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Influence of nutritional and ovarian parameters on pregnancy rates of Nelore cows artificially inseminated at fixed time

Influência de parâmetros nutricionais e ovarianos sobre as taxas de prenhez de vacas Nelore inseminadas artificialmente em tempo fixo

Influencia de los parámetros nutricionales y ováricos en las tasas de preñez de vacas Nelore inseminadas artificialmente en tiempo fijo

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Abstract

This experiment aimed to evaluate the effect of weight gain, body condition score and antral follicle count on the fertility of cows subjected to fixed-time artificial insemination. To this study, it was used 194 Nelore, pluriparous cows, between the 30th and the 45th day of the postpartum period. The cows were weighed, was classified by the body condition score and a transrectal ultrasound was performed in the ovaries to identify the *corpus luteum* and for counting the antral follicles. Then, the females were submitted to a hormonal protocol to perform the artificial insemination in fixed time. Thirty days after the artificial insemination, the cows were weighed again and the pregnancy was diagnosed by transrectal ultrasound. The pregnancy rate did not interact (P>0.05) with the body score condition, nor the presence of the *corpus luteum*, neither with the antral follicles count. The weight gain between the artificial insemination moment and the pregnancy diagnosis showed an interaction (P = 0.02) with the pregnancy rate. The weight gain average between the FTAI and the pregnancy diagnosis was 9.0 kg. The animals that gained up to 73 kg, showed higher pregnancy rate. In conclusion, the weight gain between the artificial insemination in fixed time and the pregnancy diagnosis influenced the pregnancy rate.

Keywords: Body condition score; Bovine reproduction; Antral follicle population; Weight gain.

Resumo

O objetivo do experimento foi avaliar o efeito do ganho de peso, escore de condição corporal e contagem de folículos antrais na fertilidade de vacas inseminadas artificialmente em tempo fixo. Para este estudo foram utilizadas 194 vacas Nelore, pluríparas, aos 30 a 45 dias pós-parto. As vacas foram pesadas, tiveram o escore corporal avaliado e foi feita ultrassonografia transretal dos ovários para identificação de corpo lúteo e para a contagem dos folículos antrais. Em seguida, as fêmeas passaram por um protocolo hormonal para realização inseminação artificial em tempo fixo. Trinta dias após a inseminação artificial foi realizada nova pesagem das vacas e o diagnóstico de gestação por meio de ultrassonografia transretal. A taxa de prenhez não foi influenciada (P>0,05) pelo escore corporal, presença de corpo lúteo

e contagem de folículos antrais. O ganho de peso entre a inseminação artificial e o diagnóstico de gestação apresentou interação (P=0,02) com a taxa de prenhez. O ganho de peso médio entre o início do protocolo de inseminação artificial em tempo fixo e o diagnóstico de gestação foi de 9,0 Kg. As vacas em ganharam até 73Kg de peso vivo, tiveram maior taxa de prenhez. Em conclusão, o ganho de peso entre o momento da inseminação artificial em tempo fixo e o diagnóstico de gestação influenciou a taxa de prenhez.

Palavras-chave: Escore de condição corporal; Ganho de peso; População de folículos antrais; Reprodução bovina.

Resumen

El objetivo fue evaluar el efecto del aumento de peso, el puntaje de condición corporal y el conteo de folículos antrales sobre la fertilidad de vacas inseminadas artificialmente en tiempo fijo. Para este experimento se utilizaron 194 vacas Nelore, pluríparas, entre los 30 y 45 días posparto. Las vacas se pesaron, se evaluó su puntuación corporal y se realizó una ecografía transrectal de los ovarios para identificar el cuerpo lúteo y contar los folículos antrales. Luego, los animales se pasarán por un protocolo hormonal para la realización de la inseminación artificial en tiempo fijo. Treinta días después de la inseminación artificial se volvió a pesar a las vacas y se diagnosticó la gestación mediante ecografía transrectal. La tasa de embarazo no interactuó (P> 0.05) con la puntuación corporal o con la presencia de cuerpo lúteo o con el recuento de folículos antrales. El aumento de peso entre la inseminación artificial y el diagnóstico de embarazo que muestra interacción (P = 0,02) con una tasa de embarazo. La ganancia de peso promedio entre el inicio del protocolo de inseminación artificial por tiempo fijo y el diagnóstico de gestación fue de 9.0 Kg. Las vacas que ganaron hasta 73 kg de peso vivo, tuvieron una mayor tasa de gestación. En conclusión, el aumento de peso entre el momento de la inseminación artificial en tiempo fijo y el diagnóstico de gestación influyó en la tasa de preñez.

Palabras clave: Aumento de peso; Población de folículos antrales; Puntuación de la condición corporal; Reproducción bovina.

1. Introduction

The Fixed Time Artificial Insemination (FTAI) is an extremely useful tool in livestock, since it optimizes reproductive management (Baruselli et al., 2017; Bó et al., 2018). In such a way, this biotechnology provides the insemination of cows at the beginning

of the breeding season, regardless of the cyclical state and thus eliminates the need for estrus detection and ensures greater genetic gain to the herd and profitability to livestock (Baruselli, Reis, Marques, Nasser, & Bó, 2004; Oosthuizen et al., 2020; Taponen, 2009).

The zebu animals are highly adapted to the climatic conditions of Brazil, although the climate seasonality interferes to the pasture production and has a negative impact on the beef cattle reproduction (Reese et al., 2020; Silva et al., 2017). Thus, the cows go through the period of greatest nutritional demand during the final third of gestation and the postpartum period under reduced food availability, and so, inadequate nutrition can affect the follicular function of females, and prolong the anestrus (Baruselli et al., 2017).

In this context, the Body Condition Score (BCS) can be a useful indicator of the energy and reproductive status of cows in the postpartum period (Ayres et al., 2009; DeRouen et al., 1994). Studies have shown that the BCS can influence the pregnancy rates of Nelore cows submitted to FTAI protocols (Ferreira, Miranda, Figueiredo, Costa, & Palhano, 2013; Moraes, Morotti, Costa, Lunardelli, & Seneda, 2019).

Experiments showed it is possible to associate some reproduction biotechniques to the physiological findings, such as the antral count follicles (AFC) in cattle (Evans et al., 2012), even the AFC demonstrates high variability between individuals and high individual repeatability.

Considering these aspects, this study aimed to evaluate the effect of weight gain between the hormonal treatment moment and the pregnancy diagnosis, the body condition score classification and the antral follicle count on the pregnancy rate of Nelore cows submitted to fixed time artificial insemination protocol during the postpartum period.

2 Methodology

2.1 Location and animals

The experiment took place during the 2017/2018 breeding season, between November 2017 and March 2018. The study was carried out on a rural property, located in Jundiai do Sul, Paraná State, Brazil (latitude 23º 26 '12" South, longitude 50º 14 '51 "West). The region's climate is considered humid subtropical (Cfa) according to the Köppen-Geiger classification, characterized by hot and humid summers (Beck et al., 2018).

This study used 194 pluriparous Nelore cows (*Bos indicus*), between the 30th and the 45th day of the postpartum, because this moment is considered a suitable period for obtaining a 12-month interval between the deliveries, and so to achieve a better economic result.

These cows were managed extensively on *Uruchola brizantha* pasture. And all the animals received water and mineral supplement containing phosphorus at 9% of concentration *ad libtum*.

2.2 Assessment of cyclicity, antral follicle count and weighing of cows

The cows were submitted to two ultrasound exams in 14 days apart, using a 7.5 mhz transrectal linear transducer (Myndray® 2200 vet, China). The cows cyclicity was determined by the presence of an ovarian *corpus luteum* (CL) in at least one of the two ultrasounds performed. In the first ultrasound examination, the animals underwent a gynecological evaluation, including external inspection and transrectal palpation to assess possible abnormalities in the genital tract. In the second ultrasound examination to assess the cyclicity, the cows were fasted for approximately 12 hours and the antral follicles count was also performed by the complete visualization of the ovaries by transrectal ultrasound, and all the antral follicles greater or equal to 3 mm diameter had being counted (Burns, Jimenez-Krassel, Ireland, Knight, & Ireland, 2005; J. L. H. Ireland et al., 2008).

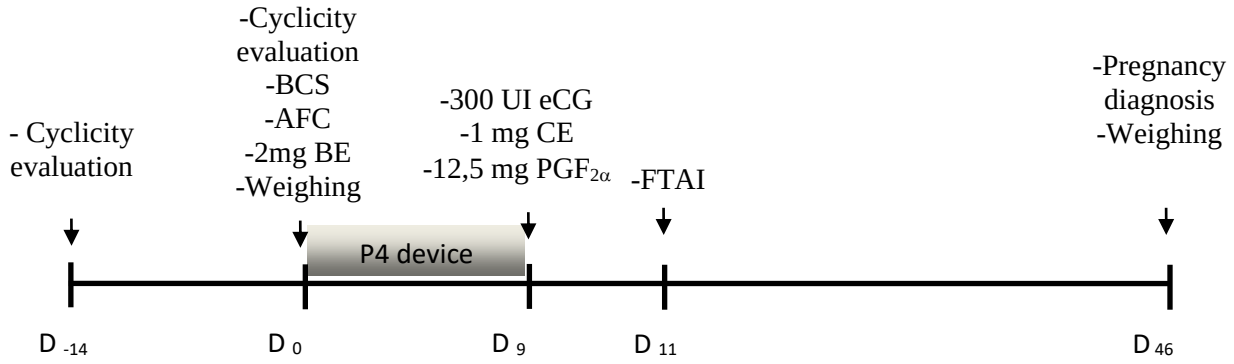
The cows were weighed on an electronic scale (KM3-N, Coimma, Dracena, São Paulo) and the body condition score (scale 1 to 5) was assessed as proposed by Ayres et al. (2009). The ultrasound exam, AFC and BCS evaluations were performed by a single Veterinarian practice. It was included in the experiment cows without abnormalities in the genital system, with or without *corpus luteum* and BCS between 2.25 and 4.25.

2.3 Hormonal treatment and pregnancy diagnosis

A second ultrasound examination (D0) was performed. At the same day (D0), the females received 2 mg of estradiol benzoate (Estrogin®, Farmavet, Brazil) by intramuscular (IM) application, and an intravaginal device containing 1.9 g of progesterone (CIDR, Zoetis ®, Brazil). Nine days (D9) later, 12.5 mg IM of dinoprost (Lutalyse®, Zoetis, Brazil) was administered and the progesterone devices were removed, simultaneously to the 350 IU eCG IM (Novormon®, Zoetis, Brazil) and 1 mg of estradiol cypionate (ECP®, Zoetis, Brazil) injections.

The Artificial insemination was performed in the D11, 50 to 54 hours after the removal of the intravaginal device. The cows were inseminated with frozen-thawed semen from a single Nelore bull. The pregnancy diagnosis was conducted by ultrasound in the 35th day after FTAI (D46). The cow was considered pregnant when it was visualized a vesicle with an embryo with heartbeat in the uterus. Still at the D46 was performed the second weighing, preceded by fasting for approximately 12 hours (Figure 1).

Figure 1. Schematic representation of ultrasound exams, hormonal treatment for fixed-time artificial insemination (FTAI), pregnancy diagnosis and weighing of post-partum Nelore cows.



BE: Estradiol benzoate; eCG: Equine chorionic gonadotropin; CE: Estradiol cypionate; PGF 2α:Dinoprost tromethamine; AFC: antral follicles count; BCS: assessment of body condition score; FTAI: Fixed time artificial insemination.
Source: Authors.

2.4 Variables and Statistical Analysis

The data obtained were submitted to the Kolmogorov-Smirnov test to verify the normality in its distribution. The comparison of means between the groups of low and high AFC was carried out through the analysis of variances. The cyclicity was analyzed by logistic regression, and so the AFC and BCS were considered as independent variables. The pregnancy rate (PR) was determined by the ratio of pregnant cows in the gestation diagnosis to the total number of inseminated cows (PR = N pregnant cows / N inseminated cows). The results for comparing the pregnancy rate were analyzed by logistic regression, in which pregnancy was considered a dependent variable and cyclicity, AFC, weight gain (WG) and BCS were classified as independent variables. All statistical tests were performed using the Software SigmaStat (1999), considering statistical significance when $P < 0.05$.

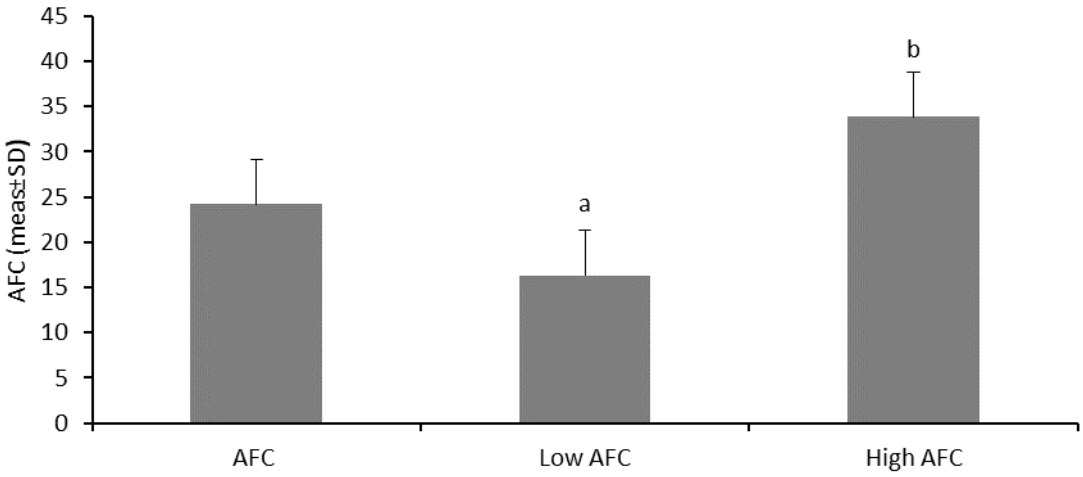
2.5 Ethical aspects

This experiment was submitted to the Ethics Committee of the Universidade Estadual do Norte do Paraná - UENP, and it was approved under the protocol number 05/2016. All procedures followed the Brazilian federal law No. 11,794 of October 8, 2008.

3 Results

The cows were grouped using the AFC mean (mean= 24) as a parameter, forming groups of low AFC (AFC≤ 24; N = 107) and high AFC (AFC≥24; N = 87). The AFC average was 24.1 ± 10.7 follicles, the frequencies of cows with high AFC was 33.8 ± 7.5 follicles, and the low AFC cows had 16.3 ± 5.0 follicles (Figure 2).

Figure 2. Antral follicles count in the postpartum Nelore cows (n = 194) and distribution of cows in the experimental groups with low AFC (n = 107) and high AFC (n = 87) observed in the second ultrasound exam (D0). Each column represents the mean ± standard deviation.

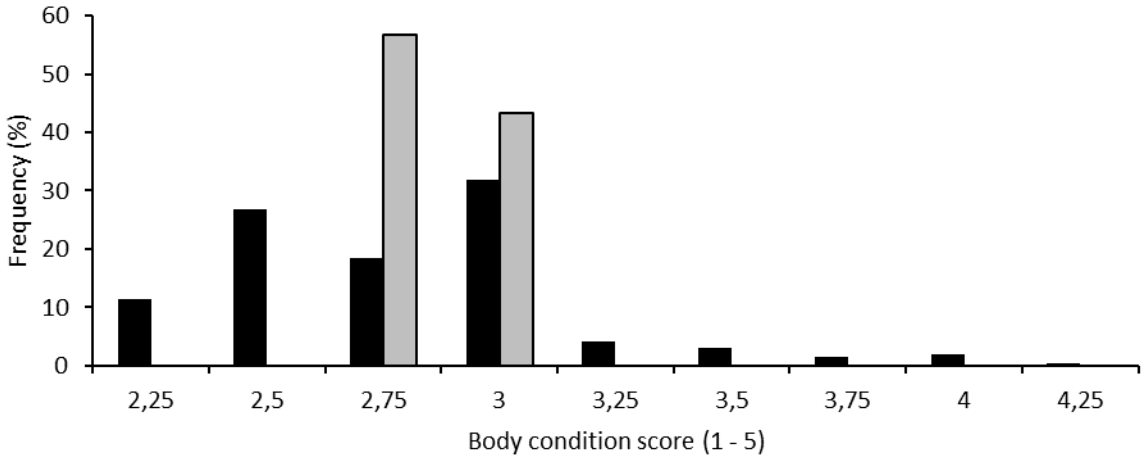


a, b - represent a statistically significant difference between the mean AFC Low and AFC High (P<0.001).
Source: Authors.

The animals have shown different frequencies in the postpartum BCS. So it was observed that 11.3% (n = 22) of the animals had BCS = 2.25; 27.0% (n = 52) shown BCS of 2.5; BCS = 2.75 was found in 18.5% (n = 36) of the cows; 32.0% (n = 62) had BCS = 3.0; BCS = 3.25 was observed in 4.1% (n = 8); 3.1% (n = 6) of the cows had BCS = 3.5; while 1.5% (n = 3) showed BCS = 3.75; 2.0% (n = 4) of the animals had BCS = 4.0; and only 0.5% (n = 1) showed BCS = 4.25. The BCS ranged from 2.25 to 4.25, so it was formed the group

BCS≤2.75 (n = 110) which represents 56.7% of the animals, and the BCS≥3.0 (n = 84) group formed by 43.2% of the cows (Figure 3).

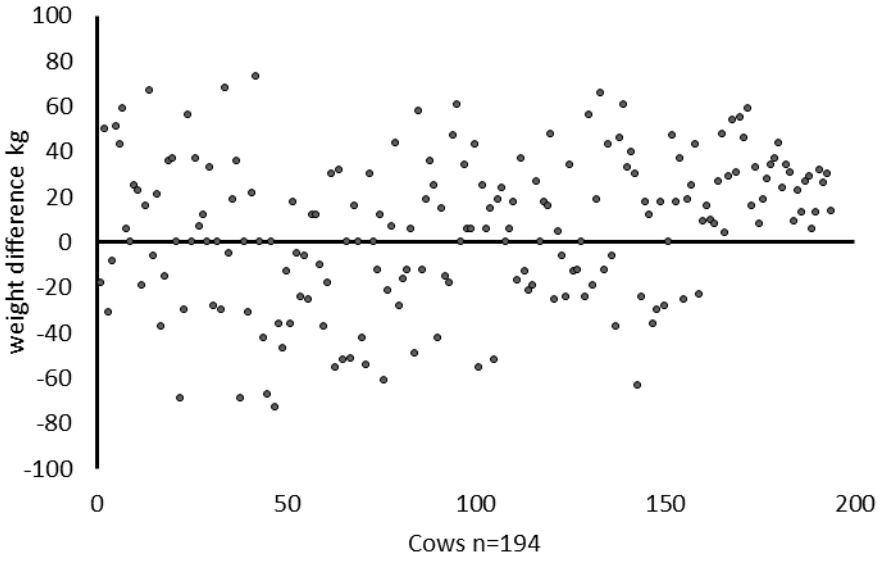
Figure 3. Frequency of postpartum Nelore cows with different BCS.



Black columns represent the distribution of the cows BCS. Gray columns represent the BCS clustered into BCS≤2.75: 56.7% (n = 110) and BCS≥3.0: 43.2% (n = 84).
Source: Authors.

The weight gain (WG) was obtained by the difference between the final weighing in D46 and the initial weighing in D0 (WG = weight D46 - weight D0). It was observed that 67 animals suffered a weight loss, up to 73 kg, while 111 cows maintained or gained weight. However, this variable did not present a normal distribution and, therefore, was represented by the median. The WG showed an average value of 9 kg (minimum: -73 kg, maximum: 73 kg), between D0 and D46, and the distribution of cows (Figure 4).

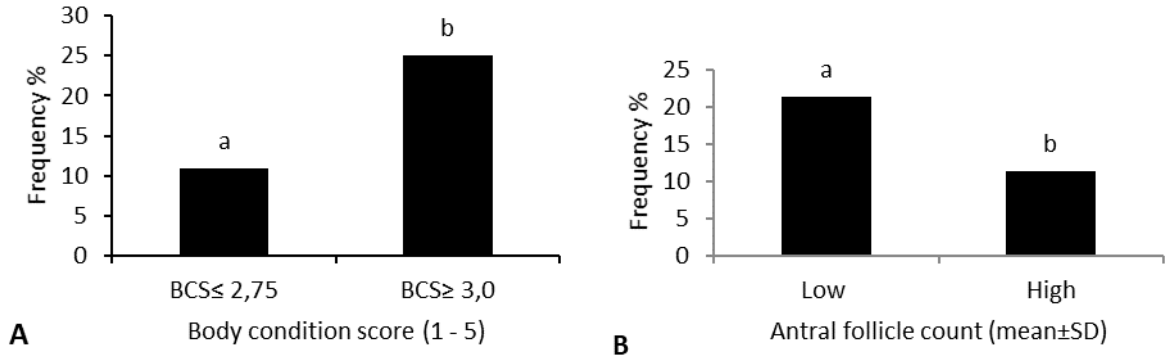
Figure 4. Distribution of postpartum Nelore cows that showed maintenance or weight gain (n = 127) or weight loss (n = 67), according to the weight variation between the beginning of the hormonal protocol (D0) and the diagnosis of pregnancy (D46).



Source: Authors.

The cows were considered cyclic when presented a CL (n = 33) in one of the ultrasound exams performed, while the others were considered in anestrus (n = 161). Cyclicity was influenced by the BCS and the AFC (P <0.05). Cows with BCS ≥3.0 had higher cyclicity than cows with BCS≤2.75 (P = 0.007), while animals with low AFC showed higher cyclicity than cows with high AFC (P = 0.02). The frequencies of cyclic cows, according to the main effects (Figure 5).

Figure 5. (A) Effect of body condition score (BCS) on the cyclicality of post-partum Nelore cows. (B) Effect of antral follicle count (AFC) on the cyclicality of postpartum Nelore cows.



a, b- Different letters represent statistical difference between the effects of BCS and AFC on the cyclicity of cows (P <0.05).
Source: Authors.

The pregnancy rate of Nelore cows submitted to the hormonal treatment previous to the FTAI was not influenced by BCS and AFC (P> 0.05). However, the weight gain between D0 and D46 had an effect on the pregnancy rate (P <0.05), and cyclicity tended to be significant (P= 0.07). The results for pregnancy rate, considering the main effects (Table 1).

Table 1. Pregnancy rate of calving Nelore cows subjected to FTAI, according to cyclicity, weight gain, antral follicle count and body condition score.

Effect	Pregnancy rate % (n/N)		P - value
Ciclicity	Cyclic	Anestrus	0,07
	57,5(19/33)	41,6(67/161)	
Weight gain	Lost (-73 Kg)	Maintenance (0 - 73 Kg)	0,05
	35,8(24/67)	49,5(55/111)	
AFC	Low (16,3 ± 5,0)	High (33,8 ± 7,5)	0,26
	45,8 (49/107)	42,5 (37/87)	
BCS	Low (≤2,75)	High (≥3,0)	0,45
	44,5 (49/110)	44,0 (37/84)	

a, b- Different letters represent statistical difference for pregnancy rate between groups (P <0.05).
Source: Authors.

4. Discussion

The present study revealed a direct influence of the weight gain on cows' fertility, because the females that gained up to 73 Kg had a conception rate 13.7% higher than those that had not gained in weight. Studies have observed that in postpartum cows, all weight loss or gain is reflected as a variation of the body score (Bó et al., 2018; Cutaia & Bó, 2004). Thus, the daily weight gain contributes to the pregnancy success in cows inseminated at a fixed time (Baruselli et al., 2017). Hence, beef heifers have presented a positive correlation between their weight and pregnancy rate 30 days post FTAI (Baruselli et al., 2017), and still in 15 month-old heifers the weight gain was associated to their reproductive performance (Handcock et al., 2020).

Cows subjected to a poor nutrition have their hypothalamus sensitive to a negative feedback of estradiol for up to 30 days after calving (Nishimura et al., 2018; Wiltbank, Gumen, & Sartori, 2002), in addition, the presence of the calf and a low BCS may reduce the LH release frequency, which prolongs the anestrous period (Myers et al., 1989; Canfield & Butler, 1990; Wiltbank et al., 2002; Nishimura et al., 2018). These factors clarify the positive influence of BCS on the CL presence verified in this study, and also corroborates the literature data which states that the body score is the main factor responsible for the return of ovarian cyclicity in postpartum females (Nishimura et al., 2018).

Cows living under tropical conditions have a high incidence of postpartum anestrus, a condition that increases the interval between parturition and conception, and consequently has a negative effect on reproductive performance (Baruselli et al., 2004; Meneghetti et al., 2009). This situation occurs, because in tropical countries, the last third of gestation coincides to the dry season, a period of low forage supply (Silva et al., 2017). Thus, beef cows raised under an extensive system, such as the females used in this experiment, have the postpartum anestrus duration affected by their nutrition and the season (Crowe, Diskin, & Williams, 2014; Diskin & Kenny, 2016).

The pastures can be limited and low quality in postpartum periods of Nelore cows, so animals exposed to these conditions go through nutritional challenges (Moraes et al., 2019). By this means, cows can have a negative energy balance, resulting in poor reproductive performance, probably due to the decrease in the IGF - I (Insulin-like Growth Factor – I) serum concentrations (Moraes et al., 2019; Wathes et al., 2007). Studies have pointed out that the higher dry matter intake and, consequent, weight gain are correlated to the IGF - I serum concentrations increasing (Cooper-Prado et al., 2018; Velazquez, Spicer, & Wathes, 2008). The IGF-I is related to the follicular activity and to the estrogen production, playing an important role in bovine reproduction (Velazquez et al., 2008), being this, a possible justification for the higher pregnancy rates in cows with greater weight gain.

The higher occurrence of cyclicity in cows with a low AFC was another finding of the study. In contrast, another experiment found a greater correlation between the presence of ovarian CL in taurine cows with a higher AFC (Martinez, Sanderson, Quirke, Lawrence, & Juengel, 2016). However, the associations between AFC and CL occurs due to the corpus luteum be the result of morphological and biochemical transformations happened to the follicles after its ovulation (Berisha, Schams, Rodler, & Pfaffl, 2016). At the beginning of the FTAI protocol, 82.9% of the cows did not present a CL, a fact that may indicate the absence of cyclicity. There is a reduction in the cows cyclicity during the postpartum period, and so it

was found that up to 67% of bovine females may not be cycling at the breeding season beginning, which may compromise the breading season and generate a negative impact on the subsequent on the reproductive efficiency (Ferreira et al., 2018).

In this study 41.6% of the females that did not shown a CL, became pregnant after the administration of the hormonal protocol. In this context, the FTAI protocols are very useful, and are frequently performed in acyclic cows during the postpartum period, a condition in which the cow has a low LH support and reduced follicular development (Meneghetti et al., 2009; Roche, Crowe, & Boland, 1992). Thus, the administration of Equine Chorionic Gonadotropin (eCG) is an alternative that can be used in hormonal protocols, as it has an activity similar to FSH and LH in ruminants, promoting follicular growth and oocyte maturation (Ferraz et al., 2019; Thedy et al., 2018). In the present study, the 350 IU eCG administration at the moment of the P4 device removal, may have positively influenced the cows' pregnancy rates. As previously reported, the injection of 400 IU eCG in the moment of the progesterone device remoting, improved the ovulation rate in beef cattle (Núñez-Olivera et al., 2014).

The results obtained did not verify the BCS impact on the pregnancy rates, in contrast, it was demonstrated a 90% effect of the BCS, at the FTAI moment, on the pregnancy rate in cows submitted to hormonal protocols, observing greater conception rates in cows with better body conditions (Cutaia & Bó, 2004). In other studies, cows that presented BCS> 2.75 (scale from 1 to 5) showed higher pregnancy rates than cows with BCS <2.5 (Nishimura et al., 2018), and also the BCS classification have effected on pregnancy rates in zebu cows submitted to FTAI (Moraes et al., 2019).

The AFC did not have a positive influence on the pregnancy rate of Nelore cows evaluated in this experiment. And so corroborates the literature in which *Bos indicus* presenting a higher AFC had lower pregnancy rates (Moraes et al., 2019). However, studies performed in taurine cows have shown that cows with low AFC had phenotypic characteristics associated to infertility (Ireland et al., 2011).

This report demonstrates that the weight gain, between insemination and the pregnancy diagnosis, influences on pregnancy rates obtained by FTAI. However, even the weight gain being related to the IGF-I concentrations, as previously described, the effective mechanism that the weight gain exercised on the success of pregnancy rates in cows subjected to FTAI has not yet been fully clarified (Oosthuizen et al., 2020), therefor further studies are needed.

5. Conclusion

The study reaffirmed the benefit of the hormonal protocol to promoting the pregnancy in females during the postpartum anestrus. Furthermore, within the parameters analyzed, only the weight gain between the FTAI protocol and the pregnancy diagnosis showed an influence on the pregnancy rates, and thus can be considered a relevant factor to increase the FTAI efficiency in beef cattle raised extensively.

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