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Original article

Reproductive parameters of buffaloes subjected to pharmacological control of the estrous cycle: lowest cost x best benefit

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Abstract

This study aimed to evaluate the effects of equine chorionic gonadotropin (eCG), Estradiol benzoate (EB) and Gonadotropin-releasing hormone (GnRH) on follicular dynamics and luteal characteristics of buffaloes (*Bubalus bubalis*). The animals were separated into four experimental groups: EB (n=10), EB+eCG (n=10), GnRH (n=10) and GnRH+eCG (n=10). Using B-mode and Power-Doppler ultrasound, follicular characteristics of the largest follicle, from day 9 until day 11, diameter of the preovulatory follicle, follicular area, total wall area of the preovulatory follicle, growth rate of the ovulatory follicle, vascularization in the wall area of the preovulatory follicle, percentage of vascularization in the wall area of the preovulatory follicle and moment of ovulation, were evaluated for follicular dynamics. Furthermore, on day 18 of the protocol, diameter of the corpus luteum, total area of the corpus luteum, vascularization of the corpus luteum, percentage of vascularization of the corpus luteum and the serum progesterone levels were also evaluated. Statistical analyses were performed using Tukey's test, Kruskal-Wallis and repeated measures ANOVA with a 5% significance level. Statistically significant differences were found for diameter of the corpus luteum with 1.78±0.12cm in the EB+eCG group and 1.62±0.11cm in the EB group (p=0.047), as well as in the Progesterone dosages between the treatments GnRH+eCG (4.09±0.94ng/mL) and GnRH (2.80±0.70ng/mL), p=0.017. Regarding the variables assessed by Power Doppler, no statistical differences were found between the experimental groups. Finally, the price analysis revealed that the EB+eCG protocol is 19% cheaper than the GnRH+eCG protocol. Therefore, EB offers better cost/benefit on the reproductive parameters of buffaloes subjected to pharmacological control of the estrous cycle.

Keywords: *Buballus bubalis*, corpus luteum, equine chorionic gonadotrophin, follicles, progesterone

Introduction

Buffaloes (*Bubalus bubalis*) are considered seasonally polyestrous animals or seasonal breeders with negative photoperiod, mainly in subtropical and high latitude environments (Perera 2008). However, in tropical regions where the photoperiod is relatively constant, females are considered to be annual polyestrous suffering greater influence on the estrous cycle by factors such as rainfall and the availability and quality of forage (Perera 2011), environmental heat stress, social interaction, calving date and lactation (Rosa and Bryant 2003). The species is also characterized by low estrus expression which contributes to the decline in reproductive efficiency (Mirmahmoudi and Prakash 2012). Observing the species' peculiarities, reproductive biotechnologies such as artificial insemination (AI) and fixed-time artificial insemination (FTAI) may be strategies capable of mitigating such influences (Mirmahmoudi and Prakash 2014). However, the use of the conventional AI technique presents two significant difficulties in buffaloes. The first is related to inefficient detection of estrus due to the discrete observed behavior and the second is related to seasonal and nutritional anestrous that leads to decreased reproductive activity (Baruselli et al. 2013).

One way to improve reproductive efficiency is the administration of exogenous hormones capable of inducing cyclicity, synchronizing the ovulation (Baruselli et al. 2007, Carvalho et al. 2013). Stimulating the growth of the dominant follicle with gonadotropins may be a strategy to increase ovulation and pregnancy rates with synchronized ovulation protocols in buffaloes in anestrus, both in cows and calves (Carvalho 2016).

The administration of GnRH in buffaloes induces ovulation in 80% of the females, reaching satisfactory levels during synchronization of ovulation in FTAI protocols (Marugavel et al. 2009, Carvalho et al. 2014). However, the use of EB as an ovulation inducer has some advantages over GnRH, including lower cost, practical management schedule prior to FTAI, greater uterine toneand relative ease of insemination (Mirmahmoudi et al. 2014). Moreover, it also provides exposure to higher concentrations of estradiol during proestrus and estrus, which likely creates a better uterine environment for embryonic development (Bridges et al. 2012).

After the removal of an ear implant in lactating Murrah buffaloes that had received eCG administration, follicular development was favored, suggesting that the addition of eCG to the synchronization protocol may anticipate the reproductive function of calving buffaloes (Stella et al. 2018). The eCG also favors the development of the corpus luteum (CL), since it has a long half-life that allows its connection with the LH receptors of the CL, promoting greater gene expression of the angiogenic factors VEGF and FGF2 and the multiplication of luteal cells responsible for the production of progesterone, essential for the maintenance of pregnancy (Atli et al. 2017).

Thus, a better understanding of the follicular and luteal dynamics in buffaloes (Vecchio et al. 2012) is necessary for the development of new techniques and improvement of the currently used treatments for the manipulation of the estrous cycle in cyclic and acyclic animals (Yindee et al. 2011, Baruselli et al. 2013). This study aimed to estimate the best cost-benefit ratio of using eCG, EB and GnRH in follicular dynamics and corpus luteum characteristics in buffalo cows subjected to different fixed-time ovulation synchronization protocols.

Materials and Methods

Location and animals

The experiment was conducted in the Experimental Farm of Entre Rios-BA, located at a latitude of 11°56′31″ South, a longitude of 38°05′04″ West and an altitude of 162 m, between the months of October and November of the year 2018 (in these months, buffaloes are in a reproductive or non-reproductive period for the region). The property is part of the School of Veterinary Medicine and Zootechny of the Federal University of Bahia (EMEVZ/UFBA). Forty multiparous and lactating Mediterranean buffaloes were used, which were kept in pastures with predominantly Pangola grass (*Digitaria decumbens*) with mineral salt and water ad libitum. The animals were selected after gynecological examination by transrectal ultrasonography, using a linear transducer with a frequency of 7.5 MHz (Mindray, Z5 VET, Shenzhen, China) in order to evaluate the presence of reproductive abnormalities that could impair their use in the experiment. The buffaloes were evaluated according to their body condition score (BCS), on a scale of one to five (Anitha et al. 2011) and those that presented BCS ≥3 were considered suitable to participate in the experiment. All animals had had more than 2 parturitions, more than 3 months had passed since their last parturitions, and had followed the mandatory vaccine protocol, established by the Ministry of Agriculture, Livestock and Food Supply (MAPA) and had an up-to-date deworming schedule.

Institutional review board statement

The study was conducted according to ethical precepts recommended by the National Council of the

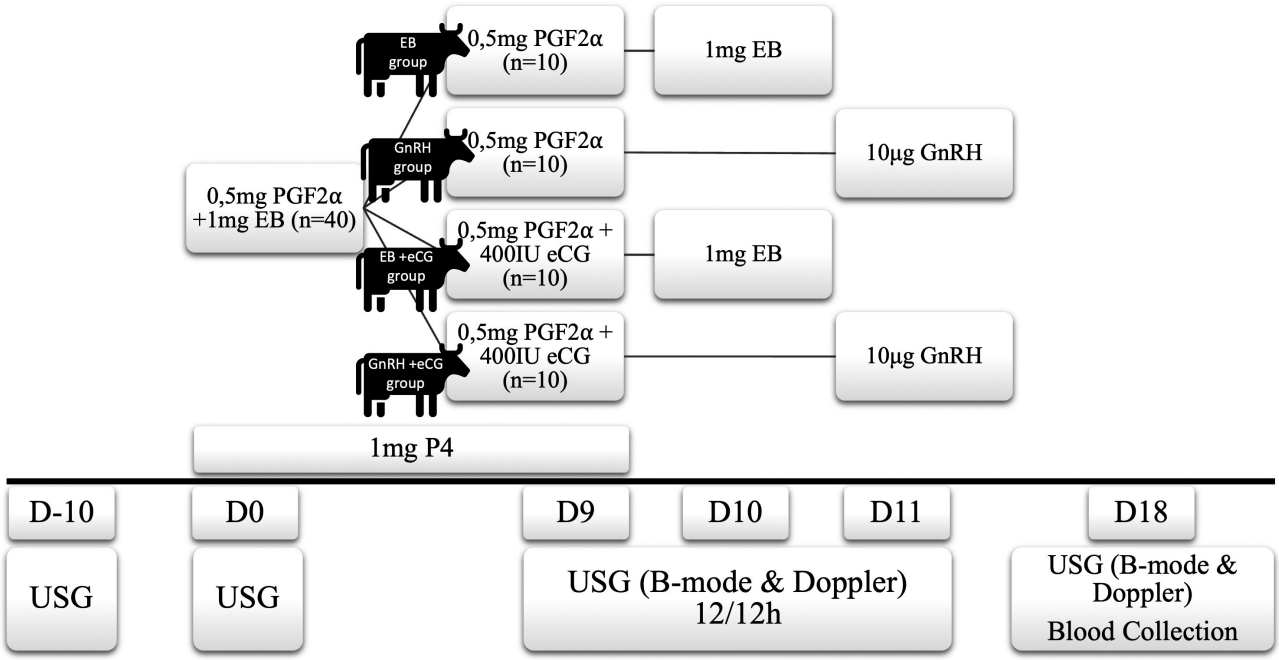

Fig. 1. Activity diagram to study the follicular and luteal dynamics of the ovulation synchronization protocol for lactating buffaloes (*Buballus bubalis*)

Control of Animal Experimentation, after approval by the Ethics Committee on the Use of Animals of the Federal University of Bahia, under number 27/2018.

Synchronization protocol

All animals were subjected to different synchronization protocols (Fig. 1) initiated on a random day of the estrous cycle, designated day 0 (D0), when they received an intravaginal device containing 1.0 g of progesterone (P4, DIB®, ZOETIS, São Paulo, Brazil) associated with 2mg of intramuscular (im) EB (Gonadiol®, São Paulo, Brazil) and 0.5mg of sodic cloprostenol (im) (Sincrosin®, Vallée S/A Produtos Veterinários, Minas Gerais, Brazil). On D9, the intravaginal P4 implant was removed and 0.5mg of sodic cloprostenol (im) was administered to all animals (n=40).

Randomly, on D9, 20 animals received 400 IU (im) of eCG (NOVORMON®, MSD Animal Health, São Paulo, Brazil). Of these, 10 animals received 10 µg (im) of EB on D10 (24 h after implant removal) and the other 10 received 10 µg of a GnRH analogue (im) (buserelin acetate, Sincroforte®, Ourofino Agronegócio, São Paulo, Brazil) on D11 (48h after implant removal), forming respectively the EB+eCG (n=10) and the GnRH+eCG groups (n=10).

Similarly, the 20 animals not treated with eCG received 1.0 mg (im) of EB on D10 and 10µg of a GnRH analogue (im) on day 11. Thus, the other two experimental groups were formed: EB (n=10) and GnRH (n=10), according to Fig. 1.

USG: Ultrasonography; D: day; PGF2α: prostaglandin F two alpha; EB: estradiol benzoate; P4: progesterone; eCG: equine chorionic gonadotropin; GnRH: Gonadotrophin Releasing Hormone.

Ultrasonographic evaluations

A portable ultrasound machine (Mindray, Z5 VET, Shenzhen, China) equipped with a linear transducer with a frequency of 7.5 MHz was used to perform ultrasound examinations during ovarian evaluation on days D-10, D0 and to register the largest diameter follicles on the days of follicular dynamics and luteal evaluation. Initially, the ovaries were located by rectal palpation following the evaluation order, first the right ovary and then the left ovary, being visualized slowly and continuously for the registration of images through ultrasound in B and Power-Doppler mode, the latter adjusted to the speed of 6 cm/s to detect movement of blood cells in small vessels at a frequency of 4.2 Mhz, with color gain patterns of 80%, pulse repetition frequency (PRF) of 0.7 KHz and evaluation depth of six centimeters.

Estimation of follicular and luteal variables

Starting on D9, ultrasound scans of the follicular structures were performed in the four treatment groups, with repetitions of the exam every 12 hours until the moment of ovulation and day D18 to evaluate the CL. The images of the two largest follicles were frozen, in B-mode, to determine the largest follicle on day nine

(LFOL D9), day ten (LFOL D10) and day eleven (LFOL D11) of the synchronization protocol. To verify the diameter of the preovulatory follicle (DPF) the value of this variable was registered, obtained from the largest follicle prior to its disappearance due to the emergence of the hemorrhagic body.

The growth rate of the ovulatory follicle (GRFOL) was obtained by dividing the difference between DPF and LFOL D9 by the growth period in days and expressed in centimeters per day (cm/day) The moment of ovulation (MOV) was the interval between the removal of the intravaginal P4 implant and the ovulation. In addition, B-mode ultrasound was used to measure the total wall area of the largest follicle on day nine (ALFOL D9), day ten (ALFOL D10) and day eleven (ALFOL D11) of the synchronization protocol and the total wall area of the preovulatory follicle (WAPF) was calculated when the preovulatory follicle was identified. The follicle images were also stored with Power-Doppler activated to quantify the vascularization in the wall area of the preovulatory follicle (VWAPF).

The data obtained by Power-Doppler ultrasonography were submitted to objective evaluation (GHETTI et al. 2016), by calculating the total area of the follicle wall in each animal, using a function of the device. Subsequently, the area of vascularization was calculated using the cursor to determine the area of vascularization on the follicle wall. The percentage of vascularization in the wall area of the preovulatory follicle (%VWAPF) was obtained by the ratio between the area of vascularization and the total follicular wall area.

Luteal images on B-mode ultrasound were frozen for the determination of the CL diameter (CLD) and total area of CL (TACL) measurements. In cavity CL, the area of the cavity was calculated and subtracted from the total area of the CL. Furthermore, with these images an objective evaluation in Power-Doppler mode (MARINO et al. 2020) and the calculation of the TACL in each animal, using a function of the device itself, were performed. To calculate the vascularization area of the CL (VCL), the cursor was used to verify the indicated vascularization and this value was then subtracted by the TACL. The percentage of vascularization of the CL area (%VCL) was determined by the ratio between the area of vascularization and the total area of the CL.

Serum progesterone (P4)

The samples for the luteal function evaluation were obtained by caudal lateral venipuncture, performed at the D18 moment of the FTAI protocol. Vacuum tubes of 10mL without anticoagulants (VACUTAINER®, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were used in the collections and packed in a thermal box with recyclable ice, kept at an average temperature of 4°C. Subsequently, the samples were centrifuged at 3,000G for 10 minutes to separate the serum, transferred to polyethylene microtubes, aliquoted and stored at -20°C until the hormone assays were performed.

Analyses of the P4 contraction were performed using chemiluminescence methodology, using the Access Immunoassay Systems Progesterone (Beckman Coulter®, Fullerton, CA, USA), in the Immunology Laboratory of the Health Sciences Institute, UFBA. The intra-assay and inter-assay coefficient of variation were 6.25% and 8.95%, respectively, and the assay's sensitivity was 0.016ng/mL.

Hormonal treatment costs

In calculating the cost for every protocol, the researchers only took into account the dosage levels of the drugs administered within each respective protocol. Standard consumables used across all groups, such as alcohol and cotton, were excluded from these figures. To evaluate protocol expenses, variables like average drug prices, vial concentrations and per-dose costs were systematically recorded. A secondary chart was then generated to outline the daily medication costs for each specific protocol. Total expenditures were derived from these figures, allowing for a comparison of price differences. By establishing the highest-priced protocol as the reference point, the relative cost savings of the remaining protocols were identified.

Experimental design and statistical analysis

Experimental design and statistical analysis. The experimental design adopted was Completely Casualized and the data were analyzed using the Statistical Package for Social Science (SPSS) version 13.0 (SPSS 2004), according to the following sequence: 1) The means and standard deviations were obtained by descriptive analysis. 2) The comparisons of the studied variables' means were performed, initially, using the Shapiro-Wilk normality distribution test. If the variable MOV did not show normal distribution, in this case the Kruskal-Wallis test was applied. 3) For other variables the analysis of variance (ANOVA) was performed, and for the comparison between the experimental groups Tukey's test was used; 4) For the comparison of POFD and POFA, between treatments throughout the follicular dynamics, ANOVA of repeated measures was used. For all analyses executed, a 5% significance level was adopted.

Table 1. Effects of the proposed synchronization of the estrous cycle in buffaloes protocols on follicular characteristics assessed by B-mode and Power-Doppler ultrasonography.

Variables	Treatments (mean±SD)				P value
	EB (n=7)	GnRH (n=6)	EB+eCG (n=7)	GnRH+eCG (n=7)	
LFOL D9 (cm)	1.15±0.19	1.23±0.22	1.18±0.23	1.13±0.22	0.387
LFOL D10 (cm)	1.25±0.23	1.36±0.20	1.20±0.21	1.26±0.28	0.811
LFOL D11 (cm)	1.39±0.26	1.53±0.07	1.33±0.18	1.49±0.14	0.495
DPF (cm)	1.49±0.15	1.57±0.06	1.38±0.17	1.54±0.13	0.100
ALFOL D9 (cm²)	0.51±0.07	0.52±0.09	0.51±0.13	0.52±0.09	1.000
ALFOL D10 (cm²)	0.68±0.09	0.63±0.08	0.64±0.13	0.65±0.06	0.807
ALFOL D11 (cm²)	0.76±0.11	0.69±0.07	0.66±0.14	0.69±0.04	0.501
WAPF (cm²)	0.75±0.11	0.72±0.06	0.70±0.11	0.70±0.05	0.671
GRFOL (cm/day)	0.09±0.02	0.10±0.03	0.07±0.04	0.11±0.04	0.300
MOV (hours)	66.86±9.44	76.00±6.20	66.86±13.61	75.43±5.86	0.142
VWAPF (cm²)	0.24±0.10	0.20±0.08	0.20±0.05	0.23±0.08	0.757
%VWAPF (%)	30.93±9.41	28.57±10.82	29.55±9.12	32.68±10.37	0.922

GnRH – Gonodotropin-releasing hormone, eCG – equine chorionic gonotropin, EB – estradiol benzoate, SD – standard deviation, LFOL – Largest follicle, D9 – day 9, D10 – day 10, D11 – day 11, DPF – diameter of the preovulatory follicle, ALFOL – follicular area, WAPF – total wall area of the preovulatory follicle, GRFOL – growth rate of the ovulatory follicle, MOV – moment of ovulation, VWAPF – vascularization in the wall area of the preovulatory follicle, %VWAPF – percentage of vascularization in the wall area of the preovulatory follicle.

Results

At the time of removal of the intravaginal P4 device, it was observed that three animals had lost it and were excluded from the evaluations. Furthermore, it was also found that, ten (10) animals did not respond to the pharmacological control protocol of the estrous cycle (not ovulate), they were also excluded from the evaluations regarding the following characteristics: DPF, WAPF, VWAPF, %VWAPF and MOV. The experimental groups were therefore defined as follows: EB (n=7), GnRH (n=6), EB + eCG (n=7) and GnRH + eCG (n=7). The overall ovulation rate of the experiment was 73%.

Follicular variables

There was no significant difference between protocols with eCG or without eCG, associated with GnRH or EB for any of the follicular characteristics evaluated (Table 1).

Assessing the variable follicular diameter over time between treatments, it showed a similar behavior, with no statistical difference between groups ($p \geq 0.05$). Likewise, the variable follicular wall area also showed no statistical difference, with similar variation ($p \geq 0.05$), indicating that the protocols used stimulated follicular growth equally.

In this study, despite the variation in the different protocols between the times of ovulation there was no statistical difference between the experimental groups ($p=0.142$). It was also evident in all groups that the MOV values were similar.

For the estimated variables VWAPF and %VWAPF (Tab. 2), there was no statistical difference between the FTAI protocols, indicating no influence of the treatments on the vascularization of the follicle wall.

Luteal variables

Table 2 describes the luteal characteristics evaluated by B and Power Doppler mode ultrasound at D18 of the FTAI used protocols. The influence of the hormone treatments used on the variables CL area (ACL) and CL vascularization (VCL and %VCL) was not observed. A statistically significant difference ($P=0.047$) was observed between the EB and EB+eCG groups regarding the CL diameter (CLD). However, this difference did not correlate with serum P4 concentrations in both groups.

A statistically significant difference ($p=0.017$) was found for P4 concentration between the GnRH and GnRH+eCG groups.

Table 3 shows the costs (US$) of each protocol per animal and the percentage savings (%) for each experimental protocol. In the treatments in which eCG was combined, there was an increase in costs per animal, with its combination with EB being approximately 21.76% cheaper than when combined with GnRH, in addition to providing the highest, statistically different values of CLD and P4.

Table 2. Effects of the proposed synchronization protocols on luteal characteristics evaluated by B and Power Doppler mode ultrasonography and progesterone (P4) dosages on day 18 of the FTAI protocol.

Variables	Treatments (mean±SD)				P value
	EB (n=7)	GnRH (n=6)	EB+eCG (n=7)	GnRH+eCG (n=7)	
CLD (cm)	1.62±0.11[a]	1.75±0.10[ab]	1.78±0.12[b]	1.71±0.09[ab]	0.047
TACL (cm²)	2.04±0.19	2.31±0.27	2.42±0.29	2.23±0.33	0.096
VCL (cm²)	0.91±0.30	0.97±0.19	0.84±0.23	0.77±0.32	0.570
%VCL (%)	44.02±11.26	42.58±9.90	34.68±7.70	33.97±11.33	0.333
P4 (ng/mL)	3.26±0.57[ab]	2.80±0.70[a]	3.87±0.56[ab]	4.09±0.94[b]	0.017

EB – estradiol benzoate, GnRH – Gonadotrophin Releasing Hormone, eCG – equine chorionic gonadotropin, CLD – corpus luteum diameter, TACL – total area of the corpus luteum, VCL – vascularization of the corpus luteum, %VCL – percentage of vascularization of the corpus luteum, P4 – progesterone.

Table 3. Cost of each hormone per day, in the FTAI protocol values of the experimental buffaloes groups, indicating the total cost per animal and the economic viability.

Day	Hormones	Protocols			
		EB	GnRH	EB+eCG	GnRH+eCG
D0	P4+PGF2α+ EB (US$)	3,94	3,94	3,94	3,94
D9	PGF2α (US$)	0.87	0.87	0.87	0.87
	eCG (US$)	0.00	0.00	3.38	3.38
D10	EB (US$)	0.19	0.00	0.19	0.00
D11	GnRH (US$)	0.00	2.52	0.00	2.52
	Value/animal (US$)	5.00	7.33	8.38	10.71
	Economic viability (%)	53.31	31.56	21.76	0.00

EB – estradiol benzoate, GnRH – Gonadotrophin Releasing Hormone, eCG – equine chorionic gonadotropin, US$ – US dollar.

Discussion

The steady development of ovarian structures signaled that the animals were cycling, with average follicular diameters consistent with findings from Raval (2020) and El-Shahat (2012) for buffalo. Murrah calves typically reach ovulatory readiness when the dominant follicle hits 0.85 cm (Abd Ellah et al. 2010); by day nine, all experimental groups had surpassed this size, confirming ovulation across the board. Furthermore, the growth rate of ovulatory follicles remained uniform across treatments (p=0.300), showing effective development and growth comparable to the 0.13 to 0.15 cm/day reported by Baruselli et al. (2007).

In studies of follicular patterns in cycling buffaloes, it was found that dominant follicles achieved average diameters of 1.30 to 1.50 cm (Baruselli et al. 1997). In this study, no statistical difference was found between ovulatory follicle diameters (p=0.100) and values across experimental groups were similar to those in the cited study. Similarly, Estradoublesynch protocol diameters (1.30 to 1.60 cm) align closely here (Mirmahmoudi and Prakash 2012).

Buffalo protocols using the same hormonal basis (P4/EB + eCG) reported ovulatory follicle diameters of 1.31, 1.37 and 1.37 cm for EB24, EB36 and GnRH48 groups, respectively, achieving satisfactory pregnancy rates (Carvalho et al. 2016). Notably, these authors conducted the experiment in the unfavorable reproductive season, underscoring the importance of eCG as gonadotrophic support. The same hormonal base applied in fixed-time AI (FTAI) protocols across any seasons demonstrated eCG's viability in both scenarios (Baruselli et al. 1997).

In contrast, in this research, in this research, eCG increased maximum diameters of pre-ovulatory follicles at the end of synchronization protocols in buffaloes under seasonal anestrus, likely because anestrous females lack the pulsatile LH release necessary for final follicular development and ovulation, making eCG supportive in such cases (Baruselli et al. 2007).

MOV durations of 69.8 h and 70.0 h using

GnRH + eCG and GnRH alone, respectively, were similar to those observed here, both achieving high ovulation rates (Baruselli et al. 2007). Ovulation times of 70.0, 78.4 and 73.6 h were found in studies using EB24, EB36 and GnRH48, respectively (Carvalho et al. 2016).

Carvalho et al. 2016 also noted that EB and GnRH produced very similar outcomes regarding follicular growth, synchronization and ovulation induction, which corroborates the present findings. Likewise, replacing GnRH with EB in bubaline FTAI protocols yielded satisfactory follicular response, ovulation and pregnancy rates (Monteiro et al. 2018).

Other studies highlight advantages of using EB instead of GnRH, such as improved estrus expression (Yousuf et al. 2015), a higher LH peak (Jacomini et al. 2014), better uterine tone and enhanced cost-effectiveness (Mirmahmoudi and Prakash 2014).

In a follicular dynamics study, preovulatory follicle blood flow measured by Power-Doppler in pregnant and nonpregnant buffaloes undergoing FTAI was unaffected by treatment (Neglia et al. 2012). Studies evaluating follicular development via Doppler ultrasonography in buffaloes are scarce; nevertheless, vascular formation in the theca layer to meet growing follicle metabolic demands has been documented (Acosta et al. 2003, Siddiqui et al. 2009, Ginther 2014). Therefore, analysis of the vascularization area can provide valuable data on follicular viability and oocyte maturity (Ginther 2014).

Moreover, adding eCG during proestrus did not enhance morphological parameters of dominant or preovulatory follicles under the tested hormone treatments.

Regarding the CLD on D18, the groups with EB (1.62 ± 0.11^{a}cm) and EB + eCG (1.78 ± 0.12^{b}cm) differed statistically from each other and were much higher than those found by Abd Ellah et al. (2010), when they also evaluated on day 18, (1.18 ± 0.15 cm); and closer to those of Monteiro et al. (2018) (1.84; 1.90 cm). When the protocol was with GnRH (GnRH - 1.75 ± 0.10 and GnRH + eCG - 1.71 ± 0.09 cm), this study found a statistical difference between the group that used only EB, higher than those of Russo et al. (2010) (1.64 cm) and Vecchio et al. (2012) (1.70 cm), but lower than those of Di Francesco et al. (2012) (1.86 cm). These findings demonstrate the effectiveness of protocols with GnRH alone and those combined with eCG, supporting the choice based on costs.

The CL area data ranged from 2.04±0.19 to 2.42± .29cm², did not differ statistically from each other and were close to those reported by Vecchio et al. (2012) (2.40cm²), but, they were lower when compared to Di Francesco et al. (2012) (2.72cm²) and Neglia et al. (2012) (2.84cm²).

The serum progesterone concentrations found on D18 for the EB, GnRH, EB+eCG and GnRH+eCG groups corresponded to the reports of Abd Ellah et al. (2010); however, a significant difference ($p=0.017$) was found between the GnRH group and the others, corroborating the need to use eCG in GnRH-based protocols (Marugavel et al. 2009, Carvalho et al. 2012).

Furthermore, eCG administered after P4 implant removal in lactating buffaloes during the nonreproductive season enhanced CL growth rate and P4 concentration (Carvalho et al. 2014). These findings align with outcomes in this study regarding DCL in EB and EB + eCG groups and P4 in GnRH and GnRH + eCG groups, which showed statistical differences.

Protocols used did not affect vascular characteristics of the CL evaluated by Power-Doppler ultrasonography. To date, no studies have applied the same protocol base combined with objective Power-Doppler analysis of the CL in buffaloes (Ghetti et al. 2016). CL wall blood flow, used to calculate resistance and pulsatility indices in buffaloes with $PGF_2\alpha$ analog under Ovsynch followed by AI, was similarly unaffected (Neglia et al. 2012).

Studies have demonstrated strong positive correlations between VEGF expression, vascular density and plasma progesterone, suggesting a close relationship between angiogenesis and CL function in this species (Papa et al. 2007). Adequate CL blood flow on day 10 after AI appears vital for luteal function and pregnancy success (Russo et al. 2010).

The evaluation of CL function development during the first two weeks post-AI and gestation progression via color-Doppler suggested a model for future foundational studies on early gestation endocrinology in buffaloes. There is potential to develop CL functionality assessment using this tool as a pregnancy predictor highlighting the need for further Doppler studies in buffaloes under FTAI (Vecchio et al. 2012). Complementarily, luteal evaluations confirm that the tested protocols promoted structurally and functionally adequate CL formation.

It is known that a crucial factor in the IATF protocol is the careful balance between low cost and high efficacy. This study identified the GnRH + eCG protocol as the most expensive option, followed by EB + eCG, GnRH and EB in order of cost. Since no significant differences were found in CLD values or P4 concentrations between the GnRH-treated groups, the addition of eCG to GnRH only increases the price by 31.56%, without improving efficiency.

However, the EB + eCG protocol showed statistically superior results to GnRH without eCG, justifying its higher price. When compared to GnRH + eCG, the EB + eCG method showed similar results but was

21.76% cheaper, making it the most cost-effective solution for achieving better reproductive rates.

Conclusion

Although the follicular morphofunctional results did not show a statistical difference between the protocols tested in this study, the luteal parameters and hormone values indicate that the cost-benefit ratio of the EB+eCG protocol is the most suitable under these experimental conditions, as it provides follicular growth, synchronization, ovulation, CL development and P4 production adequate for the maintenance of buffalo pregnancy.

Author Declarations

Ethics approval

The study was conducted according to ethical precepts recommended by the National Council of the Control of Animal Experimentation, after approval by the Ethics Committee on the Use of Animals of the Federal University of Bahia, under number 27/2018

Use of generative artificial intelligence

No artificial intelligence tools were used in the creation of the text, images, and tables. GPT Chat was used to adapt the references.

Conflict of interest

The authors declare that they have no financial, personal, or institutional relationships that could be perceived as influencing the research. Therefore, they declare that there was no conflict of interest.

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