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The effect of parity on metabolic profile and resumption of ovarian cyclicity in dairy cows

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ABSTRACT

The aims of the present study were to investigate the effect of parity on the resumption of ovarian cyclicity and to characterize metabolic factors affecting ovarian cyclicity. The study was conducted on a total of 24 Holstein-Friesian dairy cows. The cows were divided into 2 groups comprising 12 animals each, according to parity as primiparous or multiparous. The serum samples were analysed for the indicators of metabolic status: non-esterfied fatty acids (NEFA), beta-hydroxybutyrate (BHB), glucose, triglycerides, total cholesterol, and high density lipoprotein (HDL)-cholesterol. The resumption of ovarian cyclicity was determined by progesterone concentrations and gynaecological examination. The concentrations of HDL-C and NEFA were either significantly higher or lower, respectively, in relation to the resumption of ovarian cyclicity. These findings suggested that overcoming the negative energy balance (NEB) is the most important prerequisite for determining the resumption of ovarian cyclicity. This study also revealed a significantly longer period until the first service in primiparous cows as compared to multiparous cows. In conclusion, the primiparous cows were more susceptible to metabolic stress during the transition period, and their metabolic and endocrine profiles were more unbalanced compared to the multiparous cows.

Key words: negative energy balance, parity, ovarian cyclicity, dairy cows

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Introduction

The resumption of bovine ovarian cyclicity after parturition is an important physiological process for cattle breeding, and attaining pregnancy of dairy cows in the postpartum period (SAMARDŽIJA et al., 2008; KOČILA et al., 2013). In order to achieve their full reproductive potential, cows should conceive at least 3 months after the previous parturition. This requires early resumption of ovarian cyclicity and onset of oestrus postpartum (OPSOMER et al., 1998; KONIGSSON et al., 2008; GAUTAM et al., 2010). Peripartum diseases, such as afterbirth retention of foetal membranes, hypocalcaemia, endometritis, mastitis, ketosis and others, have a major impact on days open (PTASZYŃSKA, 2009; ĐURIČIĆ et al., 2011 and 2012; TURK et al., 2012; ĐURIČIĆ et al., 2014 and 2015).

High-yielding dairy cows commonly have a negative energy balance (NEB) in early lactation, as energy demands exceed their feed consumption capacity. Cows with reduced feed intake postpartum have extreme NEB status, which induces utilization of their own fat and protein reserves. This leads to increased non-esterified fatty acid (NEFA) and ketone concentrations in the blood of such cows (LACETERA et al., 2005). Several hormones and metabolic parameters, such as leptin (LIEFERS et al., 2003), insulin like growth factor -I (IGF-I) (HUSZENICZA et al., 2001) and NEFA (DUFFIELD et al., 2005) influence reproduction efficiency in dairy cows. The NEB status that occurs in dairy cows during the transition period has a negative effect on the secretion of LH from the anterior lobus of the pituitary gland and, thus, causes a certain delay in re-establishment of ovulation (ROSSI et al., 2008).

The first postpartum ovulation occurs 10-14 days after the NEB reaches its maximum, or its nadir (BUTLER, 2003). McCARTHY et al. (2005) reported that cows with high concentrations of NEFA before calving had a higher risk of developing anoestrus in the period after calving. Insulin and glucose have also been proven to play a role in the relationship between energy balance and postpartum reproductive efficiency in dairy cows. Insulin increases the ovarian response to the impact with gonadotropins and has a positive effect on the selection and better growth of follicles. Likewise, concentrations of insulin and IGF-I in plasma influence the production of adequate amounts of oestradiol, essentially required for ovulation (FORMIGONI and TREVISI, 2003). When the concentrations of IGF-I and insulin are low, the follicles do not synthesize sufficient concentrations of 17β-estradiol, or have insufficient growth. Consequently, pulsatory LH secretion is insufficient (SAMARDŽIJA et al., 2006; DOBRANIĆ et al., 2008). The concentration of progesterone in the blood of non-pregnant cows provides for secretory activity of the luteal tissue, because there are no other sources of progesterone at that time (OPSOMER et al., 1999). Therefore, the measurement of progesterone in the period after calving is a good indicator of ovarian activity resumption.

The aims of the present study were to investigate the effect of parity on the resumption of ovarian cyclicity, and to characterize metabolic factors that affect ovarian cylicity.

Materials and methods

Animals. This study was conducted on 24 Holstein-Friesian cows, aged between 2 and 7 years. The cows were kept on a commercial dairy farm located in the Koprivnica-Križevci County in North-western Croatia, near Đurđevac (coordinates: 45°59' N, 17°03' E). The cows were fed a ration composed of haylage, corn silage, hay and a complete feed mixture for dairy cows with 19% of crude protein. They were assigned into two groups according to parity (primiparous or multiparous). The first group (n = 12) comprised cows with two to five parturitions (multiparous cows) and the second group (n = 12) comprised cows without previous parturitions (primiparous cows).

Blood sampling. Blood samples were always taken after morning milking by the "Vacutainer" method from the tail vein (v. coccygea) into tubes without anticoagulant, but with clot activator. After storing at room temperature for 1 hour, blood samples were centrifuged at 1500×g for 15 minutes. Separated serum samples were collected and stored at -70 °C until used for analyses. For determination of energetic/metabolic status, the samples were taken 9 times, as follows: on Days 30, 10 and 2 before parturition, and on the Day of calving and on Days 5, 12, 19, 26 and 60 after parturition. The resumption of ovarian cyclicity was determined by progesterone concentrations on Days 5, 12, 19, 26 and 60 after parturition.

Clinical reproductive examination of cows. The cows were also subjected to vaginoscopy, rectal palpation and ultrasound examination (real time B-mode linear array scanner with a 7.5 MHz transducer, Sonovet 2000) of their reproductive organs per rectum, at the time of blood sampling. Rectal palpation of genitalia was conducted to monitor uterine involution and ovarian activity. The reproductive parameters measured were days to first ovulation, and intervals from parturition to first service.

Metabolic profile. The concentrations of glucose, triglycerides (TG), total cholesterol and high density lipoprotein cholesterol (HDL-C) were determined by standard commercial packages of reagents (Beckman Coulter Biomedical Ltd., Ireland) using the biochemical analyser Beckman Coulter AU 680. Beta-hydroxybutyrate (BHB) concentrations in the serum of cows was determined using the biochemical analyser SABA 18 (AMS, Rome, Italy) using reagents from Randox (Randox, Ireland). The concentration of D-3-hidroxybutirate was determined by the kinetic method with 3-hidroxybutitrate dehydrogenase, and the concentration of BHB was expressed in mmol/L. The non-esterified fatty acid (NEFA) concentration in the serum of cows was determined using the biochemical analyser SABA 18 (AMS, Rome, Italy) using reagents from Randox (Randox, Ireland). The reaction was performed in the presence of acyl-CoA-synthetase, adenosine triphosphate and CoA, free fatty acids in the serum which produced esters of acyl-CoA. In the second reaction, the activity of acyl-CoA oxidase produced esters which oxidate and produce H_2O_2. The concentration of NEFA was expressed in mmol/L.

Progesterone determination. The concentration of progesterone was determined using an Immulite 2000 analyser (Siemens Health care Sector AG, Germany). The principle of measurement was based on the competition binding of labelled and unlabelled antigens in a defined and limited number of binding sites. The enzyme alkaline phosphatase was used for labelling the antigens, while the unlabelled antigens were measured in the serum samples. The concentration of progesterone (P_4) was expressed in ng/mL.

Statistical analyses. Statistical analyses of data were performed using SAS 9.3. Software (2002-2010 by SAS Institute Inc., Cary, NC, USA.). The mixed model (PROC MIXED) with the repeated measure statement was used to analyse metabolic parameters. The statistical model included the fixed effects of group and time, and their interactions. The cow was the subject variable on which repeated measurements were taken. Multiple comparison tests of least-squares means were performed using the Tukey-Kramer correction. Results in are shown graphs and tables as least squared means with a 95% confidence interval. All variables were transformed with logarithms in base 10 except progesterone P_4 which was transformed using exponential transformation -0.5 (1/square root - sqrt) in order to obtain normal distribution and homoscedasticity, and after analysis the data were back transformed. The general linear model (GLM PROC) was used to analyse the variables between cows with cyclic ovarian activity and cows without cyclical ovarian activity, and in analysis of the variable of the period from the birth to the first artificial insemination. Cows with blood serum progesterone concentrations >1 ng/mL were considered to have luteal activity, and formed the cyclic group. Cows with blood serum progesterone concentrations <1 ng/mL were considered to be without luteal activity, and formed the noncyclic group.

Results

NEFA, BHB, TG, cholesterol, HDL-C and glucose concentrations. No significant differences were established in the NEFA concentrations between the multiparous and the primiparous cows. No significant differences were established in the BHB concentrations between either the groups of cows or between the days of sampling, although the primiparous cows had on average higher levels of BHB by days in comparison to multiparous cows. From analysis of cholesterol, HDL-C, and TG concentrations, no significant differences were found between the multiparous and the primiparous cows. No significant differences were established in the glucose concentrations between the multiparous and the primiparous cows (Table 1).

Table 1. Least squared means ± 95% confidence interval for metabolic parameters in the serum of primiparous and multiparous cows during the days of the experiment

Parameters	Transition period Least squared means (95% confidence interval) Days of experiment								
Group	-30	-10	-2	0	5	12	19	26	60
NEFA									
Multiparous	0.46^{a} (0.31-0.67)	0.55 (0.38-0.80)	0.32 (0.13-0.75)	1.00 (0.21-1.52)	0.89 (0.54-1.47)	0.94 (0.57-1.55)	1.24^{b} (0.82-1.87)	0.52 (0.35-0.76)	0.46 (0.31-0.70)
Primiparous	0.32^{a} (0.18-0.56)	0.36^{ac} (0.20-0.63)	0.70 (0.50-1.04)	1.20^{b} (0.85-1.71)	1.16^{bd} (0.77-1.74)	1.03^{bcd} (0.63-1.67)	0.77 (0.38-1.55)	0.57 (0.40-0.83)	0.53^{ad} (0.37-0.76)
BHB									
Multiparous	1.61 (1.20-2.15)	1.63 (1.23-2.17)	1.89 (1.42-2.21)	2.15 (1.64-2.81)	1.94 (1.31-2.89)	2.63 (1.70-4.07)	3.04 (2.08-4.44)	1.79 (1.21-2.63)	1.76 (1.13-2.72)
Primiparous	2.12 (1.68-2.67)	2.16 (1.74-2.68)	2.33 (1.86-2.91)	2.42 (1.90-3.07)	2.92 (2.21-3.86)	3.38 (2.37-4.83)	3.01 (2.01-4.50)	2.15 (1.53-3.01)	2.15 (1.51-3.08)
TG									
Multiparous	0.20 (0.16-0.25)	0.19 (0.15-0.22)	0.23^{a} (0.18-0.30)	0.17 (0.14-0.22)	0.15 (0.13-0.18)	0.15 (0.12-0.18)	0.14 (0.11-0.17)	0.13^{b} (0.11-0.15)	0.14 (0.12-0.16)
Primiparous	0.22^{a} (0.18-0.27)	0.20^{ac} (0.15-0.27)	0.13^{ad} (0.10-0.18)	0.14 (0.11-0.17)	0.12 (0.09-0.16)	0.12^{bcd} (0.09-0.15)	0.12^{bd} (0.09-0.14)	0.12^{bd} (0.09-0.14)	0.12^{b} (0.09-0.14)
Cholesterol									
Multiparous	2.68^{a} (2.32-3.09)	2.29^{a} (1.82-2.86)	2.56^{a} (2.20-2.97)	2.18^{a} (1.81-2.61)	2.41^{a} (1.81-3.20)	2.58^{ac} (1.98-3.34)	2.79 (2.10-3.69)	4.07^{bc} (3.36-4.92)	4.36^{b} (3.60-5.29)
Primiparous	2.72^{ac} (2.35-3.15)	2.74^{ac} (2.29-3.28)	2.16^{ac} (1.83-2.56)	1.78^{c} (1.42-2.22)	2.55 (1.92-3.39)	3.15^{ab} (2.52-3.95)	4.20^{ab} (2.52-3.95)	4.20^{b} (3.35-5.10)	4.25^{b} (3.49-5.18)
HDL-C									
Multiparous	1.87^{ac} (1.63-2.13)	1.61^{ac} (1.35-2.89)	1.64^{ac} (1.46-1.83)	1.52^{ac} (1.27-1.78)	1.72 ^{c} (1.31-2.18)	1.81^{c} (1.41-2.27)	1.94 (1.48-2.47)	2.70^{bc} (2.27-3.17)	2.85^{b} (2.42-3.31)
Primiparous	1.80^{ac} (1.55-2.07)	1.67^{ac} (1.42-1.93)	1.51^{c} (1.34-1.70)	1.31^{c} (1.06-1.57)	1.85 (1.42-2.34)	2.08. (1.65-2.56)	2.36^{ab} (1.87-2.91)	2.72^{b} (2.27-3.22)	2.72^{b} (2.29-3.18)
Glucose									
Multiparous	3.80^{a} (3.61-4.00)	4.03^{a} (3.60-4.50)	3.90^{a} (3.50-4.36)	4.54^{A} (3.38-6.08)	3.31 (2.96-3.69)	2.95 (2.49-3.49)	2.82^{b} (2.43-3.27)	3.15 (2.62-3.78)	3.20 (2.68-3.81)
Primiparous	3.79 (3.59-3.99)	3.87 (3.48-4.30)	3.54 (3.15-3.97)	3.52 (3.15-3.97)	3.05 (2.69-3.46)	2.84 (2.36-3.42)	2.96 (2.55-3.44)	3.17 (2.62-3.84)	3.05 (2.54-3.67)

Different superscripts indicate significante differences between days of experiment

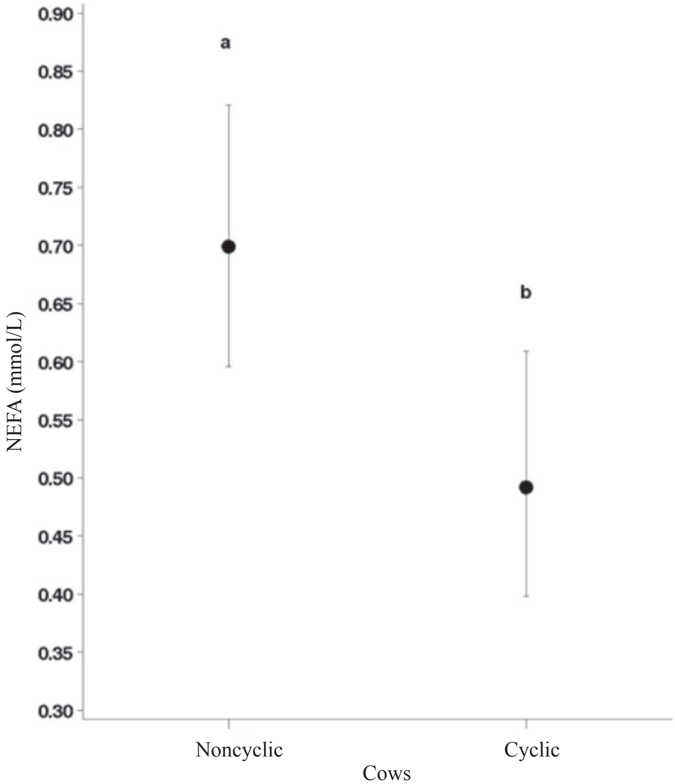

Fig. 1. Least squared means ± 95% confidence interval for concentrations of NEFA between cyclic and noncyclic cows. Different exponent indicate significant differences between cyclic and noncyclic cows (P<0.05).

Concentration of progesterone (P_4) and resumption of ovarian cyclicity. Out of 24 cows, 11 (45.8%) resumed ovarian cyclicity within 60 days after calving. Out of those 11 cows only 4 cows had normal resumption of ovarian cyclicity postpartum (2 primiparous *vs*. 2 multiparous), whereas 3 cows had a prolonged luteal phase (1 primiparous *vs*. 2 multiparous), and 4 cows had cessation of cyclicity (2 primiparous *vs*. 2 multiparous).

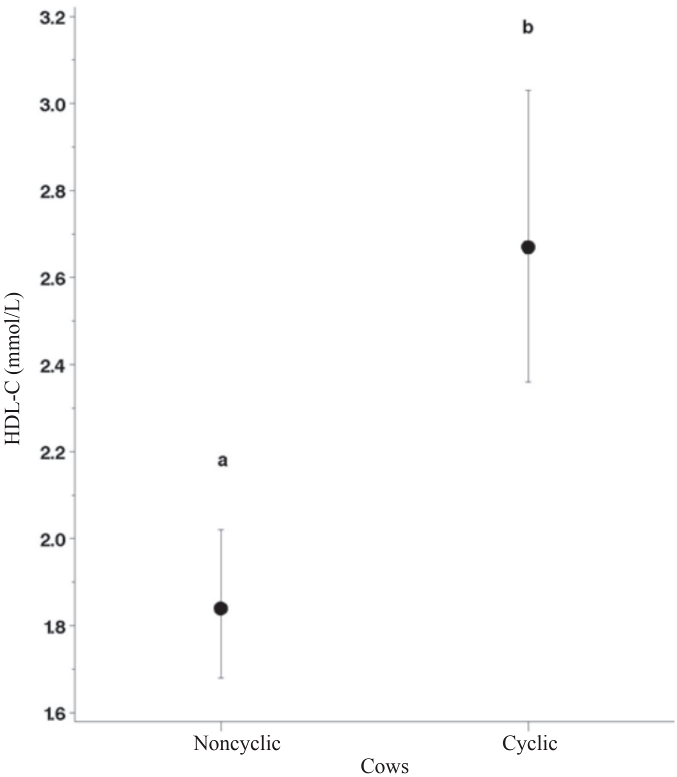

Fig. 2. Least squared means ± 95% confidence interval for concentrations of HDL-C between cyclic and noncyclic cows. Different exponent indicate significant differences between cyclic and noncyclic cows ($P<0.05$).

Changes of metabolic parameters in relation to the resumption of ovarian cyclicity. By analysis of the NEFA concentrations a significantly lower concentration of NEFA was established in the cyclic cows ($P_4>1$ ng/mL) in comparison to the noncyclic cows ($P_4<1$ ng/mL). By analysis of the HDL-C concentration a significantly higher concentration of HDL-C was established in the cyclic cows ($P_4>1$ ng/mL) in comparison to the noncyclic cows ($P_4<1$ ng/mL). No significant differences were established in concentrations of glucose, BHB, TG between cyclic and noncyclic cows.

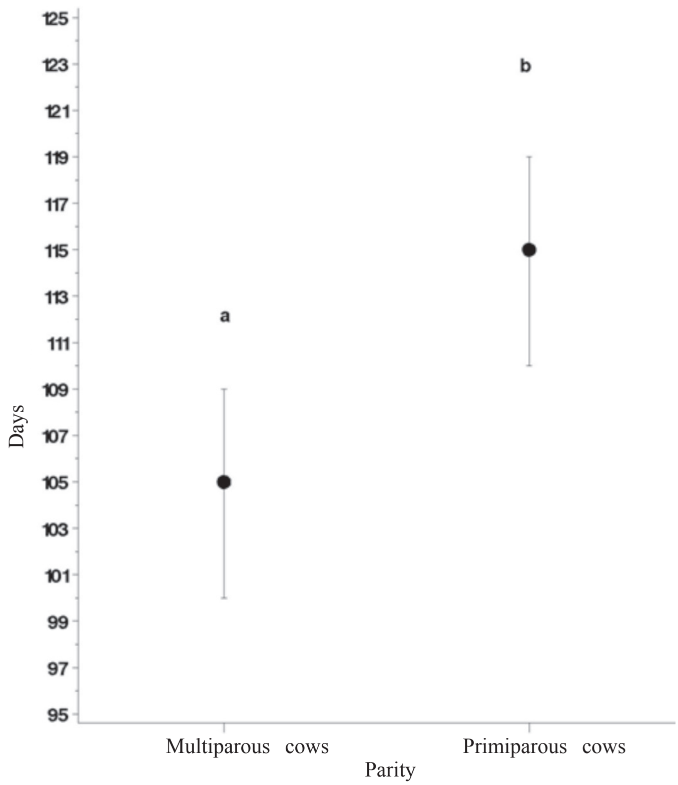

Fig. 3. Least squared means ± 95% confidence interval for multiparous and primiparous cows from calving to first service. Different exponent indicate significant differences between multiparous and primiparous cows ($P<0.05$).

Days until first service. By analysis days of the first service it was established that there was a significantly longer period until the first insemination of an average of 115 days (ranging from 110-119 days) in the primiparous compared to the multiparous cows, in which it was an average of 105 days (ranging from 100-109 days).

Discussion

In order to achieve maximal reproductive efficiency, the goal is to shorten the service and calving period. To accomplish this goal, the service period should be reduced to 80 to 120 days, and the intercalving period needs to be within 365-400 days. This requires resumption of ovarian cyclicity as quickly as possible during the puerperium, and the uterus must return to its state before pregnancy. This requires a complex endocrine interaction between the brain, liver, ovary and uterus (WATHES et al., 2007a). Parity has been also reported as an important factor affecting some metabolic and reproductive parameters (MEIKLE et al., 2004). In our study we found significantly lower concentrations of NEFA in cows with the resumption of ovarian cyclicity during the puerperium compared to acyclic cows. Accordingly, cows ovulating within 35 days of calving had higher concentrations of IGF-I, as well as higher concentrations of glucose and insulin, but lower concentrations of NEFA and BHB (HUSZENICA et al., 2001).

In addition, KOČILA et al. (2009) found that a group of acyclic cows had significantly higher concentrations of NEFA than a group with cyclic ovarian activity during the puerperium. KONIGSSON et al. (2008) obtained the opposite results to ours, where they did not find a significant difference in NEFA concentrations between the group of cyclic cows compared to acyclic cows. Moreover, the above authors did not find any significant differences between these groups in the loss of body weight, milk yield, NEB, or the concentration of leptin, but they found a significantly higher concentration of IGF-I in cyclic cows, emphasizing the role of IGF-I as the most sensitive signal of metabolic and reproductive status. It should be noted that these authors conducted a study on primiparous cows with low milk production.

REMOND et al. (1991) found a more pronounced degree of NEB in primiparous cows compared to multiparous. They explained that heifers have greater needs for nutrients for growth, but also for the growth and development of the foetus inside them, the requirements of their first lactation in life, and a reduced capacity for food intake, which is already more expressed in heifers than in cows. Heifers of dairy cattle breeds usually have their first calving at the age of 24 months. Age at first calving has a significant impact on milk production, the percentage of fat and protein in the milk, and the productivity and longevity of cows (PIRLO et al., 2000). It is important to notice that cows continually grow until the end of their third lactation, although growth is reduced once a heifers reaches the age of 450 days (COFFEY et al., 2004). Therefore, primiparous cows approaching calving are in a different metabolic status than multiparous cows (WATHES et al., 2007b; ANTONČIĆ-SVETINA et al., 2011).

The concentrations of total cholesterol and HDL-C in our study were significantly lower at the end of pregnancy and in the period of calving, in comparison to the period after the second week of lactation. PYSERA and OPALKA (2000) and TURK et al. (2004; 2005; 2008; 2013) obtained similar results. They explained that the lower concentration of cholesterol at the end of the dry period is probably due to the increased needs of the foetus for growth and development, as well as the need of the ovaries for synthesis of steroid hormones.

Furthermore, in our study we found a significantly higher concentration of HDL-C after parturition in cows with a normal return of ovarian activity, in comparison to acyclic cows, which additionally confirms the importance of cholesterol in the synthesis of sexual hormones and the role of HDL-C which transports cholesterol to the ovaries, where most of their synthesis occurs, necessary for the resumption of ovarian cyclicity following parturition. Considering the fact that higher concentrations of HDL-C and lower NEFA concentrations are significantly correlated with the resumption of ovarian cyclicity, it may be concluded that overcoming NEB is the most important event that determines the resumption of ovarian cyclicity. These results are consistent with the assertions by BUTLER (2003), who claimed that as soon as cows overcome NEB, they achieve earlier onset of cyclical ovarian activity and conception. Our research revealed a significantly longer period until the first insemination of the primiparous as compared to the multiparous cows. In addition, MEIKLE et al. (2004) attained results consistent with ours, where they found a significantly longer period until the first insemination and significantly longer duration of days open in primiparous as compared to multiparous cows.

We conclude that primiparous cows are more susceptible to metabolic stress during the transition period, and that their metabolic and endocrine profiles are more unbalanced as compared to the multiparous cows, which results in more severe and prolonged recovery from NEB.

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SAŽETAK

Cilj ovog rada bio je istražiti učinak pariteta na povratak ciklične aktivnosti jajnika i odrediti metaboličke pokazatelje koji utječu na cikličnu aktivnost jajnika. Istraživanje je provedeno na ukupno 24 krave holštajnsko-frizijske pasmine. Krave su bile podijeljene s obzirom na paritet u dvije skupine od po 12 životinja u svakoj prema paritetu na primiparne i multiparne. U uzorcima seruma analizirani su pokazatelji metaboličkog statusa neesterificirane masne kiseline (engl. akronim NEFA), betahidroksi butirat (BHB), glukoza, trigliceridi, ukupni kolesterol i kolesterol iz lipoproteina visoke gustoće (engl. akronim HDL-C). Ponovno uspostavljanje ciklične aktivnosti jajnika bilo je određeno pomoću koncentracije progesterona i ginekološkim pregledom. Utvrđena je značajno viša koncentracija HDL-C i niža koncentracija NEFA u odnosu na ponovno uspostavljanje ciklične aktivnosti jajnika. Ovi nalazi upućuju na zaključak da je nadvladavanje NEB-a najvažnije preduvjet koji određuje ponovno uspostavljanje ciklične aktivnosti jajnika. Naše je istraživanje također pokazalo da su primiparne krave imale značajno duže razdoblje do prvog osjemenjivanja nakon teljenja u odnosu na multiparne krave. Stoga se može zaključiti da su primiparne krave osjetljivije na metabolički stres tijekom prijelaznog razdoblja te da su njihovi metabolički i endokrini profili neuravnoteženiji u usporedbi s onima u multiparnih krava.

Ključne riječi: negativni energetski status, paritet, ciklična aktivnost jajnika, mliječna krava