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POSTPARTUM RESUMPTION OF CYCLIC OVARIAN FUNCTION, FIRST ESTRUS AND RE-CONCEPTION AND THEIR RELATION TO ENERGY METABOLISM IN HIGH-PRODUCING DAIRY COWS.*

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In the last few decades a continuous increase was observed in average milk production of dairy cows all over the world. Simultaneously, however, a dramatic decrease was seen in reproductive performance. This tendency is attributed to the increased incidence of bacterial complications in uterine involution, as well as to the high occurrence of ovarian malfunctions in the postpartum period. The aim of this paper is to review the physiology and pathology of the latter, really complex phenomenon. The nutritional basis of this process, that the requirements of high-producing dairy cows shift abruptly after parturition as the daily milk yield rapidly increases and the ensuing negative energy balance (NEB) will extend 10-12 weeks. In the context of the high genetic merit dairy cow, the pp NEB is the difference between the dietary intake of utilizable energy and the expenditure of energy for body mass maintenance and milk synthesis. In principle, it is a physiological phenomenon, which may, however, result in more or less severe disorders in both the metabolism and reproduction and so it may lead to great economic losses in modern dairy practice [112]. In the first 3-4 weeks after calving the NEB is highly correlated with both milk yield and the interval to first ovulation. Because the number of ovulatory estrous cycles preceding the insemination (AI) has been shown to influence the conception rate, the length of the pp interval to first ovulation provides an important parameter for assessing the effect of NEB on reproductive performance [19, 20].

Key words: high-producing dairy cows, energy metabolism, post partus, cyclic ovarian function, first estrus, re-conception

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The pathophysiology of negative energy balance in postpartum dairy cows

• Increased lipid mobilization and its consequences

During the period of NEB high-producing cows have to mobilize their body reserves, first of all their subcutaneous lipid stores (*increased* or *forced lipid mobilization*, FLM). Generally the animals overfed before calving [body condition score (BCS) at calving: 3.75] are in the most endangered position (*fat cow syndrome*). The rapid reduction in body weight (BW) and BCS are the most obvious clinical symptoms of FLM. As an early consequence, a sharp elevation can be seen in circulating levels of non-esterified fatty acids (NEFA), which is followed – quite frequently, but not in all of the cases – by increased production of OH-butyrate (BHB) and other ketone bodies (*hyperketonaemia*). Simultaneously lipids, mainly triacylglicerols (TAG) are accumulated in the liver (*fatty liver disease*). The moderate to severe forms of fatty liver (fat content: 20 %) may result in well-defined disorders in hepatocellular functions (gluconeogenesis, cholesterol, bile acid and bilirubin metabolism, inactivation of steroid hormones and insulin, synthesis of certain apolipoproteins and the 25-hydroxylation of cholecalciferol). The clearance of bromosulphothalein becomes markedly slower and the concentrations of *total bilirubin* and *total bile acids* in plasma are positively correlated with the degree of fatty infiltration in hepatocytes. Simultaneously *hypoalbuminaemia*, obviously reduced levels of *very low density lipoproteins* and *low density lipoproteins*, furthermore lower than normal concentrations of all the *lipoprotein-transported substances* (*total cholesterol*, TAG, β-*carotene* and *tocopherol*) can be detected in the peripheral blood. Although the membrane damaging effect of fatty infiltration is not obvious, moderate increases are usually observed in serum activities of *aspartate aminotransferase*, *alkaline phosphatase* and *lactate dehydrogenase*. In the more severe forms of hepatic lipidosis the hepatocellular detoxifying capacity of ruminal *ammonia* as well as of *endotoxin* absorbed from the gastrointestinal tract or liberated from any endotoxin-mediated diseases including the acute putrid (endo)metritis (APE), or certain forms of mastitis is diminished. Also the impairment of the immune system has been reported to occur, which is known to make the affected individual susceptible to bacterial complications of uterine involution (APE), as well as to *mastitis*. The FLM-induced biochemical changes predispose the cow for *retained fetal membrane*, *milk fever,* and *displaced abomasum,* as well as for various forms of *ketosis*. This latter disease is a direct consequence of NEB and FLM. The liver markedly increases its NEFA uptake from the blood. These fatty acids are either esterified to TAG, or converted into acetyl-CoA. Whether this acetyl-CoA is introduced into the Kreb's cycle depends on the availability of oxaloacetate, which is predominantly delivered from gluconeogenetic

precursors such as propionate, pyruvate, glycerol, or certain amino acids. If the supply of these precursors is inadequate, as can occur frequently during NEB, the availability of oxaloacetate for introducing acetyl-CoA into the Kreb's cycle decreases. The excess acetyl-CoA is then used for ketogenesis and the cow can develop subclinical or clinical forms of ketosis. An inadequate supply with gluconeogenic precursors seems to be the core event in this process [94, 95, 44, 45, 57, 54, 59, 106, 56, 28, 77, 49, 105, 89, 48, 144, 5, 16, 88, 139, 73, 137, 112, 113].

- **The endocrinology of negative energy balance**

The pp NEB can induce marked endocrine changes, which are known as important factors in regulation of FLM. Beside the involvement of *chatecolamines,* elevated glucagon and decreased insulin concentrations characterize the earliest changes, whereas the *growth hormone* (GH) level remains unchanged or perhaps slightly increases. Simultaneously the *GH-induced hepatic insulin-like growth factor-1* (IGF-1) liberation and the *glucagon-induced insulin responsiveness of pancreatic* β*-islets* are diminished and certain tissues lose their insulin sensitivity. All these endocrine events can shift the metabolism from an anabolic to the catabolic state [110, 109, 61, 130, 128, 90, 30, 58, 60, 136, 39, 115, 148, 50, 134, 108]. This metabolic shift may be amplified by the endocrine consequences of a simultaneous endotoxin exposure [38, 37, 70]. The peripheral tissues try to fit their current local energy metabolism to this new, NEB-induced catabolic condition through increasing the capacity of an inactivating [3,3',5'-triiodothyronine (rT_3) producing] path of thyroid hormones [thyroxin (T_4) and 3,3',5-triiodothyronine (T_3)]. So although the thyrotropin releasing hormone (or thyrotropin) induced T_4 response of the thyroid gland is only slightly altered, obviously decreased T_4 and T_3 levels and elevated rT_3 concentrations are reported to occur in the peripheral blood [26, 17, 101, 103, 109, 140, 141, 58, 99, 138]. The involvement of adrenocortical function / cortisol production was also supposed a long time ago, however, since then no existing experimental evidence has been found to confirm this hypothesis. Recently, the research on regulation of feed intake and energy metabolism has been focused on the supposed role of *leptin*. This newly identified 16 kDa cytokine like protein hormone is secreted mainly by the adipose tissue, and is believed to act through hypothalamic nerve centers in mediation of neuroendocrine responses to energy supply or deprivation. It may signal nutritional status perhaps also for the peripheral organs. This hormone is one of the primary agents communicating information about the level of peripheral energy stores to brain regions concerned with orchestrating feeding behavior, metabolism, and endocrine function so as to maintain energy homeostasis [150, 43, 68, 63]. In mice, rats, humans and also in ruminants and pig its circulating concentration may vary directly with changes in BW and percentage of body fat and leptin contributes to the regulation of body fat content. Insulin, glucocorticoids and endotoxin exposure may increase its gene expression and/or plasma level, whereas

leptin can directly inhibit cortisol synthesis by adrenal cells. So leptin and cortisol interact in a negative feedback loop. The 24-h incubation with T_3 (but not with T_4) significantly increased the expression of leptin mRNA and the levels of secreted leptin in adipocyte cultures. The leptin liberation from adipocytes is down-regulated by adrenergic stimulation and it is also supposed to interact with growth hormone and IGF-I secretion [27, 24, 62, 63, 8, 7, 41, 74, 25, 69]. Multi-species, and later species-specific assay systems allowing the exact quantification of plasma leptin in various domestic mammals were developed only in the late nineties and at the beginning of this decade. Since then an increasing body of information has been available suggesting that leptin may be associated with NEB also in ruminants including cattle [14, 24, 92, 4, 31, 36, 72, 13, 25, 69].

Ovarian function and negative energy balance in postpartum dairy cows

• Resumption of regular follicular growth. Onset of ovarian cyclicity

In almost the entire non-suckling dairy cows the FSH concentrations in plasma increase to peak values on d 4-5 after calving. This first FSH peak is followed immediately by the initiation of the first pp follicular wave producing the first dominant [>9 mm] follicle (DF). Subsequently the regular formation of new FSH waves followed by growing of new follicular cohorts and producing new DF-s is reported to proceed despite the average NEB of -7.5 Mcal/day during the first 3-week period after calving [10]. It appears that the initiation of follicular waves in early pp cows is unperturbed by NEB and occurs in response to the re-establishment of periodic FSH surges synchronized only by the end of gestation and parturition. During the NEB in early weeks of lactation LH, but not FSH appears to be deficient. Therefore regular onset of FSH dependent follicular growth seems to be insensitive to NEB [79, 116, 104, 10, 12]. The steroidogenic capability of the first DF formed during the second week after calving, as well as the factors influencing its fate, however, require further studies.

Three patterns of pp follicular development based on the fate of the first DF have been described [116, 104, 10, 11, 12]: (1) *ovulation* of the first-wave DF; (2) development of a first-wave *anovulatory* DF followed by (several other) additional waves of follicular development before the first ovulation; (3) development of a first-wave DF that becomes *cystic*. Patterns 1 (ovulatory) and 3 (cystic) are characterized by development of 17 -estradiol (E_2) producing (estrogen-active) DF, whereas pattern 2 is characterized by growth of DF that produce only low peripheral concentrations of E_2 and become atretic. In the case of pattern 1 the ovarian activity becom cyclic after the ovulation of the first-wave DF. Patterns 2 and 3 may be repeated several times, so both the regression of the (first-wave and subsequent) DF(s) and the formation of anovulatory cysts can prolong the interval to first pp. ovulation (*pp. acyclic period*). Since the early eighties it has been widely

accepted that the EB is one of the most important factors influencing the duration of this acyclic period: in non-suckling dairy cows the first pp. ovulation takes place *on about the 10th d after the nadir* of NEB [18, 22].

There are only few studies available in literature which examined the direct relationship between NEB and follicular dynamics in pp. dairy cows. *Lucy* et al. (1991) demonstrated that the number of class 3 (10-15 mm in diameter) – but not of the classes 1 (3-5 mm) and 2 (6-9 mm) – follicles increased with more positive energy balance (EB) before d 25 after calving. This observation suggests that as cows improve in EB, the movement of smaller follicles into larger size classes is enhanced. During the first pp follicular wave (d 8-14 after calving) in cows receiving three levels of dietary fat, the number of class 1 and class 2 follicles was not correlated with EB during either the 1st or 2nd wk pp, regardless of diet [11]. The development of a DF in pp dairy cows is tolerant to NEB. However, several studies demonstrated that the ultimate diameter and E_2 production of DF are influenced by metabolic factors: both of the size of DF and the E_2 level in plasma increased after EB improved from its most negative level [10, 11].

- **Metabolic and endocrine signaling mechanisms**

The effect of NEB on the time of the first pp ovulation was confirmed a relatively long time ago [18, 22]. However, the physiological signaling mechanisms informing the *hypothalamus - anterior pituitary - ovary* (HPO) axis on the current stage of EB have remained open for a while, and perhaps some details are questionable even nowadays. The restricted energy intake did not alter the pituitary GnRH receptor density in pp cows [96], but dietary energy restrictions were followed by both decreased [114] and increased [145] responsiveness to exogenous GnRH. *The loss of pulsatile LH secretion* was shown to result from prolonged inadequacy of energy supply in both of suckling beef cows, and non-suckling dairy cows [79, 23, 102]. Up to now it has been widely accepted that the re-establishment of a pulsatile LH secretion pattern conducive to preovulatory follicular development and function is a key event in the return of ovarian cyclicity in pp dairy cows experiencing NEB [12]. It appears, that pp recovery of pituitary LH content and responsiveness to GnRH is complete by d 10 after calving in dairy cows, and available evidence across species suggests a predominantly hypothalamic locus for the primary effect of decreased energy intake [96, 117]. In the early weeks of lactation the reduced activity of the GnRH pulse generator in pp dairy cows is expressed as reduced pulsatile LH support of follicular steroidogenesis necessary for induction of a preovulatory like LH surge and subsequent ovulation. However, a seemingly low LH pulse frequency (2 pulses per 6 h) is apparently adequate to sustain the morphological development of DF by the 2nd wk pp. This observation is consistent with the growth and differentiation of competent DF-s during the mid-luteal phase of the bovine estrous cycle when the LH pulse frequency is low [33].

Studies investigating the potential metabolic signals for the HPO axis have been focused primarily on blood metabolites (NEFA, glucose) and metabolic hormones (insulin:GH ratio, insulin, IGF-I) known to fluctuate during altered states of energy metabolism. Concerning the NEFA and glucose, however, quite contradictory observations were reported [66, 23, 50]. So it is generally accepted nowadays that any mechanism coupling metabolic status with HPO has to involve ultimately a hormonal component [12]. Studies of *Beam and Butler* [10, 11] confirmed the physiological importance of plasma insulin:GH ratio and the day of the EB nadir, indicating that these hormonal differences in the intermediate pp period may influence the first wave follicular function. Insulin has been shown to stimulate follicular cells in vitro also in cows [127] and small increases pp could have important effects during the very early stages of follicular development. Circulating concentrations of IGF-I and one of its binding proteins (IGFBP-2) in the peri-parturient period were good indicators of the capacity of energy-restricted cows to resume cycling after calving [108]. Furthermore, an increased insulin:GH ratio following parturition may be conducive to greater hepatic IGF-I production [91], resulting in increased amounts of this growth factor earlier after calving. Although some seemingly contradictory observations have also been reported in heifers [128], during the first 2 weeks pp *Beam* and *Butler* [10, 11] could detect significantly higher circulating IGF-I concentration in cows developing E_2-active, ovulatory first-wave DF than in those with E_2-inactive anovulatory first-wave DF. In cows the circulating IGF-I concentrations correlated with IGF-I levels in the follicular fluid of large follicles [34]. Both insulin and IGF-I are known to stimulate the in vitro steroidogenesis and proliferation of bovine thecal and granulosal cell cultures [127, 129]. Under *in vitro* conditions in bovine thecal cells the IGF-I increased the number of LH-binding sites and enhanced the LH-induced production of androstenedione and progesterone [129]. This finding confirms the in vivo observation of *Beam* and *Butler* [11] demonstrating an apparent relationship between the steroidogenic activity of first-wave DF and circulating levels of IGF-I. In human and porcine granulosa cell cultures the T_3 could also increase the estrogen-producing aromatase activity and E_2 yielding [87, 52, 143, 53]. Up to now there is only one study evaluating the effect of T_3 and T_4 on steroidogenesis of ovarian cells in cattle: *Spicer* et al. (2001) reported a direct stimulatory effect of T_3 and T_4 on thecal cell steroidogenesis. The stimulatory effect of T_3 and T_4 on androstenedione production (i.e., increases to two- to fourfold) was similar to the influence of LH on androstenedione production (i.e. increases to 4- to 9-fold). The stimulatory effect of T_3 was biphasic (i.e., low dose stimulated, high dose had no effect), however the influence of T_4 was similar at both doses. In contrast, T_3 inhibited hCG-induced androgen secretion by porcine thecal cells [53]. Thus, species differences and/or differences in culture conditions may exist with regard to thyroid hormone regulation of thecal steroidogenesis, as for granulosa cell steroidogenesis. In cattle the T_4 was a much weaker (i.e., increase to 1.3-fold) inducer of thecal cell P_4 production than was LH (i.e., increases to four- to ninefold) and its effect was only evident

at hyperthyroid levels (i.e., 100 ng/ml, but not 30 ng/ml); T_3 had no effect on granulosa and thecal cell P_4 production in this study (*Spicer* et al., 2001). The association between low E_2, negative energy balance, and low T_3 was also found in young women distance runners [149]. In contrast, diameters of dominant follicles from multiparous Brahman cows were not affected by induced hypothyroidism [32].

Through affecting the hypothalamic GnRH and pituitary gonadotrop secretions also the *leptin* has been reported to influence the genital functions in rodents, primates and recently also in ruminants. Experiences have confirmed that this hormone may be one of the long-sought indicators of nutritional status that allows reproductive processes to proceed. Results in rodents, non-human primates and also in ewes suggest that the suppression of GnRH / LH during fasting is mediated by central action of leptin in the pituitary or the brain [40, 97, 98, 93, 122]. Moreover, *Ahima* et al., [2] showed that treatment with leptin accelerates puberty in normal mice at doses that do not change body weight, and suggested that actions of leptin to regulate neuroendocrine and reproductive function in normal mice are not secondary to effects on energy balance. Low leptin induced markedly increased production of neuropeptid Y (NPY) in the hypothalamus [1], and increased NPY inhibited the gonadal axis in lab rodents and also in ruminants, so it has been postulated that impaired reproductive function in adverse metabolic condition such as fasting could be due to excessive hypothalamic NPY release [47, 118]. Several observations suggest that leptin is an important biochemical message between fat stores and the reproductive axis [1, 6, 2, 41]. Leptin receptors have been detected also in gonads. Under in vitro conditions leptin has week inhibitory effects on gonadotropin- and/or IGF-I-induced steroidogenesis of thecal and granulosa cells [128]. In conclusion, not only the hypo-, but also the hyperleptinemia can be supposed to have a negative influence on reproduction in domestic mammals including cattle [122].

In *periparturient dairy cows*, changes in the plasma concentration of leptin have been measured during the period from 35 d before to 56 d after parturition. The plasma concentration of leptin was highest during late pregnancy and declined by 50% after parturition. The plasma leptin concentration remained depressed during early lactation despite a gradual improvement in energy balance. Corresponding changes occurred in the abundance of leptin mRNA in subcutaneous white adipose tissue. The pp reduction in plasma leptin was due to the NEB because plasma leptin remained high in cows not milked after parturition. The plasma concentration of leptin was positively correlated with plasma concentrations of insulin and glucose, and negatively correlated with plasma concentrations of GH and NEFA [13]. There are only limited data available on the interrelation between circulating leptin levels and pp resumption of cyclic ovarian function in dairy cows [72, 67]. In 20 high-producing Holstein cows kept under model conditions the plasma leptin concentrations declined after parturition, reached their nadir on 10.1 2.2 days after calving, then they increased and became stable near

the time of the first ovulation on 25.9 2.0 days. The interval from calving to first ovulation correlated significantly with the interval from parturition to leptin nadir, but it was not in correlation with the prepartum, pre- and/or postovulatory leptin values [72]. The other trial [67] involved two separate experiments, which were conducted in commercial large-scale dairy herds. The pp leptin levels of cows resuming their cyclic ovarian function regularly within 35 days after calving were compared to those with delayed (after 35 days) onset of cyclicity. The rate of the latter group was 31 % in the *Exp. 2,* but 65 % in the *Exp. 1*, perhaps due to the prepartum overfeeding of these cows. Immediately after calving the plasma leptin concentrations, as well as the BCS of cows were almost the same, regardless of the forthcoming duration of pp acyclicity. In cows of *Exp. 1* the plasma leptin levels varied within a wide range with a mean of about 4 ng/ml on the first 1-3 days after calving. Up to week 5 they remained unchanged in cows ovulated within day 35, but reduced in those with a delayed onset of cyclicity. In cows of *Exp. 2* the week 1 leptin levels were lower and less variable than those of *Exp. 1*. In weeks 2 and 3 a slightly increasing tendency of leptin pattern was seen in cows resuming their ovarian cyclicity within 35 days whereas in those with delayed onset of cyclicity the leptin level remained unchanged. Up to week 5 this tendency resulted in exactly the same leptin values as seen in *Exp. 1* with significant differences between the group means in both of the weeks 5 and 10. In the early weeks of lactation the BCS and BW decreased significantly in all animals, but this reduction was more obvious in cows with a delayed onset of cyclicity. In both experiments the cows with delayed onset of cyclicity were usually characterized by lower glucose, total cholesterol, insulin, IGF-1 and T_3 levels and higher NEFA and BHB concentrations than those that ovulated within 35 days, although these differences were significant only in the early weeks of sampling and were more obvious in *Exp. 1*. The leptin levels determined at various times after calving were in strong positive correlation with each other, and correlated with BCS immediately after calving and later again in week 10, but not in week 5. Mild to moderate positive correlation of leptin with insulin and mild negative correlation with plasma metabolites related directly to energy imbalance (NEFA, BHB) were seen from week 2 (but not in the first few days after calving). Concluding these data in pp dairy cows the plasma level of leptin may interfere with the resumption of cyclic ovarian function in the early weeks of lactation, but its influence may be only permissive (e.g. a minimal concentration above a supposed threshold seems to be the prerequisite of the onset for cyclicity). It may be suggested that the lower than normal insulin, IGF-1, leptin and perhaps T_3 levels, the incorrect insulin:GH ratio and/or their combination may be the factors informing the HPO axis on the current nutritional status and body fat content. At the hypothalamic-pituitary level this signal is realized as lower than normal pulse frequency of basal GnRH / LH secretion.

Energy related factors influencing fertility in postpartum dairy cows

The detrimental effects of NEB in early lactation appear to be manifested also as reduced fertility during the pp breeding period. In normal dairy herd situations direct assessment of energy balance in individual cows is not possible, but changes in BCS provide an indirect measure. With more extensive loss of BCS, the reduction in conception rate becomes greater [15]. Cows losing one unit or more BCS (5 point scale) during early lactation are at the greatest risk for low fertility with conception rates of 17 to 38 %. Recent studies indicated that cows with marked losses in BCS (1.25 unit) were only half as likely to conceive at first AI as cows with more modest loss [51], and that the conception rate increases 10 % for every unit increase in BCS [135]. A recent large survey study found that cows with a BCS of 3.0 at the first AI were most likely to become pregnant [81].

- **Delayed onset of ovarian cyclicity**

Although reduced fertility as a consequence of NEB in early lactation may be explained by prolonged acyclicity in 30 - 36 % of cows [66, 68, 133, 107, 113, 71], our understanding of the linkage between NEB and sub-optimum conception rates in ovulatory cyclic cows remains rather speculative. One important link between NEB and lower fertility appears to be through the aforementioned effects on the timing of first postpartum ovulation. A positive association between the early commencement of ovulatory cycles and the improved conception rate to AI is well documented. Cows remaining anovulatory for 35-50 days of lactation were significantly less likely to become pregnant during lactation and would be culled [68, 22, 42, 20, 29].

- **Bacterial complications of uterine involution**

While accepting the general benefit of an early return to ovarian cycles on fertility, we should be mindful of the interaction with uterine health. Multiparous cows ovulating before 21 days postpartum exhibited poorer reproductive performance than those ovulating later in association with a high incidence of persistent corpora lutea (CLP) [123]. CLP presumably result from uterine infection and their incidence in dairy cows has increased in recent years [111]. Also a higher rate of irregularly shortened CL phases was reported to occur [65]. Evidence of uterine infection and endometritis are clearly associated with reduced fertility to AI, and must be considered part of the overall trend for lower fertility in high yielding cows [65, 81, 71, 84].

- **The effect of circulating progesterone level in already cyclic cows**

Pre-insemination period

Another important link between NEB and fertility is the carryover effect on plasma P_4 concentrations. During the first two or three pp ovulatory cycles the peak level of P_4 is reported to increase in the blood from one cycle to the next [68, 142, 136, 133], and in the early weeks of lactation the rate of this P_4 increase is reduced or moderated by NEB [142, 130]. Cows with the most negative energy status during the first 9 days postpartum still had decreased plasma P_4 levels during their third estrus cycles [142] corresponding to the start of the breeding period. Plasma P_4 concentrations in cows selected for high milk yield were 25 to 50% lower during the second and third luteal phase than in control line cows [82].

Post-ovulatory rise of progesterone in inseminated cows

The ability to produce and maintain optimum P_4 concentrations is important for fertility due to the regulatory effect of this hormone on endometrial function [120]: by 4-7 days after AI the plasma P_4 was found to be higher in cows that became pregnant than in non-pregnant cows [1, 21].

Early (by day 5 after fertilization) P_4 stimulation alters endometrial secretions and advances conceptus development [46]. Conversely, a slower rate of post-ovulatory P_4 rise through days 4-5 has been associated with lower fertility [121, 21] and decreased embryo growth by day 16 [86]. At this critical time for maternal recognition of pregnancy an inadequately developed embryo produces insufficient quantities of interferon- to inhibit the uterine luteolytic mechanism, the oxytocin receptor stimulated PGF_2 release [85], which in itself is made stronger by lower circulating P_4 [78]. In addition, a delayed rise in luteal P_4 around days 4-5 may allow pregnancy recognition and extended luteal function, but early termination of the pregnancy because the embryo has been compromised during development [80].

The physiological mechanism by which NEB early in the pp period translates into reduced P_4 production two months later has not been established. *Britt* [15] hypothesized that ovarian follicles are detrimentally affected by exposure to NEB during their early growth and development and that ovulation of affected follicles would lead to lower P_4 secretion. This hypothesis may explain the pattern of plasma P_4 concentrations in lactating cows, however, the effects of dietary intake on P_4 clearance must also be considered [64]. In sheep, high dietary energy intake increases metabolic clearance of P_4 from blood by the liver [100]. The baseline liver blood flow in lactating cows was found to be twice that in non-lactating cows and was acutely increased 20-30% with feeding [146]. High liver blood flow resulted in increased steroid metabolism and 25% lower plasma E_2 and P_4 concentrations during the estrous cycle and this was associated with a higher incidence of degenerating embryos on day 5. During early lactation total dietary intake in dairy cows increases two-fold by the beginning of the breeding

period [9]. In this situation increases in P_4 clearance due to high dietary intake (both energy and protein) may be combined with the carryover effects of NEB to result in lower plasma P_4 concentrations and to reduce fertility. The uterine environment is dependent on P_4, but may be rendered sub-optimum by effects of NEB and increased metabolic clearance in high yielding cows.

- **The quality of oocytes**

Early pp NEB may adversely impact oocytes during the 80-100 days required for follicle development and, thereby, exert another carryover effect on fertility [15]. Several studies have aspirated oocytes transvaginally from ovarian follicles over the period of 30-120 days of lactation. Cows on high energy diets produced more oocytes and oocytes of higher quality than cows on low energy diets, but there were only modest changes over the collection period in both groups [75, 55]. Cows having more severe pp NEB due to over-fatness before and at calving, produced oocytes with lower developmental capacity following in vitro maturation and fertilization procedures as compared with control cows during the period of 80-120 days of lactation [76]. Further evidence for an even more prolonged effect on oocyte development comes from the study of *Snijders* et al. [124, 125]. High genetic merit cows yielded oocytes with lower developmental potential than medium genetic merit cows during mid-lactation (125-229 days). Differences in milk yield did not affect embryo development, but blastocyst formation rates were reduced in cows with low BCS (1.5-2.5). High genetic merit cows had lower BCS than medium merit cows. Lower conception rates for the high genetic merit cows were suggested to be the result of impaired oocyte quality rather than the currently existing level of any of the metabolic parameters measured at the time of AI studied. Overall, these experiences suggest a generally adverse effect of reduced energy status during lactation on oocyte development, but whether such effects are limited in relation to the time required for follicle development remains unclear.

Conclusion / *Zaključak*

Reproductive performance in dairy cows has declined over the past several decades in association with impressive increases in milk yields. The metabolic demands of high milk production result in greater NEB, during which blood levels of glucose, insulin, IGF-I, leptin and T_3 levels are generally reduced, while TAG content in the liver and plasma NEFA (and perhaps BHB) concentrations are increased. NEB and its metabolic consequences are associated with pp follicular development and first ovulation, variability of plasma P_4 concentrations, and impair oocyte development, resulting in decreased fertility. Fertility in dairy cows reflects the cumulative influence of metabolic, endocrine, and health components that have been modified and exaggerated by selection for high milk yield, as well

as by certain diseases (bacterial complications of uterine involution, mastitis). EB seems the most important factor, but the complex interactions of all these factors must be considered and controlled if we are to improve our understanding and develop new strategies to improve fertility.

Abbreviation key: AI = artificial insemination; APE = acute putrid (endo)metritis; BCS = body condition score; BHB = βOH-butyrate; BW = body weight; CL = corpus luteum; CLP = corpus luteum persistency; DF = dominant follicle; E_2 = 17β-estradiol; EB = energy balance; FLM = forced lipid mobilization; FSH = follicle stimulating hormone; GH = growth hormone; GnRH = gonadotrop releasing hormone; HPO axis = hypothalamus - anterior pituitary - ovary axis; IGFBP-1 to 5 = insulin-like growth factor binding protein-1 to 5; IGF-I = insulin-like growth factor-I (syn.: somatomedin C); LH = luteinizing hormone; mRNA = messenger ribonucleic acid; NEB = negative energy balance; NEFA = non-esterified fatty acids; NPY = neuropeptid Y; P_4 = progesterone; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; pp = postpartum; RFM = retained fetal membrane; rT_3 = 3,3',5'-triiodothyronine; T_3 = 3,3',5-triiodothyronine; T_4 = thyroxin; TAG = triacylglicerols

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POSTPARTALNI NASTAVAK CIKLIČNE FUNKCIJE JAJNIKA, PRVI ESTRUS I PONOVNO OPLOĐENJE I NJIHOV ODNOS PREMA METABOLIZMU ENERGIJE KOD VISOKO-MLEČNIH KRAVA*

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U proteklih nekoliko decenija uočeno je stalno povećanje prosečne proizvodnje mleka kod mlečnih krava širom sveta. U isto vreme, međutim, zapaženo je dramatično smanjenje reproduktivnih rezultata. Ova tendencija je pripisana povećanom pojavljivanju bakterijskih komplikacija tokom involucije uterusa, kao i velikom broju disfunkcija jajnika u postpartalnom periodu. Cilj ovoga rada je da se razmotri fiziologija i patologija ove druge pojave, tog zaista kompleksnog fenomena. Nutriciona osnova ovog procesa je da se potrebe visoko mlečnih krava naglo promene posle partusa kada se dnevni prinos mleka naglo povećava, a posledični negativni energetski bilans se produžava na 10 do 12 sedmica. U kontekstu visoke genetske vrednosti mlečnih krava, postpartalni negativni energetski bilans je razlika između dijetetskog unosa iskoristive energije i potrošnje energije za održavanje telesne mase i sinteze mleka. U principu, to je fiziološki fenomen koji, međutim, može da rezultira u manje ili više teškim poremećajima kako u metabolizmu, tako i u reprodukciji, i tako može da dovede i do velikih finansijskih gubitaka u savremenoj proizvodnji mleka [112]. U prvih tri do četiri sedmice posle teljenja, negativni energetski bilans je u visokoj korelaciji i sa prinosom mleka i intervalom do prve ovulacije. Zbog toga što je ukazano da broj ovulatornih estrusnih ciklusa, koji prethode inseminaciji, utiče na stepen koncepcije, dužina postpartalnog intervala do prve ovulacije obezbeđuje važan parametar za procenu efekta negativnog energetskog bilansa na reproduktivne rezultate [19, 20].

Ključne reči: visoko-mlečne krave, metabolizam energije, post partus, ciklična funkcija jajnika, prvi estrus, ponovno oplođenje

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Patofiziologija negativnog bilansa energije kod mlečnih krava u postpartalnom periodu

• Povećana mobilizacija lipida i njene posledice

Tokom perioda negativnog energetskog bilansa, visoko mlečne krave moraju da mobilišu svoje telesne rezerve, pre svega supkutane rezerve lipida *(povećana ili prinudna mobilizacija lipida)*. Uopšte uzev, životinje koje su previše hranjene pre teljenja (ocena telesne kondicije: pri teljenju: 3,75) najugroženije su *(sindrom debele krave)*. Naglo smanjenje telesne mase je najočitiji klinički simptom povećane mobilizacije lipida. Kao rana posledica može da se vidi nagli porast nivoa neesterifikovanih masnih kiselina u cirkulaciji, što prati – vrlo često, ali ne u svim slučajevima – povećana proizvodnja OH-biturata i drugih ketonskih tela *(hiperketonemija)*. U isto vreme lipidi, uglavnom triacilgliceridi akumuliraju se u jetri *(bolest „masne" jetre)*. Ovi umereni do ozbiljni oblici masne jetre (sadržaj masti: 20%) mogu da rezultiraju u dobro definisanim poremećajima hepatocelularnih funkcija (glikoneogeneza, sinteza holesterola, metabolizam žučnih kiselina i bilirubina, inaktivacija steroidnih hormona i insulina, sinteza apolipoproteina i hidroksilacija holekalciferola). Eliminisanje bromosulftaleina postaje znatno sporije i koncentracije *ukupnog bilirubina i ukupnih žučnih kiselina* u plazmi su u pozitivnoj korelaciji sa stepenom infiltracije masti u hepatocite. U isto vreme, u perifernoj krvi mogu da se uoče *hipoalbuminemija*, očito smanjeni nivoi *lipoproteina veoma male gustine* i *lipoproteina male gustine*, niže i od normalnih koncentracija svih *supstancija transportovanih lipoproteinima (ukupni holesterol, triacilgliceridi, β-karoten i tokoferol)*. Iako nije uočljiv štetan efekat infiltracije masti na membranu, umerena povećanja su najčešće uočena u serumskim aktivnostima aspartat aminotransferaze, alkalne fosfataze i laktat dehidrogenaze. U težim oblicima hepatične lipidoze smanjena je hepatocelularna sposobnost detoksikacije amonijaka kod preživara kao i *endotoksina* apsorbovanog iz gastrointestinalnog trakta ili oslobođenog zbog neke bolesti usled endotoksina, uključujući akutni gnojni endometritis, ili razne oblike mastitisa. Takođe je uočeno da se javlja i poremećaj imunog sistema, za koji se zna da dotičnu jedinku čini podložnom bakterijskim komplikacijama tokom involucije uterusa, kao i *mastitisu*. Biohemijske promene indukovane pojačanom mobilizacijom lipida kod krave stvaraju predispoziciju ka zadržavanju *posteljice, mlečnoj groznici i dislokaciji sirišta*, kao i raznim oblicima *ketoze*. Ketoza je direktna posledica negativnog energetskog bilansa i pojačane moblizacije lipida. Značajno se povećava unos neesterifikovanih masnih kiselina iz krvi u jetru. Ove masne kiseline se ili esterifikuju do triacilglicerida, ili se katabolišu do acetil-koenzima A. Dostupnost oksaloacetata, koji se uglavnom dobija iz glikogenoplastičnih prekursora kao što su propionat, piruvat, glicerol, ili izvesne amino-kiseline, odrediće stepen korišćenja acetil-koenzima A u Krebsovom ciklusu. Ukoliko nije dovoljna snabdevenost ovim prekursorima, kao što se često dešava tokom negativnog energetskog bilansa, smanjuje se dostupnost oksa-

loacetata za korišćenje acetil-koenzima A u Krebsovom ciklusu. Višak acetil-koenzima A se zatim koristi za ketogenezu i kod krava mogu da se razviju supkliničke ili kliničke forme ketoze. Izgleda da je neodgovarajuća snabdevenost glikogeno-plastičnih prekursora ključni događaj u ovom procesu [94, 95, 44, 45, 57, 54, 59, 106, 56, 28, 77, 49, 105, 89, 48, 144, 5, 16, 88, 139, 73, 137, 112, 113].

Endokrinologija negativnog bilansa energije

Postpartalni negativan bilans energije može da izazove značajne endokrine promene koje su poznate kao važni činioci u regulaciji procesa mobilizacije lipida. Osim uloge *kateholamina*, najranije promene karakterišu povišene koncentracije glukagona i smanjene koncentracije insulina, dok nivo *hormona rasta* ostaje nepromenjen ili se čak blago povećava. U isto vreme, oslobađanje *hepatičnog faktora rasta-I sličnog insulinu* indukovano sa *hormonom rasta* i *insulinski odgovor* β-*ćelija pankreasa* indukovan *glukagonom* su smanjeni, a izvesna tkiva postaju manje osetljiva na insulin. Svi ovi endokrini događaji mogu da pomere metabolizam iz anaboličnog u katabolično stanje [110, 109, 61, 130, 128, 90, 30, 58, 60, 136, 39, 115, 148, 50, 134, 108]. Ova promena metabolizma može da bude pojačana endokrinim posledicama simultanog izlaganja endotoksinu [38, 37, 70]. Periferna tkiva pokušavaju da uklope svoj trenutni energetski metabolizam u ovo novo katabolično stanje prouzrokovano negativnim energetskim bilansom povećanjem kapaciteta inaktivirajućeg mehanizma (koji proizvodi 3,3,5-trijodotironin – reverzni trijodotironin – rT_3) tiroidnih hormona (tiroksin T_4 i 3,3,5-trijodotironin T_3). Stoga, iako je T_4 odgovor tireoidne žlezde izazvan oslobađajućim tireotropnim hormonom (tireotropin) samo slabo izmenjen, opisani su jasno smanjeni novoi T_4 i T_3 i povećane koncentracije rT_3 u perifernoj krvi [26, 17, 101, 103, 109, 140, 141, 58, 99, 138]. Takođe, odavno se smatralo da je uključena i adrenokortikalna funkcija (proizvodnja kortizola), međutim, od tada nije pronađena bilo kakva eksperimentalna potvrda ove hipoteze. U poslednje vreme, ispitivanja regulacije unošenja hrane i metabolizma energije se usmeravaju na pretpostavljenu ulogu *leptina*. Ovaj nedavno identifikovan proteinski hormon sličan citokinu (16 kDa) luči uglavnom masno tkivo i veruje se da deluje preko nervnih centara hipotalamusa u posredovanju neuroendokrinih mehanizama odgovornih za snabdevanje organizma energijom. Moguće je da signalizira o nutricionom statusu i za periferne organe. Ovaj hormon je jedan od primarnih činilaca koji prenosi informaciju o nivou rezervi periferne energije do regiona mozga koji treba da usaglašava ponašanje u hranjenju, metabolizam i endokrine funkcije da bi se održala homeostaza energije [150, 43, 68, 63]. Kod miševa, pacova, ljudi, a takođe i kod preživara i svinja, njegova koncentracija u krvi može da se menja direktno sa promenama u telesnoj masi i deponovanih masti u telesnim depoima. Leptin doprinosi regulaciji kaliozne masti u telesnim depoima. Izlaganje delovanju insulina, glikokortikosteroida i endotoksina može da poveća ekspresiju gena za leptin i/ili nivo leptina u plazmi, dok leptin direktno može da koči sintezu kortizola u adrenalnim ćelijama. Na taj način,

leptin i kortizol interreaguju u mehanizmu negativne povratne sprege. Inkubacija kultura adipocita 24 časa sa T_3 (ali ne i sa T_4) značajno povećava ekspresiju iRNA za leptin i nivo izlučenog leptina. Oslobađanje leptina iz adipocita je regulisano adrenergičnom stimulacijom, a veruje se i da je povezano sa lučenjem hormona rasta i IGF-I [27, 24, 62, 63, 87, 41, 74, 25, 69]. Test sistemi (za više vrsta životinja i kasnije specifični samo za jednu vrstu), koji omogućavaju preciznu kvantifikaciju leptina u krvnoj plazmi kod različitih vrsta domaćih životinja, razvijeni su tek kasnih devedesetih godina i na početku ove dekade. Od tada, dostupno je sve više podataka koji nagoveštavaju da je leptin možda povezan sa negativnim energetskim bilansom i kod preživara, uključujući i goveda [14, 24, 92, 4, 31, 36, 72, 13, 25, 69].

Funkcija jajnika i negativan bilans energije kod postpartalnih mlečnih krava

• Nastavak regularnog rasta folikula. Početak ciklične aktivnosti jajnika

Kod skoro svih mlečnih krava koje ne doje telad koncentracije FSH u plazmi se povećavaju do najviših vrednosti četiri do pet dana posle teljenja. Ovaj prvi „*peak*" FSH odmah prati pojava prvog postpartalnog talasa folikula koji proizvodi prvi dominantni (>9mm) folikul. Zapaženo je da se zatim javljaju pravilni novi talasi FSH koje prati rast novih populacija folikula i proizvodnja novih dominantnih folikula, uprkos prosečnom negativnom bilansu energije od –7,5 Mcal/dan tokom prvog tronedeljnog perioda posle teljenja [10]. Čini se da pojavu folikularnih talasa u ranom postpartalnom periodu krava ne remeti negativan bilans energije i da se javlja kao odgovor na ponovno uspostavljanje periodičnih porasta koncentracije FSH koji su sinhronizovane samo sa završetkom gestacije i porođaja. Čini se da je tokom negativnog bilansa energije u prvim sedmicama laktacije deficitaran LH, a ne FSH. Prema tome, izgleda da negativni energetski bilans ne utiče na regularan početak rasta folikula koji zavisi od FSH [79, 116, 104, 10, 12]. Međutim, neophodna su dalja istraživanja steroidogene sposobnosti prvog dominantnog folikula formiranog tokom druge sedmice posle teljenja, kao i činilaca koji utiču na njegovu sudbinu.

Opisana su tri oblika razvoja folikula u postpartalnom periodu u zavisnosti od sudbine prvog dominantnog folikula [116, 104, 10, 11, 12]: (1) *ovulacija* dominantnog folikula prvog talasa; (2) razvoj *anovulatornog* dominantnog folikula prvog talasa koga prate (više drugih) dodatni talasi razvoja folikula pre prve ovulacije; (3) razvoj dominantnog folikula prvog talasa koji postaje *cističan*. Oblici 1 (ovulatorni) i 3 (cistični) karakterišu se razvojem dominantog folikula koji proizvodi 17 -estradiol, dok oblik 2 karakteriše rast dominantnog folikula koji proizvode samo niske periferne koncentracije 17 -estradiola i postaju atretični. U slučaju oblika 1, ovarijalna aktivnost postaje ciklična posle ovulacije dominantnog folikula prvog talasa. Oblici 2 i 3 mogu da budu ponovljeni više puta, tako da i regresija

dominantnog folikula prvog talasa i onih sledećih i formiranje anovulatornih cista može da produži interval do prve postpartalne ovulacije (pp. *aciklični period*). Od ranih osamdesetih godina opšte je prihvaćeno da je balans energije jedan od najvažnijih činilaca koji utiču na trajanje prvog acikličnog perioda: prva postpartalna ovulacija kod mlečnih krava koje ne doje telad, javlja se oko *10. dana posle nadiranja negativnog energetskog bilansa* [18, 22].

Postoji svega nekoliko radova u literaturi u kojima se ispitivao direktan odnos između negativnog energetskog bilansa i folikularne dinamike kod postpartalnih mlečnih krava. Lucy i sar. [83] pokazali su da se broj folikula klase 3 (prečnika 10 do 15 mm) – ali ne i klase 1 (3 do 5 mm) i 2 (6 do 9 mm) povećao sa većim pozitivnim energetskim balansom pre 25. dana posle teljenja. Ovaj podatak ukazuje da, kako se kod krava popravlja energetski balans, pojačano je i pomeranje folikula iz manjih u veće klase. Tokom prvog postpartalnog folikularnog talasa (8 do 14. dan posle teljenja) kod krava koje dobijaju tri nivoa masti u ishrani, broj folikula klasa 1 i 2 nije u korelaciji sa energetskim balansom tokom 1. ili 2. nedelje postpartalno, bez obzira na vrstu ishrane [11]. Razvoj dominantnog folikula kod postpartalnih mlečnih krava podnosi stanje negativnog energetskog bilansa. Međutim, nekoliko autora je ukazalo da su krajnji prečnik dominantnog folikula i proizvodnja 17 -estradiola pod uticajem metaboličkih činilaca: i veličina dominantnog folikula i nivo 17 -estradiola u plazmi povećali su se pošto se energetski balans poboljšao posle svog najizraženijeg negativnog nivoa [10, 11].

Metabolički i endokrini signalni mehanizmi

Uticaj negativnog energetskog bilansa na vreme prve postpartalne ovulacije potvrđen je relativno davno [18, 22]. Međutim, fiziološki signalni mehanizmi koji obaveštavaju osovinu *hipotalamus-prednji režanj hipofize-jajnik* (HHJ) o trenutnoj fazi energetskog bilansa ostali su nepoznati neko vreme, a neki detalji su čak i danas pod znakom pitanja. Ograničeno unošenje energije nije izmenilo gustinu hipofiznih GnRH receptora kod krava postpartalno [96], ali su ograničenja energije u obroku pratila smanjeni [114], kao i povećani [115] odgovor na egzogeni GnRH. Ustanovljeno je da *gubitak pulsatorne sekrecije LH* rezultira iz produženog neadekvatnog snabdevanja energijom i kod tovnih rasa krava koje doje telad i kod mlečnih krava koje ne doje telad [79, 23, 102]. Do sada je opšte prihvaćeno da ponovno uspostavljanje sheme pulsatorne sekrecije LH koja uzrokuje predovulatorni razvoj i funkciju folikula predstavlja ključni događaj u povratku ovarijalne cikličnosti kod postpartalnih mlečnih krava koje su prošle kroz fazu najnižeg stepena balansa energije [12]. Izgleda da se postpartalni oporavak hipofiznog sadržaja LH i odgovora na GnRH završava do 10. dana posle teljenja kod mlečnih krava, a dostupni podaci po vrstama sugerišu da se primarni efekat smanjenog unošenja energije ispoljava uglavnom preko hipotalamusa [96, 117]. U prvim nedeljama laktacije smanjena aktivnost GnRH pulsatorna kod

postpartalnih mlečnih krava ispoljava se kao smanjena pulsatorna aktivnost LH folikularne steroidogeneze koja je neophodna za indukovanje rasta LH sličnog predovulatornim, kao i pratećoj ovulaciji. Međutim, naizgled niska pulsatorna frekvencija LH (2 pulsa na 6 časova) očito je dovoljna da podrži morfološki razvoj dominantnog folikula do 2. sedmice *post partum*. Ovo zapažanje je u skladu sa rastom i diferencijacijom kompetentnog dominantnog folikula tokom srednje lutealne faze estrusnog ciklusa krava kada je frekvencija LH pulsa niska [33].

Istraživanja potencijalnih metaboličnih signala za osovinu hipotalamus – prednji režanj hipofize – jajnik uglavnom su usmeravana na metabolite u krvi (neesterifikovane masne kiseline, glikoza) i metaboličke hormone (odnos insulin:hormon rasta, insulin, IGF-I), za koje se zna da variraju tokom izmenjenih stanja energetskog metabolizma. Međutim, što se tiče neesterifikovanih masnih kiselina i glikoze, postoje veoma kontradiktorna mišljenja [66, 23, 50]. Stoga je danas opšteprihvaćeno da bilo koji mehanizam, koji vezuje metabolički status sa osovinom hipotalamus – prednji režanj hipofize – jajnik na kraju mora da uključuje i hormonsku komponentu [12]. Istraživanja Beama i Butlera [10, 11] potvrdila su fiziološku važnost odnosa insulin:hormon rasta u plazmi i u dane najniže vrednosti negativnog energetskog bilansa, što ukazuje da ove hormonske razlike u intermedijalnom postpartalnom periodu mogu da utiču na prvi talas folikularne funkcije. Ustanovljeno je da insulin stimuliše folikularne ćelije *in vitro* i kod krava [127], a mala produženja postpartalnog perioda mogu da imaju važan uticaj tokom vrlo ranih faza razvoja folikula. Koncentracije IGF-I i jednog od njegovih vezujućih proteina (IGFBP-2) u krvi u peripartalnom periodu bili su dobri pokazatelji sposobnosti krava sa ograničenom energijom da nastave ciklus posle teljenja [108]. Osim toga, povećani odnos insulin:hormon rasta posle porođaja može da izazove veću proizvodnju hepatičnog IGF-I [91], što rezultira u povećanim količinama ovog faktora rasta ranije nego inače posle teljenja. Iako su izneta i neka naizgled kontradiktorna zapažanja kod prvotelkinja [128], tokom prve dve sedmice postpartalno, Beam i Butler [10, 11] uočili su značajno veću koncentraciju IGF-I u krvi krava koje su proizvodile 17 -estradiol, ovulatorni dominantni folikul prvog talasa nego kod onih krava sa 17 -estradiol-anovulatornim dominantnim folikulom prvog talasa. Kod krava koncentracije IGF-I u krvi bile su u korelaciji sa nivoima IGF-I u folikularnoj tečnosti velikih folikula [34]. Zna se da i insulin i IGF-I stimulišu *in vitro* steroidogenezu i proliferaciju goveđih kultura teka ćelija i granulozu ćelijskih kultura [127, 129]. U *in vitro* uslovima, IGF-I poveća broj mesta koja vezuju LH u goveđim teka ćelijama i pojača proizvodnju androstenediona i progesterona indukovanu LH [129]. Ovaj nalaz potvrđuje zapažanje Beama i Butlera [11] *in vivo*, koji su pokazali očigledan odnos između steroidogene aktivnosti dominantnih folikula prvog talasa i nivoa IGF-I u krvi. U kulturama granuloza ćelija čoveka i svinja T_3 takođe može da poveća aktivnost aromataze koja proizvodi estrogen i time prinos 17 -estradiola [87, 52, 143, 53]. Do sada postoji samo jedna studija u kojoj se procenjuje efekat T_3 i T_4 na steroidogenezu ovarijalnih ćelija kod krava: Spicer i sar [126] primetili su direktan stimulatorni efekat T_3 i T_4 na steroido-

genezu teka ćelija. Stimulatorni efekat T_3 i T_4 na proizvodnju androstenediona (tj. povećanje dva do četiri puta) bio je sličan uticaju LH na proizvodnju androstenediona (tj. povećanje četiri ili devet puta). Stimulatorni efekat T_3 bio je u dve faze (niska doza je stimulirala, visoka nije imala efekta), dok je uticaj T_4 bio sličan kod obe doze. Nasuprot tome, T_3 je inhibirao hCG-indukovanu sekreciju androgena u teka ćelijama svinja [53]. Prema tome, mogu da postoje razlike između vrsta i/ili uslova kulture u odnosu na regulaciju tekalne steroidogeneze tireoidnim hormonima, kao i kod steroidogeneze granuloza ćelija. Kod krava, T_4 je bio mnogo slabiji stimulator (tj. povećanje do 1,3 puta) progesterona koji proizvodi teka ćelija nego što je to bio LH (tj. povećanje četiri do devet puta), i njegov efekat je bio uočljiv samo na hipertiroidnim nivoima (100 ng/ml, ali ne i 30 ng/ml); T_3 nije imao uticaja na proizvodnju progesterona granuloza i teka ćelija u ovim istraživanjima [126]. Ustanovljeno je da postoji odnos između niskog 17 -estrogena, negativnog energetskog bilansa i niskog T_3 i kod mladih žena atletičarki koje se takmiče u trčanju [149]. Nasuprot tome, izazvani hipotireoidizam nije uticao na prečnik dominantnih folikula kod multiparnih krava rase brahman [32].

Izazivanjem hipotalamične GnRH i pituitarne gonadotropne sekrecije, zapaženo je i da *leptin* utiče na genitalne funkcije glodara, primata i nedavno ustanovljeno, preživara. Iskustva su potvrdila da bi ovaj hormon mogao da bude jedan od dugo traženih indikatora nutricionog statusa koji omogućava nastavak reproduktivnih procesa. Rezultati kod glodara, primata i kod ovaca nagoveštavaju da je supresija GnRH/LH tokom gladovanja posredovana centralnim dejstvom leptina u hipofizi ili mozgu [40, 97, 98, 93, 122]. Osim toga, Ahima i sar [2] ukazali su da tretman leptinom ubrzava pubertet kod normalnih miševa u dozama koje ne utiču na promene telesne mase i sugerisali da dejstva leptina u regulaciji neuroendokrine i reproduktivne funkcije kod normalnih miševa nisu podređena efektima energetskog balansa. Niska koncentracija leptina je izazvala značajno povećanu proizvodnju neuropeptida Y u hipotalamusu [1], a povećani neuropeptid Y inhibirao je gonadnu osovinu kod laboratorijskih glodara, kao i preživara, pa se zaključilo da oštećena reproduktivna funkcija u lošim uslovima metabolizma kao što je gladovanje, može da bude posledica prevelikog oslobađanja neuropeptida Y u hipotalamusu [47, 118]. Nekoliko zapažanja sugeriše da leptin predstavlja važnu biohemijsku poruku između depoa masti i reproduktivne osovine [1, 6, 2]. Receptori za leptin su otkriveni i u gonadama. U *in vitro* uslovima, leptin ima slabe inhibitorne efekte na steroidogenezu teka ćelija i granuloza ćelija indukovanu gonadotropinom, i/ili IGF-I [128]. Dakle, može da se pretpostavi da ne samo hipoveć i hiperleptinemija mogu negativno da utiču na reprodukciju domaćih životinja, uključujući krave [122].

Promene koncentracije leptina u plazmi peripartalnih mlečnih krava merene su tokom perioda od 35. dana pre do 56. dana posle teljenja. Koncentracija leptina u plazmi bila je najveća tokom kasne faze graviditeta i smanjila se za oko 50 posto posle teljenja. Koncentracija leptina u plazmi ostala je niska tokom rane laktacije i pored postepenog poboljšanja energetskog statusa životinja.

Odgovarajuće promene dogodile su se u velikoj količini iRNA za leptin u supkutanom belom adipoznom tkivu. Postpartalno smanjenje leptina u plazmi bilo je posledica negativnog energetskog bilansa zato što je leptin plazme ostao visok kod krava koje nisu muzli posle porođaja. Koncentracija leptina u plazmi bila je u pozitivnoj korelaciji sa plazma koncentracijama insulina i glikoze i u negativnoj korelaciji sa plazma koncentracijama hormona rasta i neesterifikovanih kiselina [13]. Postoji ograničen broj podataka o međuodnosu između nivoa leptina u krvi i postpartalnom nastavku ciklične funkcije jajnika kod mlečnih krava [72, 67]. Koncentracije leptina u plazmi kod 20 visoko-mlečnih krava holštajn rase držanih pod uzornim uslovima smanjile su se posle porođaja, dostigle najniži nivo 10,1 2,2 dana posle teljenja, a zatim su se povećale i postale stabilne blizu vremena prve ovulacije 25,9 2,0 dana. Interval od teljenja do prve ovulacije je bio u značajnoj korelaciji sa intervalom od porođaja do najnižeg nivoa leptina, ali nije bio u korelaciji sa vrednostima leptina pre porođaja, pre i/ili posle ovulacije [72]. Drugo istraživanje [67] uključilo je dva različita eksperimenta koji su obavljeni sa velikim komercijalnim mlečnim stadima. Nivoi postpartalnog leptina kod krava koje su redovno nastavile cikličnu funkciju jajnika u 35 dana od teljenja upoređeni su sa onima kod kojih je odloženo pojavljivanje cikličnosti (posle 35 dana). Procenat za drugu grupu bio je 31 posto u eksperimentu 2, a 65 posto u eksperimentu 1, možda zbog prejake ishrane ovih krava pre porođaja. Odmah posle teljenja, koncentracije plazma leptina, kao i ocena telesne kondicije bili su skoro isti, bez obzira na buduće trajanje postpartalne acikličnosti. Kod krava u eksperimentu 1 nivoi plazma leptina su varirali u širokom rasponu sa srednjom vrednošću od oko 4 ng/ml prvih jedan do tri dana posle teljenja. Ostali su nepromenjeni do pete sedmice kod krava koje su ovulirale 35. dana, a smanjeni kod onih krava sa odloženim početkom cikličnosti. Kod krava u eksperimentu 2, nivoi leptina prve sedmice bili su niži i manje promenljivi od onih iz eksperimenta 1. Tokom druge i treće sedmice, uočeno je blago povećanje promena u leptinu kod krava koje su nastavile ovarijalnu cikličnost u okviru 35 dana, dok su nivoi leptina ostali nepromenjeni kod onih krava sa odloženim pojavljivanjem cikličnosti. Do pete sedmice, ova tendencija je rezultirala potpuno istim vrednostima leptina kao i kod eksperimenta 1, sa značajnim razlikama između srednjih vrednosti grupa i pete i desete sedmice. U ranim sedmicama laktacije ocena telesne kondicije i telesne mase značajno su se smanjile kod svih životinja, ali je ovo smanjenje bilo očiglednije kod krava kod kojih je odloženo javljanje cikličnosti. U oba eksperimenta, krave sa odloženom pojavom cikličnosti najčešće su imale niže koncentracije glikoze, ukupnog holesterola, nivoa insulina, IGF-I i T_3 i više koncentracije neesterifikovanih masnih kiselina i OH-butirata, nego one krave koje su ovulirale u okviru 35 dana, mada su ove razlike bile značajne samo tokom prvih sedmica uzorkovanja i bile su izraženije u eksperimentu 1. Nivoi leptina ustanovljeni u različitom vremenu posle teljenja bili su u snažnoj pozitivnoj korelaciji jedni sa drugima, i u korelaciji sa ocenom telesne kondicije odmah posle teljenja i kasnije ponovo tokom desete sedmice, ali ne i pete sedmice. Blaga do umerena pozitivna

korelacija leptina sa insulinom i blaga negativna korelacija sa plazma metabolitima koji se direktno odnose na energetski disbalans (neesterifikovane masne kiselina i OH-butirata) uočeni su od druge sedmice (ali ne u prvih nekoliko dana posle teljenja). Iz ovih podataka može da se zaključi da kod postpartalnih mlečnih krava nivo leptina u plazmi može da utiče na nastavak ciklične funkcije jajnika u prvim sedmicama laktacije, ali taj uticaj može da bude samo kao podstrek (na primer, minimalna koncentracija iznad pretpostavljenog praga izgleda da je preduslov za pojavu cikličnosti). Moglo bi da se pretpostavi da su niži, nego obično, nivoi insulina, IGF-I, leptina i možda T_3, nepravilan odnos insulin:hormon rasta i/ili njihova kombinacija, mogu da budu činioci koji obaveštavaju osovinu hipotalamus – prednji režanj hipofize – jajnik o trenutnom nutricionom statusu i sadržaju telesnih masti. Ovaj signal na hipotalamo-hipofiznom nivou ostvaruje se kao pulsna frekvencija bazalne sekrecije GnRH/LH koja je niža od normalne.

Faktori koji utiču na plodnost postpartalnih mlečnih krava koji su povezani sa energijom

Izgleda da se štetni efekti negativnog energetskog bilansa tokom rane laktacije odražavaju i u smanjenoj plodnosti tokom postpartalnog perioda osemenjavanja. U uslovima normalnih mlečnih stada, direktna procena energetskog statusa pojedinačnih krava nije moguća, ali promene u oceni telesne kondicije pružaju indirektnu ocenu. Sa većim gubitkom telesne kondicije, i smanjenje stepena koncepcije je sve izraženije [15]. Krave koje gube jednu ili više jedinica ocene telesne kondicije (na skali od 5) tokom rane laktacije imaju najveći rizik niske plodnosti sa uspehom koncepcije od 17 do 38 posto. Nedavnim istraživanjima je ustanovljeno da su krave ocenjene sa izraženim gubitkom telesne kondicije (1,25 jedinica) imale samo upola mogućnosti za oplođenje kod prve veštačke inseminacije, nego krave sa skromnijim smanjenjem [50]. Nedavna velika studija je ukazala da krave sa ocenom telesne kondicije od 3,0 imaju najveće mogućnosti da koncipiraju pri prvom veštačkom osemenjavanju [81].

- **Odložena pojava cikličnosti jajnika**

Iako se smanjena plodnost kao posledica negativnog energetskog bilansa tokom rane laktacije može da objasni produženom acikličnošću kod 30 do 36 posto krava [66, 68, 133, 107, 113, 71], naše razumevanje veza između negativnog energetskog bilansa i suboptimalnih rezultata koncepcije kod ovulatornih krava ostaje prilično spekulativno. Jedna važna veza između negativnog energetskog bilansa i smanjene plodnosti je izgleda kroz pomenute efekte na pojavu prve ovulacije posle porođaja. Postoji mnogo podataka o pozitivnom odnosu između rane pojave ovulatornih ciklusa i povećanog oplođenja kod veštačkog osemenjavanja. Krave koje ostaju bez ovulacije duže od 35 do 50 dana tokom lak-

tacije imaju značajno manje izgleda da ostanu steone tokom laktacije i biće isključene iz proizvodnje [68, 22, 42, 20, 29].

• **Bakterijske komplikacije involucije uterusa**

Prihvatajući opštu korist ranog povratka na ovarijalne cikluse posle oplođenja, treba obratiti pažnju na interakciju sa zdravljem uterusa. Krave koje rađaju više od jednog teleta i ovuliraju pre 21. dana posle teljenja pokazale su slabije proizvodne rezultate od onih krava koje ovuliraju kasnije uz visoko pojavljivanje perzistentnih žutih tela [123]. Veruje se da je perzistentno žuto telo rezultat infekcije uterusa i njihova pojava kod mlečnih krava je povećana poslednjih godina [111]. Takođe je uočeno veće pojavljivanje nepravilno skraćenih faza žutog tela [65]. Dokazi o infekciji uterusa i endometritisu jasno su povezani sa smanjenim oplođenjem posle veštačkog osemenjavanja i moraju da se posmatraju kao deo sveukupnog trenda ka manjoj plodnosti visoko mlečnih krava [65, 81, 71, 84].

• **Uticaj nivoa progesterona u cirkulaciji kod već cikličnih krava**

Period pre inseminacije

Još jedna važna veza između negativnog energetskog bilansa i plodnosti je preneti efekat na koncentracije progesterona u plazmi. Tokom prva dva do tri postpartalna ovulatorna ciklusa najviši nivo progesterona se povećava u krvi od jednog ciklusa do drugog [68, 142, 136, 133], a tokom prvih sedmica laktacije stepen ovog povećanja progesterona je smanjen ili ga je ublažio negativan energetski bilans [142, 130]. Krave sa najizraženijim negativnijim energetskim statusom tokom prvih 9 dana posle porođaja još uvek su imale smanjene nivoe progesterona u plazmi tokom trećeg estrusnog ciklusa [142] u odnosu na početak perioda osemenjavanja. Koncentracije progesterona u plazmi kod krava odabranih zbog visokog prinosa mleka bile su 25 do 50 posto niže tokom druge i treće lutealne faze nego kod krava iz kontrolne proizvodnje [82].

Postovulatorno povećanje progesterona kod osemenjenih krava

Sposobnost za proizvodnju i održavanje optimalnih koncentracija progesterona važna je za plodnost zbog regulatornog efekta ovog hormona na funkciju endometrijuma [120]: ustanovljeno je da je plazma progesterona viši 4 do 7 dana posle veštačkog osemenjavanja kod krava koje su gravidne, nego kod onih krava koje nisu [1, 21].

Rana stimulacija progesterona (do 5. dana posle fertilizacije) menja endometrijalnu sekreciju i ubrzava razvoj ploda [46]. Nasuprot tome, manja brzina porasta postovulatornog progesterona tokom četvrtog do petog dana dovedena je u vezu sa smanjenom plodnošću [121, 21] i smanjenim rastom embriona do 16. dana [86]. U ovo kritično vreme za majčino prepoznavanje

trudnoće, nedovoljno razvijen embrion izaziva nedovoljne količine interferona-da bi inhibirale uterini luteolitični mehanizam, oslobađanje PGF_2 stimulisano preko oksitocinskog receptora [85], koje samo postaje jače sa nižim progesteronom u cirkulaciji [78]. Osim toga, odloženo povećanje lutealnog progesterona oko četvrtog do petog dana može da omogući prepoznavanje trudnoće i produženu lutealnu funkciju, ali i rani prekid trudnoće zbog toga što je oštećen embrion tokom razvoja [80].

Još uvek nije utvrđen fiziološki mehanizam kojim se negativni energetski bilans rano u postpartalnom periodu pripisuje smanjenoj proizvodnji progesterona dva meseca kasnije. Britt [15] izneo je hipotezu da na ovarijalne folikule štetno utiče izlaganje negativnom energetskom bilansu tokom ranog rasta i razvoja i da bi ovulacija oštećenih folikula mogla da uzrokuje nižu sekreciju progesterona. Ova hipoteza bi mogla da objasni shemu koncentracija progesterona u plazmi kod krava u fazi laktacije, ali moraju da se uzmu u obzir i efekti ishrane na eliminisanje progesterona [64]. Kod ovaca, visok unos energije kroz obrok povećava metaboličko eliminisanje progesterona iz krvi kroz jetru [100]. Ustanovljeno je da je osnovni protok krvi u jetri kod krava u laktaciji dvostruko veći, nego kod krava koje nisu u laktaciji i da se ishranom naglo povećao za 20 do 30 posto [146]. Visok protok krvi u jetri rezultirao je povećanim katabolizmom steroida i 25 posto nižim koncentracijama 17 -estradiola i progesterona u plazmi tokom estrusnog ciklusa, a to je dovedeno u vezu sa većom pojavom degenerativnih embriona petog dana. Tokom rane laktacije, ukupno unošenje hrane kod mlečnih krava dvostruko se povećava do početka perioda osemenjavanja [9]. U ovakvoj situaciji, povišeno eliminisanje progesterona usled visokog dijetetskog unosa (i energije i proteina) može da bude kombinovano sa prenetim efektima negativnog energetskog bilansa i da rezultira u nižim koncentracijama progesterona u plazmi i smanjenoj plodnosti. Uterinska sredina zavisi od progesterona, ali može da postane suboptimalna pod uticajem negativnog energetskog bilansa i povećanim metaboličkim eliminisanjem kod visoko mlečnih krava.

- **Kvalitet ovocita**

Rani postpartalni negativni energetski bilans može štetno da utiče na ovocite tokom 80 do 100 dana koji su neophodni za razvoj folikula i time da ima još jedan prenet uticaj na plodnost [15]. U nekoliko istraživanja obavljena je aspiracija ovocita transvaginalno iz ovarijalnih folikula tokom perioda od 30 do 120. dana laktacije. Krave koje su jele hranu visoke energetske vrednosti proizvodile su više ovocita, i to boljeg kvaliteta, nego krave koje su imale hranu male energetske vrednosti, ali bilo je samo malo promena tokom perioda sakupljanja kod te dve grupe [75, 55]. Krave koje su imale jači postpartalni negativni energetski bilans usled jače gojaznosti pre i kod teljenja proizvele su ovocite sa nižom sposobnošću za razvoj posle *in vitro* sazrevanja i fertilizacije, u poređenju sa kontrolnim kravama tokom perioda od 80 do 120 dana laktacije [76]. Dalji dokazi o još

više produženom uticaju na razvoj ovocita mogu da se nađu u radovima Snijdersa i sar [124, 125]. Krave visoke genetske vrednosti davale su ovocite sa manjim potencijalom za razvoj nego krave srednje genetske vrednosti u toku srednjeg perioda laktacije (125-229. dana). Razlike u prinosu mleka nisu uticale na razvoj embriona, ali brzina formiranja blastocista bila je smanjena kod krava sa niskom ocenom telesne kondicije (1,5-2,5). Krave visoke genetske vrednosti imale su manju ocenu telesne kondicije od onih krava srednje genetske vrednosti. Predloženo je da je manje uspešna fertilizacija kod krava visoke genetske vrednosti pre rezultat lošeg kvaliteta ovocita, nego trenutnog nivoa bilo kod parametra metabolizma izmerenog u vreme veštačkog osemenjavanja. Sve u svemu, ova iskustva sugerišu opšteštetan uticaj statusa smanjene energije tokom laktacije na razvoj ovocita, ali ostaje nejasno da li su takvi efekti ograničeni u odnosu na vreme koje je potrebno da se folikul razvije.

Zaključak / *Conclusion*

Reproduktivni rezultati mlečnih krava smanjivali su se tokom poslednjih nekoliko dekada u odnosu na impresivna povećanja prinosa mleka. Zahtevi metabolizma kod visoke proizvodnje mleka rezultiraju većim negativnim energetskim bilansom tokom koga su nivoi glikoze, insulina, IGF-I, leptina i T_3 u krvi uglavnom smanjeni, dok se sadržaj triacilglicerida u jetri i koncentracije neesterifikovanih masnih kiselina (i možda OH-butirat) u plazmi povećavaju. Negativan energetski balans i njegove konsekvence po metabolizam povezane sa postpartalnim razvojem folikula i prvom ovulacijom, variranju koncentracija progesterona u plazmi, koče razvoj ovocita, što uzrokuje smanjenu plodnost. Plodnost mlečnih krava odražava kumulativni efekat metaboličkih, endokrinih i zdravstvenih komponenti koje su modifikovane i prenaglašene selekcijom za visok prinos mleka, kao i izvesnim bolestima (bakterijske komplikacije involucije uterusa, mastitis). Izgleda da je energetski balans najvažniji činilac, ali kompleksne interakcije svih ovih činilaca moraju da se uzmu u obzir i kontrolišu ukoliko želimo da poboljšamo naše razumevanje i da razvijemo nove strategije za poboljšanje plodnosti.

РУССКИЙ

ПОСЛЕРОДОВОЕ ПРОДОЛЖЕНИЕ ЦИКЛИЧЕСКОЙ ФУНКЦИИ ЯИЧНИКОВ, ПЕРВЫЙ ЭСТРУС И ПОВТОРНОЕ ОПЛОДОТВОРЕНИЕ И ИХ ОТНОШЕНИЕ К МЕТАБОЛИЗМУ ЭНЕРГИИ У ВЫСОКО МОЛОЧНЫХ КОРОВ

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В истекших несколько десятилетий земечено постоянное увеличение среднего производства молока у молочных коров кругом мира. В то же самое время, между тем, замечено драмматическое уменьшение репродуктивных результатов. Эта тенденция приписана увеличенному явлению бактериальных ослжнений в

течение инволюции матки, словно и большом числе дисфункций яичников в послеродовом периоде. Цель этой работы рассмотреть физиологию и патологию этого другого явления, этого действительно комплексного феномена. Питательная основа этого процесса,что нужды высоко молочных коров резко изменяются после родов, когда дневной выход молока резко увеличивается, а последовательный отрицательный энергетический баланс ОЭБ продолжается на 10-12 недель. В контексте высокой генетической стоимости молочных коров, послеродовой (пр)ОЭБ разница среди диететического вноса используемой энергии и потребления энергии для содержания массы тела и синтеза молока. В принципе, это физиологический феномен, который между тем, может являться результатом в более или менее тяжёлых расстройствах как в метаболизме так и в репродукции, и так может привести и до больших финансовых убытков в современном производстве молока (Rkkwamsuk и сотр., 1999. г.). В первых 3-4 недели после теления, ОЭБ в высокой корреляции и с выходом молока и с интервалом до первой овуляции. Вследствие этого, что показано, что число овуляторных эструальных циклов, предыдущие осеменению (ОС) влияет на степень зачатия, длина пр интервала до первой овуляции обеспечивает важный параметр для оценки эффекта ОЭБ на репродуктивные результаты (Butler, 2000 и 2001).

Ключевые слова: высоко-молочные коровы, метаболизм энергии, после родов, циклическая функция яичников, первый эструс, повторительное оплодотворение