REPRODUCTIVE EFFICIENCY OF NELLORE (*BOS INDICUS*) COWS SUBJECT TO BOTH FTAI AND HOMEOPATHIC SUPPLEMENTATION

EFICIÊNCIA REPRODUTIVA EM VACAS NELORE (BOS INDICUS) SUBMETIDAS À IATF E A SUPLEMENTAÇÃO HOMEOPÁTICA

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ABSTRACT: The aim of this work was to determine the effect of homeopathic supplementation on both ovarian dynamics and conception rate in Nellore cows subjected to fixed-time artificial insemination (FTAI). Cows (n = 150) were randomly distributed to the control (CG) and the homeopathy group (HG). The HG cows were supplemented with Pró-cio in the mineral salt for 60 days and both experimental groups were further subjected to FTAI. Cows were evaluated for ovarian dynamics (n = 16), progesterone (P4) concentration (n = 16), and conception rates (n = 150). Ovarian dynamics determined by ultrasonography and showed similar findings for CG and HG, respectively. Thus follicular diameter (8.7 ± 1.0 mm vs. 10.0 ± 0.8 mm), mean pre-ovulatory follicle volume (0.46 ± 0.15 mL vs. 0.61 ± 0.12 mL), and mean follicular growth (3.65 ± 1.41 mm vs. 4.60 ± 1.21 mm) did not differ between groups. Moreover, corpus luteum diameter was similar between groups (CG: 16.28 ± 0.7 mm vs. HG: 15.6 ± 0.8 mm; $P > 0.05$), although P4 levels did differ (CG: 2.55 ± 0.85 ng mL^{-1} vs. HG: 6.52 ± 1.19 ng mL^{-1}; $P < 0.05$). The conception rate after FTAI was not affected by homeopathic supplementation (CG: 74.67 %, and did HG: 77.33 %; $P > 0.05$). In conclusion, the homeopathic supplementation Pró-cio increases P4 concentrations but does improve the reproductive efficiency of Nellore cows subject to FTAI.

KEYWORDS: Corpus luteum. Follicle. Homeopathy. Ovarian dynamics.

INTRODUCTION

The efficiency of cattle production systems is directly related to its adequate nutritional, sanitary, and reproductive management (FERREIRA-SILVA et al., 2017; TATCHER, 2017). The application of assisted-reproduction technologies increases the overall herd genetic merit by delivering more offspring from the most productive animals (HANSEN, 2014). For such approaches, the control of estrous cycles is paramount and is typically attained by hormone-based protocols (BISINOTTO et al., 2014; STEVENSON; BRITT, 2017).

Despite the efficiency of synthetic compounds for estrous cycle control, hormone or pharmacologically-based protocols remain expensive and are not aligned with the increasing demand for hormone-free practices (MARTIN; KADOKAWA, 2006; SILVA FILHO et al., 2015; FERREIRA-SILVA et al., 2017). Thus, the investigation of alternatives for estrous cycle control remains as an attractive research venue. Under such context, homeopathy became a widespread practice in organic farming (HEKTOEN, 2005; LIMA et al., 2016). Homeopathy approaches have also been used in numerous species to treat different diseases by replacing antibiotics, anti-diarrheic, and anti-helminthics products (VARSHNEY; NARESH, 2005; CHAGAS et al., 2008; SOTO et al., 2008; WERNER et al., 2010; MATHIE; CLAUSEN, 2015; DOEHRING; SUNDRUM, 2016; ORJALES et al., 2016).

For animal reproduction applications, homeopathy can be used for increasing endogenous hormone production (RAJKUMAR et al., 2006), to improve semen parameters (GERHARD; WALLIS, 2002; LOBREIRO, 2007; SOTO et al., 2009), to treat ovarian pathologies (RAJKUMAR et al., 2006; FIDELAK et al., 2007), and further increase pregnancy rates (WILLIAMSON et al., 1991;

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KUMAR et al., 2004; SOTO et al., 2010). Despite these promising evidences, the efficacy of homeopathic supplementation for improving cattle reproduction needs further investigation.

There is a growing necessity worldwide to increase the fertility rates of commercial beef cattle herds, concomitant with the need for reducing costs while diminishing the utilization of hormone-based protocols. Therefore, the aim of this work was to evaluate the ovarian dynamics and conception rates in Nellore cows subjected to both homeopathic supplementation with Pró-Cio® and fixed-time artificial insemination (FTAI).

MATERIAL AND METHODS

Location

Experiments were carried out in Currais, Piauí state, Brazil. The geographic coordinates of the farm are latitude 09º 00' 25" South and longitude 44º 24' 39'' West. The altitude is 320 m, with a mean annual temperature of 26.4 °C, and mean annual rainfall of 996 mm^3. Animals were raised in cultivated pastures (i.e., Brachiaria brizantha and Andropogon gayanus) and had free access to water and mineralized salt.

Animal Selection and Management

Multiparous Nellore (*Bos indicus*) cows used had aged from 3 to 5 years, had delivered within 60 days, and displayed a body condition score between 2.5 and 3.5 in a 1 to 5 scale, as described by BROSTER and BROSTER (1998). The cows were subject to clinical and gynecological examination as recommended by GRUNERT and BIRGEL (1982). Based on these criteria, selected cows (n = 150) were randomly allocated to experimental groups (i.e., Control Group - CG and Homeopathy Group - HG).

The homeopathic supplement (Pró-Cio®; Real H Nutrição e Saúde Animal) was added to the mineralized salt (20 g per animal each day) to HG cows throughout the experiment (60 days). After the initial 30 days of homeopathic supplementation (adaptation period), cows were subject to an FTAI protocol.

On day 0 (D0), cows of both groups received an intravaginal device (Sincrogest®; Ourofino Agronegócio) containing 1 g progesterone and 2mg estradiol benzoate (Sincrodiol®; Ourofino Agronegócio) by an intramuscular (IM) shot. On day 8 (D8), cows were treated with 500 IU (IM) equine chorionic gonadotropin (Sincro eCG®; Ourofino Agronegócio), 500 µg Cloprostenol (Cloprostenol Sódico; Sincrocio®; Ourofino Agronegócio), and 1.0 mg EB at the moment of intravaginal device removal. Forty-four hours after intravaginal implant removal, the FTAI was carried out with frozen-thawed semen from bulls of proven field fertility (semen successfully used for FTAI). The conception rate was determined 40 days after FTAI, as previously described by Pereira et al. (2016). The conception rate was calculated by dividing the total number of pregnant cows by the total number of cows subject to FTAI and further multiplied by 100. Figure 1 is a schematic representation of the experimental design of the study.

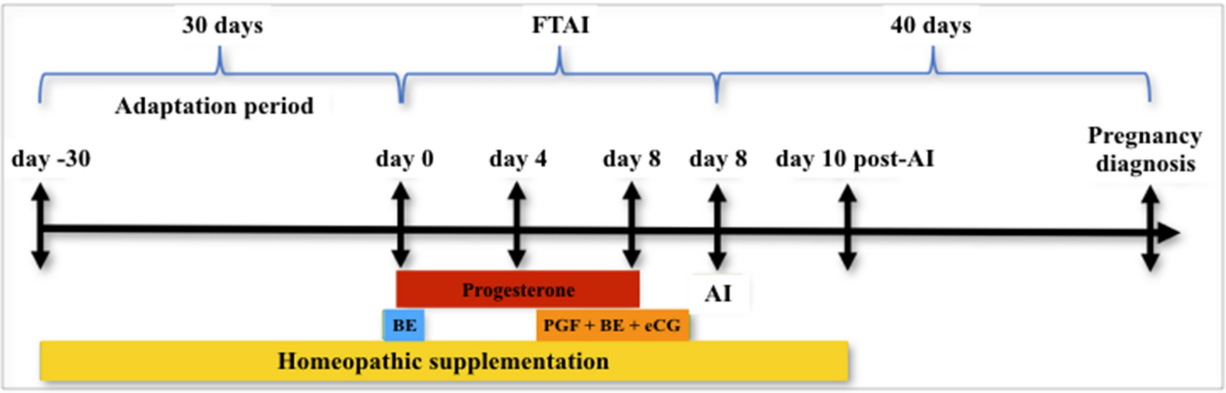

Figure 1. Experimental design to determine the effect of homeopathic supplementation with Pró-Cio® on the reproductive efficiency of Nellore (*Bos indicus*) subjected to fixed-time artificial insemination (FTAI). AI: artificial insemination. BE: benzoate estradiol. eCG: Equine chorionic gonadotropin. PGF: prostaglandin 2 alpha.

Ovarian dynamics

The ovarian dynamics was carried out by ultrasonography. One technician performed all analysis using a single device (Sono Vet R3, Madison) equipped with a linear transducer (7.5 MHz). The ovarian activity analysis was performed in eight cows of each group, as previously described (SILVA FILHO et al., 2015). The traits that were analyzed: number of follicles, mean diameter (vertical and horizontal mean diameters) of the dominant follicle, co-dominant and pre-ovulatory follicles, follicular volume, and further corpus luteum diameter (if found). The exams were carried out at the onset of homeopathic supplementation and in seven-day intervals afterward until estrous synchronization for FTAI. During synchronization, the examinations were carried out on days D0, D4, D8, and D10 and ten days after the FTAI protocol (for corpus luteum measurement).

Progesterone Analysis

Blood samples were obtained by jugular vein puncture with a 21 G needle into heparin-containing tubes (Becton Dickinson, Rutherford, NJ). The blood was centrifuged (2,500 g for 10 minutes) for plasma retrieval and stored at - 20 °C. The determination of progesterone (P4) concentration was performed by chemiluminescence (Coat-a-Count; Diagnostics Products Corporation). The coefficient of variation of the test was 4.25 %, and the minimum detectable level was 0.08 ng mL^{-1}.

Statistical Analysis

The data generated during each exam was analyzed using the SPSS 16.0. Initially, the data was subject to both critical and consistency analysis (frequencies, distributions, and homogeneity). Moreover, pre-ovulatory follicle diameter and volume, follicular growth between D4 and the day of FTAI, the quantity of recruited follicles on D4, and corpus luteum diameter 10 days after FTAI were determined by analysis of variance (ANOVA). The pregnancy rate and frequency of cows with a new follicular wave were evaluated by the chi-square test. The significance level was 5 %.

RESULTS

Table 1 contains the data relative to the ovarian activity of HG and CG cows. It can be observed that mean POF diameter, mean POF volume, follicular growth (between D4 and D10, and FTAI day), and mean corpus luteum diameter after FTAI did not differ between groups (P > 0.05; Table 1).

Table 1. Mean values ± SEM for recruited follicles on D4, diameter and volume of the pre-ovulatory follicle on D10, follicular growth between D4 and D10, and corpus luteum diameter at day 10 post-IATF in Nellore cows under homeopathic supplementation with Pró-Cio (HG) and the control group (CG).

Ovarian Activity		
Variable	**Group**	
	HG	CG
Follicles recruited on D4	10.37 ± 0.94	12.75 ±1.66
Mean pre-ovulatory follicle diameter (mm)	10.0 ± 0.8	8.7 ± 1.0
Mean pre-ovulatory follicle volume (mL)	0.61 ± 0.12	0.46 ±0.15
Mean follicular growth between D4 and D10 (mm)	4.60 ± 1.21	3.65 ± 1.41
Mean Corpus Luteum diameter post-FTAI (mm)	15.60 ± 0.8	16.28 ± 0.7

No statistical difference was found between groups (P > 0.05).

The distribution of ovarian follicles according to their size is shown below (Figure 2). There was no difference (P > 0.05) between groups for follicle size interval. Moreover, mean follicular growth during the FTAI protocol was also determined (Figure 3).

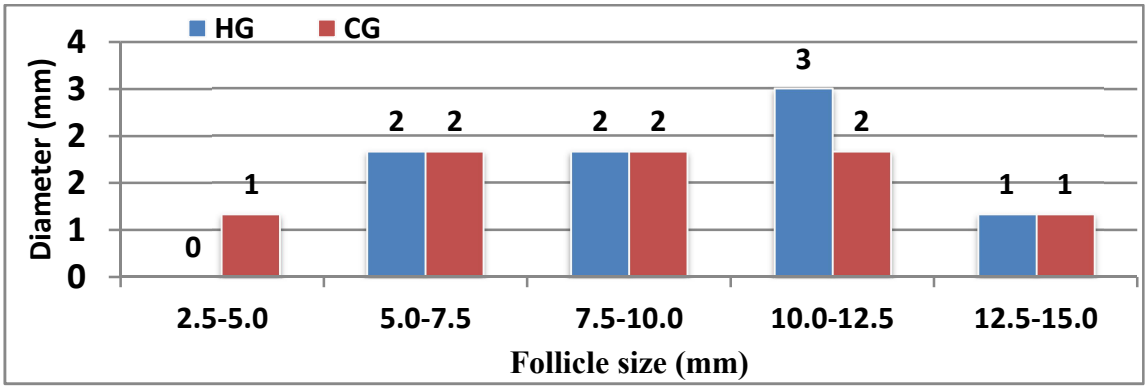

Figure 2. Follicle size in Nellore cows (*Bos indicus*) under homeopathic supplementation with Pró-Cio (HG) or the control group (CG).

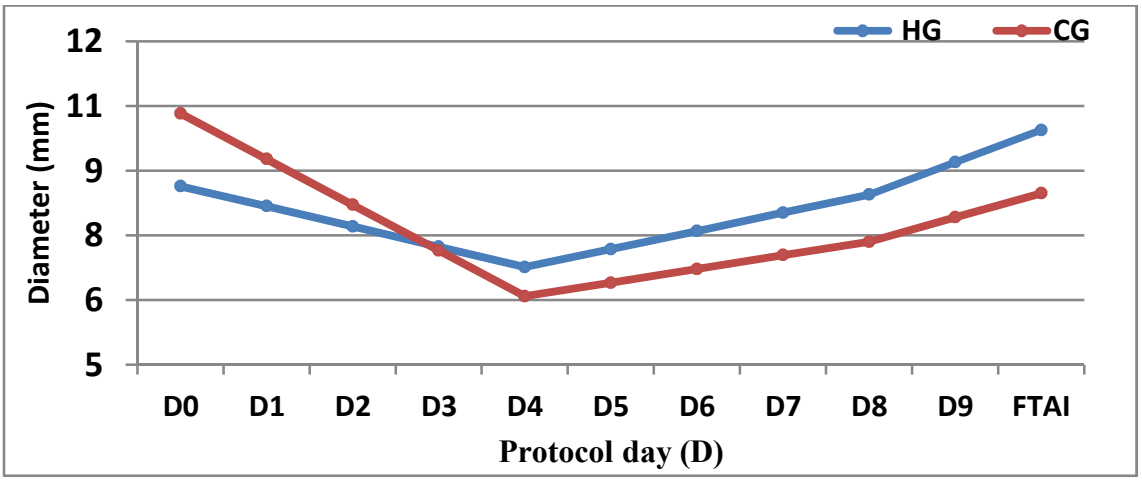

Figure 3. Follicular growth during the FTAI protocol (from day 4 until day 10) in Nellore cows (*Bos indicus*) under homeopathic supplementation with Pró-Cio (HG) or the control group (CG).

Figure 4 displays the data relative to mean corpus luteum (CL) diameter and P4 concentration in both HG and the CG at day 10 post-FTAI. No difference was found (P > 0.05) between groups for CL diameter, but P4 levels were higher (P < 0.05) on the HG than in the CG. The conception rate in the HG was 77.33 % (58/75) and 74.67 % (56/75) in the CG, with no difference (P > 0.05) between groups.

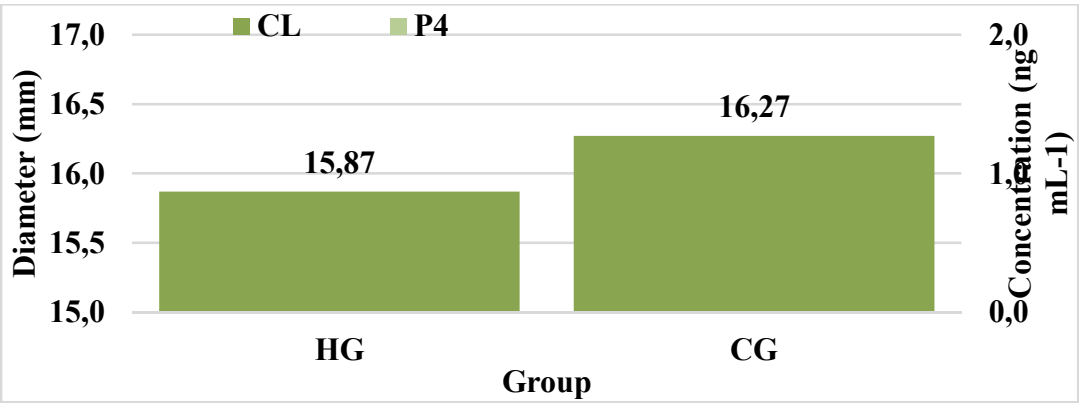

Figure 4. Relationship between Corpus Lutem diameter (CL) and progesterone concentration (P4) at day 10 post-FTAI in Nellore cows (*Bos indicus*) under homeopathic supplementation with Pró-Cio (HG) or the control group (CG).

DISCUSSION

The diameter of pre-ovulatory follicles correlates with ovulation potential (SARTORI et al., 2001; GIMENES et al., 2008), with CL diameter (VASCONCELOS et al., 2001), and ultimately with conception rate (PERRY et al., 2007). Collectively, these parameters interplay to establish cow fertility and thus lead to determine herd overall reproductive performance.

The data described above showed that homeopathic treatment with Pró-cio® did not lead to increased follicular development. Therefore, it can be ruled out that the protocol does not induce affect the cyclicity status. This reasoning is not supported by findings from RAJKUMAR et al. (2006), who found that a homeopathic supplement could be used for reverting cow anestrus. The increased estradiol concentration could be due to the addition of different plants (i.e., Pulsatilla spp. and Aletris farinosa) to this homeopathic supplement (BOERICKE, 2001; DUKE et al., 2002). In buffaloes, combinations of homeopathic products also reduced anestrus incidence (KUMAR et al., 2004).

Pre-ovulatory follicles in HG cows reached 7.5 mm or more, while follicles from some animals had reached 12 mm. This fact was suggestive that conception rates would be increased by improving ovulation rates, since dominant follicle dilation in *Bos indicus* occurs when reaching 7 mm (GIMENES et al., 2008). This reasoning is also supported by the relationship between pre-ovulatory follicle size, CL diameter and the P4 production potential. Within smaller follicle sizes, the resulting lower P4 concentration would not enough for blocking prostaglandin synthesis by the endometrium (VASCONCELOS et al., 2001) and thus inhibit premature luteolysis (BRIDGES et al., 2005).

The number of follicles that were recruited on D4 was not increased by the homeopathic supplementation. Comparison with previous data is somewhat difficult due to the varying methodological approaches and definition of minimum antral follicle size (FERREIRA et al., 2004; STARBUCK-CLEMMER et al., 2007; SÁ FILHO et al., 2008). However, some reports have pointed out to differences in reproductive physiology between *Bos indicus* and *Bos taurus* (i.e., mean recruited follicles and dominant follicle size). Such differences could be due to some physiological parameters, such as higher IGF-I and insulin serum concentrations and elevated FSH-responsiveness (SILVA-SANTOS et al., 2011; SARTORI; BARROS, 2011).

The follicle size in HG was higher than the CG cows, but CL was indistinguishable between groups. More recent reports have shown that the P4 production is not exclusively dependent on CL diameter, but is also influenced by its vasculature. In *Bos indicus* cows, the estrus P4 concentration is approximately 1 ng mL^{-1} but can reach 4.5 ng mL^{-1} ten days later (ADEYEMO; HEATH, 1980). Since P4 concentrations differed between groups, independently of CL diameter, other factors (e.g., blood flow fluctuations, the presence of luteotropic or luteolytic factors, and different availability of P4 precursors) may have accounted for such difference (WILTBANK, 1994).

Conception rates were not affected by the homeopathic supplementation. This result may be context-dependent or breed-dependent since Penteado et al. (2005) found the homeopathic product may increase cyclicity and pregnancy rates in dairy cattle. Another important factor is that eCG favors pregnancy rates after FTAI in postpartum cows (BARUSELLI et al., 2004) and thus may have masked the beneficial effect of homeopathic supplementation. Another applausive concern is that some animals may not have ingested enough homeopathic supplement during the experiment. However, this fact can be ruled out due to two reasons. Firstly, cows subjected to homeopathic supplementation displayed higher P4 levels, suggesting a physiological response from all cows under such conditions. Secondly, supplementation of mineral sat with different compounds influences reproductive traits in both cows and bulls (JUCHEM et al., 2010; REIS et al., 2014).

In conclusion, the homeopathic supplementation Pró-cio increases P4 concentrations but does improve the reproductive efficiency of Nellore cows subject to FTAI.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Committee

The experiment was approved by "Comissão de Ética e Experimentação Animal - CEUA" from the Universidade Federal Rural de Pernambuco (Protocol 060/2013).

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RESUMO: O objetivo foi determinar o efeito da suplementação homeopática na dinâmica ovariana e taxa de concepção em vacas Nelore cows submetidas à inseminação artificial em tempo fixo (IATF). As vacas (n = 150) foram distribuídas aleatoriamente nos grupos controle (GC) e grupo homeopático (GH). As vacas do GH foram suplementadas com Pró-cio® no sal mineral mineral por 60 dias. Ambos os grupos foram submetidos à IATF. As vacas foram avaliadas quanto à dinâmica ovariana (n = 16), concentração de progesterona (P4; n = 16) e taxa de concepção (n = 150). A dinâmica ovarina foi determinada por ultra-sonografia e mostrou resultados semelhantes para o GC e o GH, respectivamente. Portanto, para diâmetro folicular (8,7 ± 1,0 mm vs. 10,0 ± 0,8 mm), volume médio do folículo pré-ovulatório (0,46 ± 0,15 mL vs. 0,61 ± 0,12 mL) e crescimento folicular médio (3,65 ± 1,41 mm vs. 4,60 ± 1,21 mm) não diferiram entre os grupos. Além disso, o diâmetro do corpo lúteo foi semelhante entre os grupos (CG: 16,28 ± 0,7 mm vs. HG: 15,6 ± 0,8 mm; $P > 0.05$), apesar dos níveis de P4 diferirem (CG: 2,55 ± 0,85 ng mL^{-1} vs. GH: 6,52 ± 1,19 ng mL^{-1}; $P < 0.05$). A taxa de concepção após a IATF não foi afetada pela suplementação homeopática (GC:74.67 % vs. GH: 77.33 %; $P > 0.05$). Em conclusão, a suplementação homeopática com Pró-cio aumenta a concentração de P4 mas não melhora a eficiência reprodutiva de vacas Nelore cows submetidas à IATF.

PALAVRAS-CHAVE: Corpo lúteo. Folículo. Homeopatia. IATF.

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