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Postpartum hormone and energy profiles and their influence on the resumption of ovarian cyclicity in Curraleiro Pé-Duro cows

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ABSTRACT

The objective of this study was to evaluate hormone and energy profiles in the postpartum period and to correlate these profiles with the resumption of ovarian cyclicity, as well as characterizing the postpartum short estrous cycle of Curraleiro Pé-Duro cows. Twelve Curraleiro Pé-Duro cows were examined via rectal palpation and ultrasound at 10 days postpartum, and subsequently examined daily to evaluate the resumption of ovarian cyclicity as well as every five days in order to evaluate uterine involution. Upon analysis of the data, it was possible to observe the formation of two distinct groups, one of which was comprised of those animals which returned to cyclicity within 60 days postpartum and another comprised of those animals which returned to cyclicity more than 105 days postpartum. Therefore, animals were divided into two groups; precocious, designated Ov Group, and delayed, designated NOv Group, wherein the cut-off time for all tests was 60 days postpartum. Statistically significant differences ($P < 0.01$) between the groups occurred only regarding the day of 1st ovulation, which in the Ov Group averaged 51.4 ± 9.3 days and in the NOv Group averaged 138.3 ± 19.8 days postpartum. The other postpartum short estrous cycle variables assessed did not show statistically significant differences ($P > 0.05$) between the groups. NEFA, BHBA and thyroxine concentration levels did not differ ($P > 0.05$) between the groups in any of the statistical analyses. However, in the analysis comparing growth curves, triglycerides levels were higher for the Ov Group ($P = 0.04$) and cholesterol levels were higher for the NOv Group ($P = 0.02$). In this experiment, a small influence of a negative energy balance between the groups was observed, suggesting that these animals can present significant genetic variability due to natural selection, as evidenced by the formation of groups of animals with precocious and delayed reproductive characteristics.

1. Introduction

The postpartum period has very important effects on the metabolic and reproductive physiology of the animals. During this period, also known as the puerperium, cows undergo the anatomical and hormonal changes necessary for the restoration of the entire genital tract, in order to prepare for a new gestation [1–3]. Additionally, due to high energy demand following parturition, the cows have very significant nutritional requirements during this period.

The reproductive efficiency of cattle herds is directly linked to uterine involution and resumption of ovarian cyclicity in the postpartum period [1,2,4–6]. Concurrently, several factors may adversely impact the postpartum period, including negative energy balance (NEB), energy and lipid profiles, postpartum hormone levels, and metabolic diseases, among others [7,8]. In counterpoint to the search for reproductive efficiency, the majority of Curraleiro Pé-Duro cattle continue to be raised in a system characterized by low reproductive intensity and limited technical sophistication, in a manner almost unchanged for nearly 500 years since the time of the colonization of Brazil. Little is known regarding the phenotypical characteristics of this breed other than that they are small animals (both male and female) with low nutritional requirements,

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and are able to gain weight and body score condition while feeding in low quality pasture [9,10]. Additionally, relatively little is known regarding the reproductive characteristics of the breed, apart from some recent studies including Teixeira et al. [9] and Tortorella et al. [10] which described reproductive characteristics including easy and rapid labor, as well as characteristics associated with follicular growth and deviation of the first follicular wave.

Thus, the use of techniques such as the assessment of energy and hormone profiles can be very important for monitoring nutritional and metabolic patterns in postpartum cows [11]. Nutritional balance in domesticated animals is known to be largely dependent on physiological condition and reproductive stage [12]. Therefore, the evaluation of energy profiles, taking into account metabolites of energy and lipid mobilization, can be used as a tool for predicting metabolic disorders such as NEB, which are very important for the resumption of postpartum ovarian cyclicity. Additionally, energy profiles are widely known to be closely linked to metabolic hormones such as thyroxine (T4) and this connection directly impacts gonadotropins, hormones and follicular growth factors (in folliculogenesis) [13]. In light of the foregoing considerations, the objective of this study was to evaluate hormone and energy profiles in the postpartum period and to study the effects of these profiles on the resumption of ovarian cyclicity, as well as to characterize the postpartum short estrous cycle of Curraleiro Pé-Duro cows.

2. Material and methods

2.1. Experimental location and animals

This research was undertaken at the Sucupira Experimental Farm, which is owned by Embrapa Genetic Resources and Biotechnology Center, located to the southwest of the city of Brasília - DF (15°52′ to 15°56′S and 48°00′ to 48°02′W), with altitudes ranging from 1050 to 1250 m. The local climate is *Koppen Aw*, with dry winters and rainy summers. The farm has a total area of 1763 ha, including areas with local vegetation, pastureland, and cropland.

Fifteen Curraleiro Pé-Duro cows ranging from 3 to 8 years old with mean bodyweights of 329 ± 34 kg and body condition scores of 2.8 ± 0.3 (ranging 2.5 to 3.5 on a scale from 1 to 5) were selected via a process which included gynecological examination, transrectal palpation and ultrasonography before the beginning of the experiment in order to obtain confirmation of cyclicity and to ensure the absence of diseases or abnormalities in their reproductive tracts. The animals were allowed to graze freely (*Brachiaria brizantha*) with mineral supplements and water provided *ad libitum* throughout the experimental period. Unfortunately, the breed studied in this research is currently at risk of extinction, which is why only a limited number of these animals were available for the study.

The estrus cycle of the cows had been previously synchronized, and on D0 a progesterone implant (P4 - CIDR - Pfizer Animal Health, São Paulo-SP, Brazil) was inserted and 2 mg of estradiol benzoate (EB - Estrogin - Farmavet, São Paulo-SP, Brazil) was administered; on D8, 150 μg of D-cloprostenol (PGF_{2a} - Veteglan - Hertape Calier Animal Health, Juatuba, Minas Gerais, Brazil) was administered and the P4 implant was subsequently withdrawn; on D9, 1 mg of EB was administered and artificial insemination was conducted on D10 approximately 18 h after the application of the 1 mg dose of EB. Semen doses from three previously selected bulls were used. It is important to note that the fertility of these bulls had already been proven at the Conservation Center of the Embrapa Genetic Resources and Biotechnology Center.

Thirty days after artificial insemination, the cows were examined via rectal palpation and ultrasonography (Aloka scanner B with transrectal transducer of 7.5 MHz - Aloka CO. - Tokyo, Japan) for confirmation of pregnancy. Following this examination, those cows which had not impregnated were resynchronized and again inseminated with semen doses from the same bulls and again examined 30 days after artificial insemination. After this period, those cows which had still not impregnated were paired with bulls at the Sucupira Experimental Farm and, after 30 days, the examination was once again performed. In total, twelve cows were determined to have impregnated by the end of the breeding season (from February to April) and those were the cows used in this study.

2.2. Evaluation of the resumption of ovarian cyclicity and uterine involution

The twelve Curraleiro Pé-Duro cows were examined via transrectal palpation and ultrasonography ten days postpartum, and subsequently examined daily to evaluate the resumption of ovarian cyclicity as well as every five days in order to evaluate uterine involution. The involution process was considered complete when complete symmetry between gravid and nongravid uterine horns was observed, and the cows were considered cyclical when they first ovulated.

The data parameters evaluated in this part of the experiment included: Number of days required for complete uterine involution (macroscopic); Number of days until 1st ovulation; 1st ovulation follicle size; Number of days until visualization of corpus luteum; Maximum size of corpus luteum; Duration of the postpartum short estrus cycle; and 2nd ovulation follicle size. The cows were monitored daily until the onset of the second ovulation, following which the animals were no longer subjected to ultrasound examinations and no further blood samples were collected. A follicular cyst appeared in one cow approximately 90 days after parturition, and that cow was subsequently excluded from the experiment.

Upon analysis of the data, the formation of two distinct groups was clearly observed: One group consisting of those animals returning to cyclicity within 60 days after parturition, and the other consisting of those animals which only returned to cyclicity more than 105 days postpartum. Consequently, the group containing the precocious animals was identified as the Ov Group (for ovulation) and the group containing those animals which showed a delayed return to cyclicity was identified as the NOv Group (for non-ovulation), with the cut-off for the remaining analyses being 60 days postpartum.

2.3. Sampling

Following the pregnancy diagnosis, which occurred thirty days after artificial insemination or natural mating, the average duration of the gestation period for the cows was estimated to be between 270 and 290 days (±280 days), and the animals were monitored constantly until the day of parturition. Blood samples were collected from the cows using vacuum tubes which did not contain anticoagulant on the following days: the day of parturition, 5 days (D5), 15 days (D15), 30 days (D30), 45 days (D45), and 60 days (D60) postpartum. For the assessment of progesterone levels (P4), blood was collected daily after the first ovulation to evaluate the P4 level curve throughout the short estrous cycle. This curve was determined using blood collected on the day of the first ovulation (D0a); on the three days following the first ovulation (D1, D2 and D3); on the three days prior to the second ovulation (D-1, D-2 and D-3); and, finally, on the day of the second ovulation (D0b), for correction of the P4 level curve throughout the short estrous cycle, due to the animals showing estrus cycles of different lengths.

After collection, the blood samples were centrifuged at 3000 G

for 15 min. The serum was separated and stored in microtubes at a temperature of −20 °C until biochemical and hormonal assays could be performed, using the methods reviewed by Russell and Rousel [14].

2.4. Biochemical and hormonal assays

Hormonal assays were conducted using commercial radioimmunoassay (RIA) kits which evaluated progesterone (P4-Coat-A-Count Progesterone RIA Kit - Siemens Healthcare Diagnostics - California - USA) and total thyroxine (T4-Coat-A-Count Total T4 Thyroxine RIA Kit - Siemens Healthcare Diagnostics - California - USA). Biochemical assays were conducted using commercial colorimetric and kinetic enzyme assay kits, and the results were examined in duplicate samples, using a semi-automated spectrophotometer (Analisador Bioquímico Bioplus Bio200S — Bioplus Produtos para Laboratórios LTDA - Brazil) featuring 7 color filters and a detection range from 340 nm to 670 nm.

The blood metabolites examined were non-esterified fatty acids (NEFA - Non-esterified fatty acids Assay - Randox Laboratories Ltd - County Antrim - United Kingdom), beta-hydroxybutyrate (BHBA - Ranbut Assay - Randox Laboratories Ltd - County Antrim - United Kingdom) Triglycerides (Trig - Bioclin Triglicérides Monoreagente - Quibasa Química Básica Ltda — Belo Horizonte — Minas Gerais - Brazil) and Cholesterol (Chol - Bioclin Colesterol Monoreagente - Quibasa Química Básica Ltda — Belo Horizonte — Minas Gerais - Brazil), in accordance with the respective manufacturer's recommendations.

2.5. Statistical analysis

The data sets obtained from the two groups (Ov and NOv) were evaluated for normality using the Shapiro-Wilk test, and all the data sets were considered normal.

The data collected for uterine involution and resumption of ovarian cyclicity was analyzed using ANOVA and mean comparison (Tukey) tests with a confidence interval of 95%. The data regarding P4 level throughout the short estrus cycle was evaluated using the growth curve comparison method (CompareGrowthCurves, statmod). Statistical analyses were performed using the R Core Team, 2013 software.

The results of the energy profile (NEFA, BHBA, Trig and Chol) and thyroxine (T4) assays throughout the postpartum period were evaluated using the growth curve comparison method for pattern comparison between the two groups of concentration curves as a whole throughout the postpartum period, and also using the Survival Analysis method to evaluate the effect of energy metabolites and T4, and the interaction thereof, on the return to ovarian cyclicity or not.

To further evaluate the effects of the metabolites and T4 on the experimental groups, an analysis of variance (ANOVA) was performed, taking into consideration the effect of the days, metabolites, and experimental groups to determine the influence of the metabolite on group*day interaction.

3. Results

The results presented in Table 1 show the different variables evaluated in order to characterize the postpartum period (uterine involution, resumption of ovarian cyclicity and evaluation of short estrus cycle), and are divided into the results for Groups Ov and NOv. As described previously, the animals were divided into groups due to their significant dissimilarities regarding interval until the resumption of ovarian cyclicity, where one group returned to cyclicity within 60 days postpartum (Ov Group) and the other group returned to cyclicity only after 105 days postpartum (NOv Group). In Table 1, a statistically significant difference ($P < 0.01$) between the groups can be observed only in regards to the number of days until the occurrence of the 1st ovulation, which in the Ov Group averaged 51.4 ± 9.3 days and in the NOv Group averaged 138.3 ± 19.8 days. Thus, the significant differences between the cows in the interval until occurrence of the 1st ovulation constituted the reason for dividing them into two groups. The other variables did not significantly differ ($P > 0.05$) between groups.

Despite the significant differences between the Ov Group and the NOv Group regarding the number of days until the 1st day of ovulation, the progesterone level curve (P4) of Curraleiro Pé-Duro cows in the postpartum short estrus cycle was similar ($P = 0.62$) between the groups (Fig. 1). It was observed that, in the postpartum short estrous cycle, the maximum production of P4 occurred at D3, and functional luteolysis of the corpus luteum (CL) occurred two days before the second ovulation at D-2 due to the sharp drop-off in P4 levels.

In assessing the comparison of growth curves (CompareGrowthCurves, statmod, R Core Team, 2013) for NEFA and BHBA, there were no statistically significant differences ($P > 0.05$) between groups, even for those curves showing major differences in the concentrations of these metabolites. Survival analyses of the metabolites assessing the influence of NEFA and BHBA on the resumption of ovarian cyclicity (Ov Group versus NOv Group) also did not show statistically significant differences ($P > 0.05$) between groups. There were also no statistically significant differences ($P > 0.05$) for group*day*metabolite interactions for NEFA, BHBA or between groups (Figs. 2 and 3).

The assessment of the survival analysis for metabolites involving the influence of triglycerides and cholesterol on the resumption of ovarian cyclicity (Ov Group versus NOv Group) showed no statistically significant differences ($P > 0.05$) between groups. When combining, in this analysis, the influence of NEFA and BHBA, there were no statistically significant differences ($P > 0.05$) regarding the resumption of ovarian cyclicity between groups. In the analysis of variance there was no statistically significant difference ($P > 0.05$) in the interaction of group*-day*metabolite for triglycerides and cholesterol between groups. The evaluation of average triglyceride concentration by comparing growth curves showed statistically significant differences ($P = 0.04$) between the groups, in which the Ov Group showed higher concentrations than the NOv Group (Fig. 4). In the comparison of growth curves for cholesterol concentration, the NOv Group showed a higher concentration ($P = 0.02$) than the Ov Group (Fig. 5).

Although the T4 concentration curves (Fig. 6) appear to be very different, no statistically significant difference ($P > 0.05$) was found in the comparison of the growth curves, nor was any statistically significant difference found in any of the thyroxine assays performed for in the Ov and NOv groups of Curraleiro Pé-Duro cows. By combining the T4 assay with a survival analysis including energy metabolites, no effect of T4 on the resumption of ovarian cyclicity was observed between the groups (combining T4 with NEFA, BHBA, triglycerides and cholesterol).

4. Discussion

In the Curraleiro Pé-Duro cows evaluated in this experiment, macroscopic uterine involution, as observed using ultrasound, occurred at 45 days postpartum, on average, which is consistent with other published studies on this subject [5,6] (Table 1). Furthermore, as uterine involution is a process that comprises tissue loss, tissue repair and peristaltic muscular contractions [7], and as none of the cows showed a prolonged period of uterine

Table 1
Mean ± SD of the variables for the assessment of uterine involution, resumption of ovarian cyclicity and evaluation of short estrus cycle of Curraleiro Pé-Duro cows divided into two groups: Group Ov (returned to cyclicity within 60 days postpartum) and Group NOv (returned to cyclicity more than 105 days postpartum).

	Group Ov	Group Nov
Days to complete uterine involution	44,2 ± 3,0	45 ± 5,2
Days until 1st ovulation	51,4 ± 9,3[b]	138,3 ± 19,8[a]
1st ovulation follicle size (mm)	16,1 ± 2,9	16,9 ± 2,0
Days until visualization of corpus luteum	2,4 ± 0,5	2,8 ± 0,9
Maximum size of the corpus luteum (mm)	21 ± 4,6	17,3 ± 2,7
Length of the postpartum short estrus cycle (days)	7,4 ± 1,1	8,2 ± 3,4
2nd ovulation follicle size (mm)	17,4 ± 2,0	15,4 ± 2,7

Different letters on the same line represent statistical differences ($P < 0.01$) in the Tukey test.

involution, it may be hypothesized that all of the cows underwent this process in an efficient manner.

The cows of the Ov Group produced their 1st postpartum ovulation within 60 days after parturition, which was a far better result than that seen in the cows of the NOv Group ($P < 0.01$), which only produced their 1st postpartum ovulation 105 days after parturition. Pelssier [15], Zain et al. [16], and Stevenson et al. [17] reported that, in order to achieve maximum reproductive efficiency, cows must return to cyclicity within no more than 90 days postpartum, given the fact that their pregnancy is calculated to have an average duration of 280 days. Thus, if the cows return to cyclicity within a maximum of 90 days, it is possible that each cow can produce one calf per year. As approximately 50% (5 of 11) of the Curraleiro Pé-Duro cows in this study produced their 1st postpartum ovulation within 60 days postpartum, it can be concluded that these animals have great potential to be selected for their reproductive efficiency characteristics, given the fact that they came from a conservation herd that has not undergone any selective breeding programs. The other parameters evaluated in Table 1 serve as the characterization parameters for the postpartum short estrous cycle in Curraleiro Pé-Duro cows, since there is no information in previously published literature regarding this physiological data. Some of these parameters, such as follicle sizes and size of the corpus luteum, were recently evaluated by Tortorella [9,18], but these studies were based on data obtained from cows or heifers which were not in the postpartum period.

Fig. 1 shows the P4 average concentration curves of the Ov and NOv groups of Curraleiro Pé-Duro cows throughout the postpartum short estrous cycle. P4 concentration curves were similar between groups ($P > 0.05$), which was expected due to the similarities in both the sizes of the CLs ($P > 0.05$, 21 ± 4.6 mm and 17.3 ± 2.7 mm for the Ov and NOv groups, respectively) and the duration of the postpartum short estrous cycle ($P > 0.05$, 7.4 ± 1.1 days and 8.2 ± 3.4 days for the Ov and NOv groups, respectively). Taylor et al. [19] found a high correlation between low P4 concentrations, long periods of anestrus postpartum and delays in uterine involution [20], and these animals showed P4 levels which were lower than the values normally observed in Curraleiro Pé-Duro cows. Moreover, no influence of P4 concentrations and the duration of postpartum short estrous cycles on the resumption of ovarian cyclicity was observed in this study.

Several studies show NEFA and BHBA to be metabolites of lipid mobilization in the organisms of ruminants [11,21–25]. Several other studies have connected NEFA and BHBA with a negative energy balance (NEB) and the influence thereof on the postpartum [26–33]. As observed in this experiment, NEFA and BHBA did not

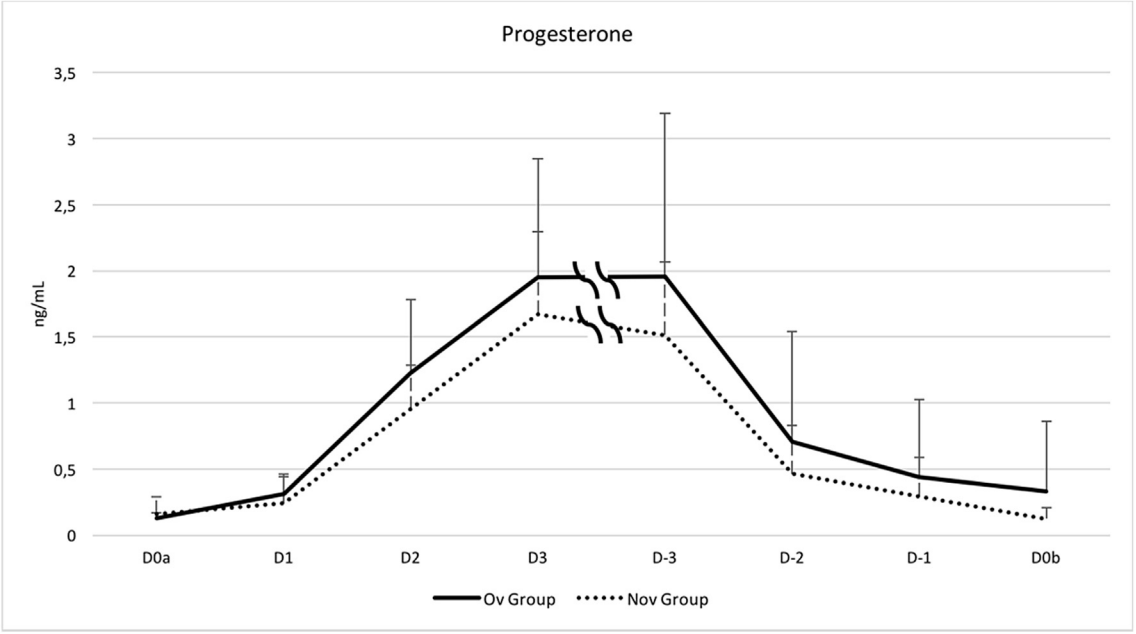

Fig. 1. Average progesterone (P4 ng/ml) concentration curves of Curraleiro Pé-Duro cows during the postpartum short estrous cycle, measured on the day of first ovulation (D0a) on the three days following the first ovulation (D1, D2 and D3); on the three days prior to the second ovulation (D-1, D-2 and D-3); and, finally, on the day of the second ovulation (D0b). Divided into two groups: Group Ov (returned to cyclicity within 60 days postpartum) and Group NOv (returned to cyclicity more than 105 days postpartum).

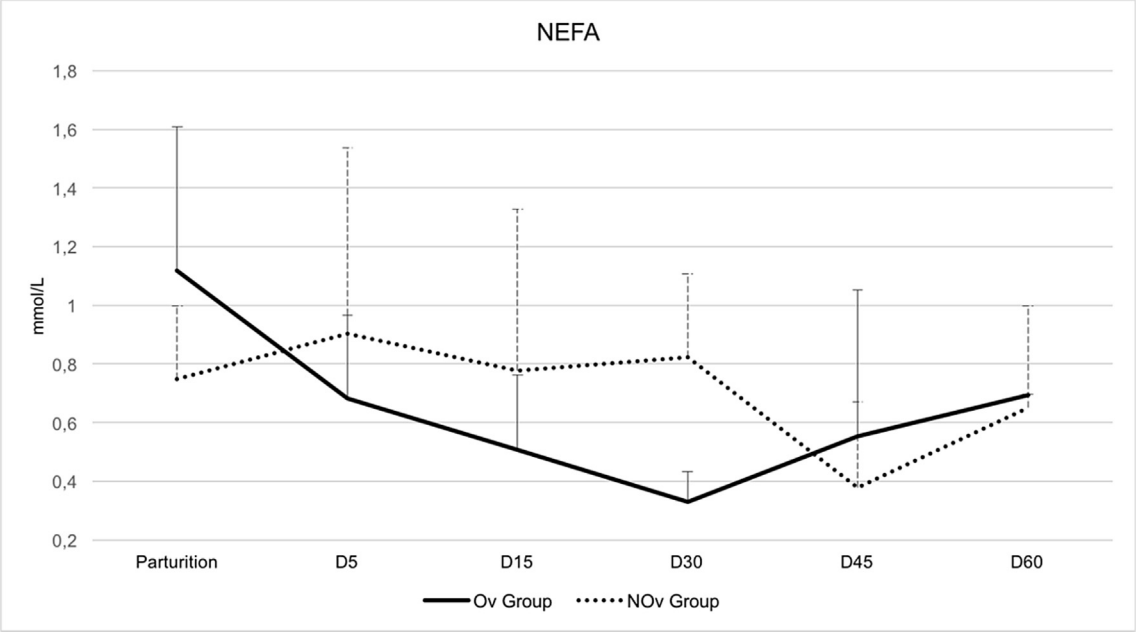

Fig. 2. Average non-esterified fatty acids (NEFA in mmol/L) concentration curves in Curraleiro Pé-Duro cows from the day of parturition until 60 days postpartum (D60), divided into two groups: Group Ov (returned to cyclicity within 60 days postpartum) and Group NOv (returned to cyclicity more than 105 days postpartum).

directly affect the resumption of ovarian cyclicity and there were no statistically significant differences ($P > 0.05$) between the groups or between days or days*group interaction. According to Vap and Weisser [34], NEFA concentrations above 0.6 mEq/L (0.6 mmol/L) and BHBA concentrations above 1.4 mmol/L are indicators of a negative energy balance and consequent risk of metabolic diseases in the postpartum period. In the Curraleiro Pé-Duro cows studied in this experiment, a very prominent decrease ($P > 0.05$) could be observed in NEFA and BHBA concentrations, primarily in the Ov Group, which varied thus: from 1.11 ± 0,48 mmol/L to 0.51 ± 0.25 mmol/L NEFA concentration from the day of parturition to D15 respectively; and from 1.5 ± 0.85 mmol/L to 0.67 ± 0.13 mmol/L BHBA concentration from the day of parturition to D15 respectively (Figs. 2 and 3). For the NOv Group, NEFA concentrations remained above the risk levels cited by Vap and Weisser [34] until D45 when they start to decline, while BHBA concentrations were low initially, then started to increase around D15, and then declined at D45. Therefore, as can be seen in the results, it is probable that Curraleiro Pé-Duro cows do not have a uniformly negative energy balance in the postpartum, however

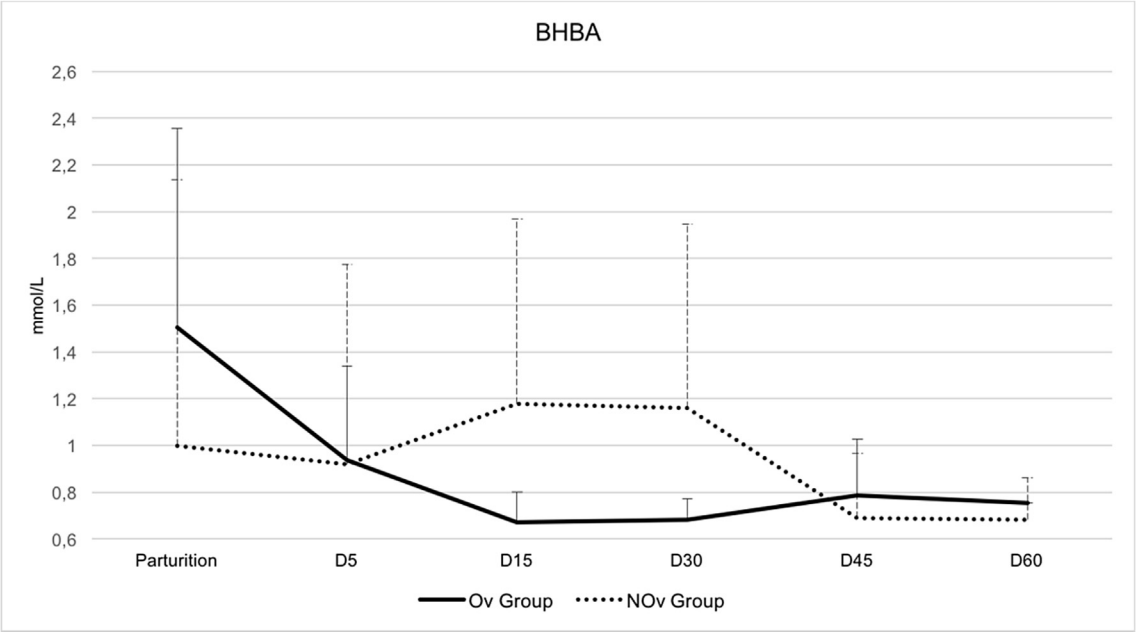

Fig. 3. Average beta-hidroxybutirate (BHBA in mmol/L) concentration curves in Curraleiro Pé-Duro cows from the day of parturition until 60 days postpartum (D60), divided into two groups: Group Ov (returned to cyclicity within 60 days postpartum) and Group NOv (returned to cyclicity more than 105 days postpartum).

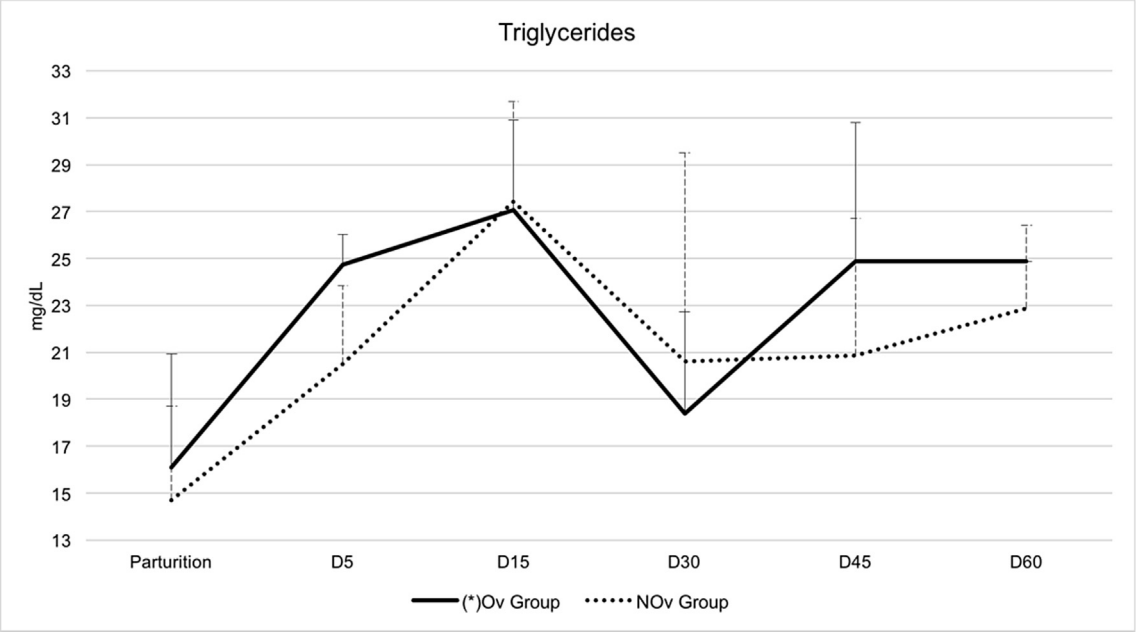

Fig. 4. Average triglycerides (mg/dL) concentration curves in Curraleiro Pé-Duro cows from the day of parturition until 60 days postpartum (D60), divided into two groups: Group Ov (returned to cyclicity within 60 days postpartum) and Group NOv (returned to cyclicity more than 105 days postpartum). (* in front of the name of the group indicates statistically significant difference ($P < 0.05$) in the evaluation of growth curves).

NEFA and BHBA concentrations were higher in the Ov Group in comparison to the NOv Group initially, whereas later, on D15 and D30, these concentrations were lower in the Ov Group than in the Nov Group, where the concentrations are increasing. It is possible that the clearance rate of NEFA and BHBA was higher for the Ov group, which would have caused this group become non-ketogenic before the NOv Group, resulting in a positive impact on folliculogenesis [13] thereby allowing the first ovulation to occur earlier, even if not by a statistically significant difference ($P > 0,05$). Another possible explanation for the difference observed in the resumption of ovarian cyclicity between the Ov and NOv groups may be the lack of genetic improvement in conservation herds, due to the natural selection processes which the animals have undergone throughout the development of this breed [35–38], as occurs in the herd at the Conservation Center for Curraleiro Pé-Duro cattle [39], which consequently results in the animals showing significant genetic variability and which can produce both animals with high breeding value characteristics such as reduced postpartum anestrus, as well as animals which have prolonged postpartum anestrus.

In contrast to the statistical similarity ($P > 0.05$) of NEFA and

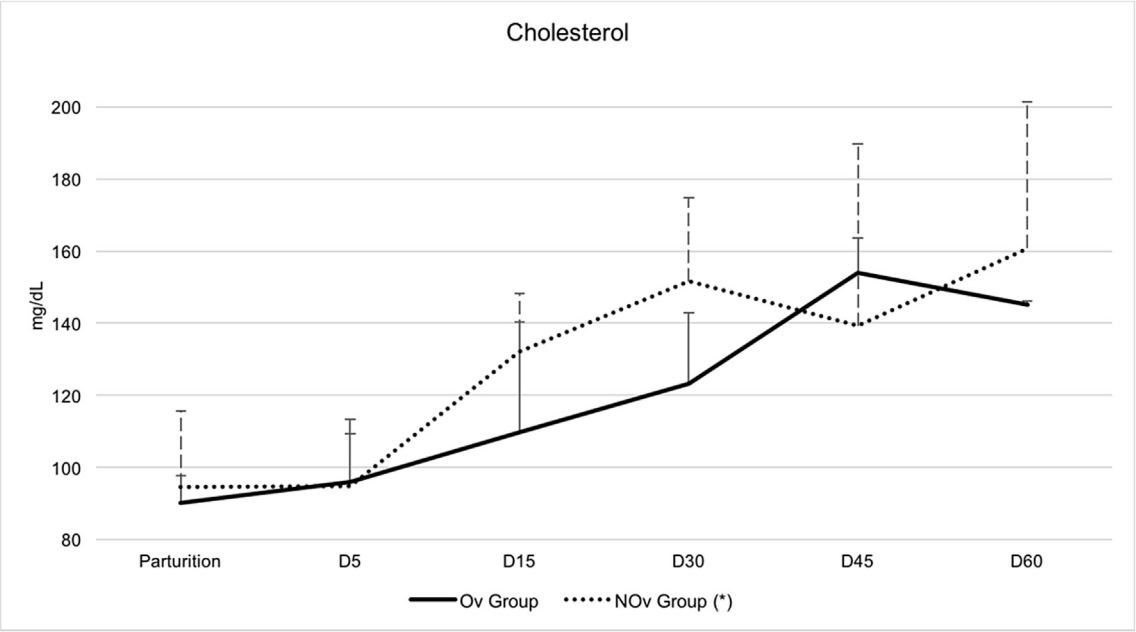

Fig. 5. Average cholesterol (mg/dL) concentration curves in Curraleiro Pé-Duro cows from the day of parturition until 60 days postpartum (D60), divided into two groups: Group Ov (returned to cyclicity within 60 days postpartum) and Group NOv (returned to cyclicity more than 105 days postpartum). (* In front of the name of the group means statistical difference ($P < 0.05$) in the evaluation of growth curves).

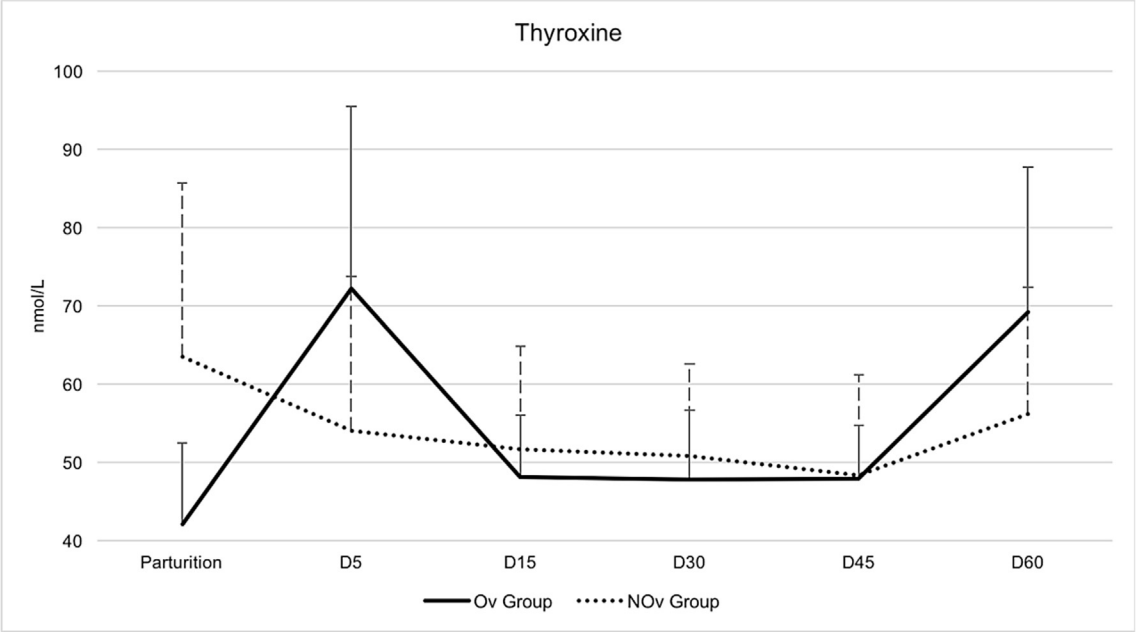

Fig. 6. Average thyroxine (T4 in nmol/L) concentration curves in Curraleiro Pé-Duro cows from the day of parturition until 60 days postpartum (D60), divided into two groups: Group Ov (returned to cyclicity within 60 days postpartum) and Group NOv (returned to cyclicity more than 105 days postpartum).

BHBA effects on 1st ovulation between the Ov and NOv groups, triglycerides showed a higher average concentration (Compare Growth Curves test) in the Ov Group than in the NOv Group. Some studies suggest that triglycerides do not directly affect follicular fluid formation and the resolution of postpartum cyclicity [40,41], but rather that low-density lipoproteins have a greater effect on follicular steroidogenesis. In contrast to the abovementioned research, the Curraleiro Pé-Duro cows that returned to cyclicity with the 1st postpartum ovulation occurring earlier (Ov Group) presented higher average triglyceride concentrations than the NOv group where the 1st postpartum ovulation occurred later. It is possible that a greater accumulation of triglycerides by the cows of the Ov Group resulted in a decrease of NEB, at a certain point, which can be seen in the NEFA and BHBA curves, even if these did not show statistically significant differences ($P > 0.05$), such as those observed by Karimian et al. [42], when working with different lipid diets. However, the fact remains that the decrease in NEFA and BHBA concentrations was more prominent in the Ov Group than in the NOv Group ($P > 0.05$). Even though the triglyceride concentrations were higher in the Ov Group, no statistically significant difference in NEFA and BHBA concentrations was ascertained between the groups. These results may be attributed to one or both of the following causes: a) the small number of animals, six per group, preventing the curves from showing statistically significant differences; b) preexisting differences between the animals due to high genetic variability, as they are the result of natural selection processes, and the herd from which they originated has not undergone any selective breeding programs.

In cows, the occurrence of ovulation during the postpartum period is influenced by the capacity of the hypothalamic-pituitary axis to secrete gonadotropins [43]. Furthermore, the rate of metabolism of lipids, particularly cholesterol, can influence reproductive performance in cows due to the latter being an ovarian steroid precursor [44,45]. Therefore, it is to be expected that those cows which have higher cholesterol concentrations in the postpartum period will produce greater quantities of ovarian hormone precursors, and thus produce larger amounts of ovarian hormones, particularly 17b-estradiol, and consequently resume postpartum ovarian cyclicity more rapidly. Guédon et al. [41] demonstrated a clear correlation in Limousine cows between a higher concentration of cholesterol and an earlier resumption of ovarian cyclicity. On the other hand, Meikle et al. [32] working with Holstein cows at pasture, did not ascertain a direct correlation between high serum cholesterol and precocity in the resumption of ovarian cyclicity. In contrast to these studies, a higher concentration of cholesterol was found in the Curraleiro Pé-Duro cows of the NOv Group ($P < 0.05$) when compared to those in the Ov Group. Day et al. [46] reported that high concentrations of estradiol in postpartum cows could delay the resumption of ovarian cyclicity due to the negative feedback cycle that occurs in the pulsatile secretion of luteinizing hormone (LH), and that prior exposure to P4 is required, as occurs in the postpartum short estrous cycle, for proper maintenance of LH pulsatility and consequently the proper resumption of ovarian cyclicity. As previously outlined, cholesterol is a precursor of steroid hormones, particularly estradiol. Thus, it is probable that the high cholesterol concentrations in the NOv Group may have influenced the hypothalamic-pituitary axis and LH pulsatility due to the high conversion rate of cholesterol molecules into 17b-estradiol.

T4 has a direct effect on lipolysis and on the increase in basal metabolism due to increased oxygen consumption, via oxidative phosphorylation, as well as the use of ATP, since it increases the activity of the Na+/K + -ATPase pumps which are present in almost all cells [47]. Therefore, the similarity in T4 concentration ($P > 0.05$) between the Ov and NOv groups may partially explain the similarity between the groups in both NEFA and BHBA concentrations. However, T4 concentrations appeared to have no effect on triglyceride or cholesterol concentrations. Huszenicza et al. [48] reported that low T4 concentrations in postpartum cows may delay the resumption of ovarian cyclicity. However, thyroxine concentrations for both groups in this experiment were within the normal concentration range for postpartum cows, as was also reported by Doornenbal et al. [49]; Hemingway et al. [50]; and Kafi et al. [51]. This corroborates the suggestion that T4 did not influence the resumption of ovarian cyclicity in Curraleiro Pé-Duro cows.

5. Conclusion

In this experiment, there was a significant difference between the group of cows that resumed postpartum ovarian cyclicity early and those in the group where this occurred later. A small difference in negative energy balance was observed between the groups, suggesting that these animals may have a high level of genetic variability due to the natural selection processes which they have undergone, which is substantiated by the formation of groups of animals with precocious and delayed reproductive characteristics.

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