a significant difference $P < 0.05$ was found for plasma P4 concentration (Table 1). This difference was not found between G-GnRH and G-hCG animals when taking into account the non-pregnant animals. Pregnant animals of both groups (G-GnRH and G-hCG) had higher ($P < 0.05$) plasma P4 concentrations when compared to non-pregnant animals (Table 1).

Table 1. Conception rates and plasma progesterone (P4) concentration of 80 buffaloes (*Bubalus bubalis*) selected for blood collections and treated with GnRH/$PGF_{2\alpha}$/GnRH (G-GnRH) and with GnRH/$PGF_{2\alpha}$/hCG (G-hCG) for fixed-time artificial insemination.

Groups (n)	Conception rate (%)	P4 (ng/ml)	P4 (ng/ml) of pregnant animals	P4 (ng/ml) of non-pregnant animals
G-GnRH (36)	61.1 (20/36)	2.94 ± 1.51^{b}	3.54 ± 1.05^{bc}	2.19 ± 1.69^{ad}
G-hCG (44)	58.7 (25/44)	4.02 ± 2.34^{a}	4.66 ± 1.73^{ac}	3.18 ± 2.78^{ad}

[a, b] Means within a column with different superscripts differ ($P < 0.05$).
[c, d] Means within a row with different superscripts differ ($P < 0.05$).

Discussion

Unexpectedly, no differences in conception rates were found between groups G-GnRH and G-hCG, which did not support our hypothesis. This result may be due to the good BCS of the animals (≥ 3.0) and may have contributed to the satisfactory results when employing the FTAI. According to Cavalieri and Fitzpatrick (1995), BCS plays an important role in ovarian cyclicity and consequently, on the response of the animals to treatments that aim to control the estrous cycle and ovulation. Geary *et al*. (1998), working with cows, and Baruselli *et al*. (2000), working with buffaloes, obtained higher conception rates in animals treated with GnRH when animals showed good BCS. Another factor that could explain the lack of differences between groups is the period of the year within which the present experiment was performed. It is well known that buffalo exhibit a reproductive seasonality influenced by photoperiod (Zicarelli, 1997). This study was performed during the breeding season, when pregnancy rates are usually higher. Therefore, the positive effect of the treatment with hCG could have been unnoticed.

Females from G-hCG showed higher plasma P4 concentrations when compared to G-GnRH. This may due to a corpus luteum (CL) that formed after the ovulation induced by hCG. This CL had higher steroidogenic properties and higher capacity of secreting P4 than the CLs of the animals treated with GnRH, as observed in cows (Chenault *et al*., 1990; Schmitt *et al*., 1996a, b). The LH surge induced by the administration of GnRH in the Ovsynch protocol has a shorter amplitude than the endogenous surge, which may limit the formation and growth of the CL (Chenault *et al*., 1990; Macmillan *et al*., 2003). Ambrose *et al*. (1998), working with non-milking Holstein cows, verified that the prolonged GnRH release promoted by an implant

induced an LH surge of greater amplitude stimulating the formation of CLs of greater size and functionality than a single GnRH injection. This may be an explanation for what happened to buffalo females treated with hCG. This gonadotropin has a longer half life than GnRH and may remain in the circulatory system for a longer duration (Schmitt *et al*., 1996a). Our results disagree with a recent experiment performed by Campanile *et al*. (2007), in which no differences in plasma P4 concentrations were observed between buffaloes treated with GnRH and hCG. However, in the previous experiment, treatments as well as P4 analysis were performed on different days.

Despite the lack of differences in conception rates of the groups synchronized with GnRH/$PGF_{2\alpha}$/GnRH and GnRH/$PGF_{2\alpha}$/hCG, pregnant females from G-hCG had higher plasma P4 concentrations than pregnant females from the G-GnRH group. It was also verified that pregnant females of both G-GnRH and G-hCG groups had higher plasma P4 concentrations than non-pregnant females from the G-GnRH and G-hCG groups, respectively. This result agrees with previous results in buffalo and cattle. In buffalo, a higher P4 concentration was found on Day 20 after FTAI in pregnant than in non-pregnant animals (Campanile *et al*., 2007). In cattle, according to Binelli *et al*. (2001), the circulating P4 concentration seems to be positively correlated to the pregnancy rate. Some authors verified this in pregnant cows when compared to non-pregnant animals; in pregnant cows there was a higher P4 concentration in milk (Bullman and Lamming, 1978; Lamming *et al*., 1989; Mann *et al*., 1999) and in blood plasma (Fonseca *et al*., 1983; Folman *et al*., 1990; Mann *et al*., 1995).

In conclusion, when using the Ovsynch protocol for FTAI in buffaloes during the breeding season, the substitution of the last GnRH administration with the hCG increased the plasma P4 concentration. However, no positive effect on the conception rate was observed. Therefore, in periods characterized by a decreased length of daylight, either treatment could be used.

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