Artykuł przeglądowy *Review*

Development of ovarian follicles, quality of oocytes and fertility of cows in view of a negative energy balance in the transition period

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Summary

Energy metabolism in cows during the first three weeks after calving has a decisive effect on the incidence of the first estrus postpartum, the length of the voluntary waiting period and interpregnancy period. Additionally, the negative energy balance (NEB) is usually promoted by the relatively one-sided selection of cows towards high production – accepting the occurrence of NEB after calving as well as excessive body condition of cows before calving. In case of a rapid body condition loss in cows after calving, connected with fat mobilization, characteristic of cows in excessive body condition during drying off, ovarian follicles are not properly formed. Disorders of the ovulation process as well as a lower secretion of progesterone are more frequently recorded in those cows. As a result of an energy deficit at the beginning of lactation, the frequency of GnRH pulses from the hypothalamus is lower in high-producing cows. A lack of pulsation secretion of LH as an effect of the prolonged lack of adaptation of a sufficient amount of energy to the needs of the female was found both in beef and dairy cows. The recurrence of LH pulses, stimulating the onset of ovarian activity and the pre-ovulatory development of ovarian follicles, is crucail to the return of cyclical activity in the postpartum period in cows suffering from NEB. An energy deficit causes the incidence of a dysfunction of the reproductive system in the form of persistent ovarian failure, a delayed onset of normal ovarian activity, and the appearance of the first corpus luteum postpartum. The number of estrus cycles occurring before effective insemination is also reduced and both the voluntary waiting period and interpregnancy period are longer. An energy deficit in the postpartum period results in disorders in the functioning of the endometrium. Disturbed energy-consuming processes of endometrium remodeling lead to delays in uterine involution. Reduced uterine contractility and changes in its environment constitute causes for the retention of the placenta and postpartum metritis; in cows with NEB it was a reduced mitogen response of lymphocytes to cytohaema gglutinin. Moreover, a reduced phagocytic activity of macrophages was found in the milk of cows, in which high contents of ketone compounds were found in their plasma. Similarly, the activity of lymphocytes and neutrophiles was reduced in the environment with an addition of β-hydroxybutyric and acetoacetic acids, as well as elevated PUFA levels and a reduced blood leptin concentration.

Keywords: cow, negative energy balance, development of ovarian follicles, oocytes, embryos

For several decades the fertility of high-producing dairy cows has been observed to decrease worldwide (6, 10, 16). According to Hungarian data the calving interval in cows extended in the period from 1970 to 2006 from 399 to 438 days (6). It results from an estimation that every day of extension of the interpregnancy period brings losses ranging from $2.5 to $5 (28). In Great Britain only 35% of healthy born calves came from pregnancy after the first insemination (10). In the rather unanimous opinion of many authors the deterioration of fertility in most cases is caused by a conflict between the metabolic needs of cows, resulting from high production, and the capacity to satisfy them as a result of limited feed uptake after calving (5, 22). Thus, in high-producing cows an energy deficit occurs together with several adverse consequences connected with reduced productivity, metabolic and hormonal disorders, as well as different forms of fertility disorders. Hormonal disorders in the postpartum period are manifested in a low blood concentration of the gonadotropin-releasing hormone (GnRH) and the luteinizing hormone (LH). Consequently disorders develop in the postpartum development of ovarian follicles, impaired uterine involution, delayed ovulation as well as the dysfunction of local defence mechanisms. This may result in an elevated frequency of postpartum metritis, clinical cases of endometritis and the appearance of ovarian cysts.

Causes and consequences of negative energy balance (NEB)

The energy metabolism in cows during the first three weeks after calving has a decisive effect on the date of the first estrus postpartum, the length of the voluntary waiting period and the interpregnancy period. Additionally, a negative energy balance (NEB) is usually promoted by a relatively one-sided selection of cows towards high production – accepting the occurrence of NEB after calving as well as an excessive body condition of cows before calving (2, 29, 33). Fat cows are characterised by a poorer appetite after calving, compensated for by the mobilisation of their own adipose tissue, a rapid loss of body condition and body weight as well as considerable deviations from the appropriate metabolic profile (12, 41). In the case of a rapid loss of body weight in cows after calving, connected with fat mobilisation, characteristic of cows with an excessive body condition during drying off – ovarian follicles after calving do not develop properly (29). In such cows disorders of the ovulation process and a lower progesterone secretion are recorded more frequently, together with the tendency towards a deep and long-term energy deficit (9, 41). In turn, Brydl et al. (6) reported that the percentage of cows with the subclinical fat mobilisation syndrome increased during the last decade from below 2.5% to 26% in the year 2006.

Issues related to causes of negative energy balance have been repeatedly discussed in the available literature. The occurrence of an energy deficit is caused by endogenous and exogenous factors. The latter include high milk yields of cows, the specific character of energy metabolism in ruminants and an excessive body condition during drying off (body condition score BCS > 4.0) and disorders of hormone metabolism (6, 11). The risk of NEB incidence in cows at the beginning of lactation is connected with difficulties in consumption of large amounts of feed. In the first week after parturition cows consume by 30-35% less feed in comparison to the period between the 8^{th} and 12^{th} week of lactation (full feed consumption capacity). The other group of factors (exogenous) is related with a too low concentration of energy in the feed ration, low feed intake and excessive body weight loss in the last 3 weeks before calving. NEB is also a consequence of poor quality feeds used in the feeding of cows and a too low proportion of concentrates in the feed ration. Cows consuming more feed and characterised by a higher dry matter uptake at an earlier time eliminate post-parturition NEB, produce more milk and have lower body weight losses, while ovulation appears in such animals earlier after parturition in comparison to cows consuming less dry matter in that period. As a consequence cows obtain the missing portion of energy from their own fat reserves, formed in the drying-off period. A long-term energy deficit may lead to a body weight loss and a deterioration of milk yields. Such conditions also contribute to the incidence of metabolic diseases, ketosis, retention of placenta and abomasum translocation (29). Primary ketosis is a typical disorder in high-producing cows in the periparturient period. This condition is caused by an insufficient amount of glucose in the blood of cows. In the period of deep NEB energy is mobilised from the adipose tissue reserves. Free fatty acids released to meet energy demand are not completely oxidised in the liver, without oxalacetic acid originating from glucose. This results in the formation of harmful ketone compounds, such as acetones, acetoacetic acid and β-hydroxybutyric acid, at the limited capacity of their energy utilisation (41). The accumulation of large amounts of β-hydroxybutyric and acetoacetic acids in the organism causes a shift in the buffer capacity of blood. This may lead to disorders in the acid-base balance and the formation of subclinical metabolic acidosis, relatively frequently accompanying cases of spontaneous ketosis in dairy cows (34).

Development of ovarian follicles after calving

In dairy cows the first ovarian follicles appear around the fifth day after calving. Within a relatively short period characteristic follicle waves appear, completed with the domination of one of the follicles. This form of ovarian activity is independent of the purpose type of cattle and coincides in time with an increase in the serum FSH concentration (32). The first dominant follicle (DF) with a diameter of more than 9 mm appears under physiological conditions at the earliest after day 12 postpartum (15, 37, 39). It is accompanied by a considerable increase in the production of estrogens, dynamic uterine involution most intensive approx. 2 weeks after parturition, and an enhanced production of prostaglandin (18). In the later period regular formations of new FSH waves occur shortly before the onset of the next follicular wave and the appearance of the next follicle. The first ovulation is recorded approx. at 15-27 days after parturition; however, it is relatively rarely accompanied by estrus (34). Ovulation connected with full estrus symptoms in dairy cows takes place after the second and more frequently third or fourth wave completed with the domination of a follicle (1). In comparison to dairy cattle, ovulation in beef cows occurs later. This is caused by e.g. suckling calves being left with their mothers or an extended period of an insufficient energy balance. In such instances in cows with a poorer body condition until the moment of the first post-partum ovulation there may be around a dozen follicle waves (39). There is evidence confirming a thesis that ovulation appears preferentially in the contralateral ovary in relation to the horn in which the pregnancy was developed and the corpus luteum was found. On the other hand, the presence of the dominant follicle on the ypsilateral ovary in relation to the previously pregnant horn – between day 14 and 28 pp. – has a significant effect on fertility, contributing to a shortening of the voluntary waiting period (37). Literature sources – based on the fate of the first dominant follicle – describe three potential ways of post-parturition development of ovarian follicles: i.e. 1) the first follicle wave completed with the DF ovulation; 2) development of the non-ovulating dominant follicle of the first wave occurring after several additional waves preceding the first ovulation; and 3) the development of the dominant follicle, being transformed into a cyst. The first and the third ways are characteristic of ovarian follicles producing large amounts of 17-β estradiol (E2), whereas the second situation is typical of follicles producing slight amounts of this hormone and undergoing atresia. In the first case ovarian activity is initiated after the ovulation of the dominant follicle of the first wave. In case of the second and third waves cows may return to service many times, while the anoestrus period up to the first ovulation postpartum is considerably extended (4, 15). It needs to be stressed at this point that an active blood flow probably exists both in ovulating and non-ovulating dominant follicles (DF) in the first follicle wave after parturition (17).

The relationship of a negative energy balance in the transition period with the development of ovarian follicles

The state of energy nutrition in the transition period is commonly considered a major factor affecting the development of follicle waves after calving, the date of ovulation and fertility in cows. It is a justified hypothesis that the above mentioned fertility disorders occur in animals in which the blood glucose concentration is lower than 2.9 mmol/l (52 mg/dl). In the period of energy deficit cows with a high productivity potential may be lacking as much as approx. 500 g glucose/day (22). Under such conditions the constantly deepening energy deficit contributes to functional disorders in ovaries, the uterus, neurohormonal regulation centres and to metabolic disorders. As a consequence this leads to a considerable deterioration of fertility indexes, shortening of productive lives in cows and increased culling rates. There is a hypothesis that as a result of energy deficit at the beginning of lactation in high-producing cows the frequency of released GnRH pulses from the hypothalamus is reduced. Although the effect of NEB on the date of the first postpartum ovulation has long been confirmed, the physiological signaling mechanism informing the hypothalamus-pituitary-ovary axis (HPO) on the present status of NEB remains open and its certain elements are being repeatedly questioned. A lack of pulse LH secretion, as an effect of an extending failure to supply a sufficient amount of energy to meet the requirements of a female, was found both in beef and dairy cows (7, 20). The re-appearance of LH pulses stimulating the onset of ovarian activity and pre-ovulation development of ovarian follicles is a key to the return of cyclical activity in the postpartum period in cows suffering from NEB (2, 7, 8). A decrease in the amount of circulating LH causes an inhibition of follicle growth and thus a decreased synthesis of estrogens. An inadequate amount of estrogens is insufficient for the occurrence of the full, external symptom of estrus. Such cows are inseminated generally at inappropriate dates, usually after ovulation.

Many authors have claimed that energy deficit causes the incidence of functional disorders of the reproductive system, such as long-term ovarian dysfunction or a delay in the onset of normal ovarian activity, as a consequence a delayed occurrence of the first corpus lutem post partum (24, 41). Moreover, the number of estrus cycles before the first effective insemination decreases and the voluntary waiting period and pregnancy interval are extended (3, 33). It was shown that the diameter of the dominant follicle in cows with post-parturition energy deficit is significantly smaller than in cows fed a balanced feed ration (9, 13). From studies conducted on heifers it can be claimed that a reduction of the level of energy in the feed ration by 40% of their daily requirement resulted in the inhibition of the growth of dominant ovarian follicles and a reduction of the maximum dimensions of dominant follicles, leading to anoestrus in 60% of cows. An improvement of feeding resulted in the appearance of a new, large dominant follicle in the period from 13 to 15 days, the presence of which was connected with an increase in serum concentrations of IGF-I, estradiol and LH release (9). Immediately after calving the level of IGF-I was higher and it increased faster in cows with normal ovarian activity than in those in which ovarian cysts, inactive ovaries and regressive corpus luteum were detected (41). From recent studies it result that during an acute energy deficit after calving a complex insulin-dependent growth factor (IGF) system is involved in the recruitment of ovarian follicles. It moreover the reduced expression of mRNA proteins binding the insulin-dependent growth factor II (IGFBP-2 mRNA) may change the availability of the insulin-dependent growth factor I (IGF-I) in the blood circulation and locally produced IGF-II, required for the modulation of ovarian follicles in the pre-recruitment stage and the return of normal post-parturition activity of ovaries (24).

Effects of a negative energy balance after calving and uterine involution

An energy deficit in the post-parturition period results in disorders in uterine involution and the functioning of the endometrium. Disturbances in energy-consuming processes of endometrial transformation lead to delayed uterine involution. Reduced contractibility of the uterus and changes in its environment constitute a cause for placental retention and post-parturition metritis. In cows with NEB it was the reduced immune mitogenic response of lymphocytes to cytohemagglutinin. Moreover, a reduced phagocytic activity was observed in the milk of cows, in which a high content of ketone compounds was recorded in the blood serum (19). Similarly, the activity of lymphocytes and neutrophiles was reduced in the case of excessive amounts of β-hydroxybutyric and acetoacetic acids as well as elevated levels of PUFAs and a reduced blood leptin concentration (19, 23, 34). Cows with lower levels of insulin, IGF-I, T4 and T3, more frequently exhibited disorders of uterine involution, *metritis puerperalis* and clinical *endometritis* than was observed for cows with normal or slightly changed levels of these metabolites and hormones (36). From recent studies it can be claimed that appropriate levels of both IGF-I and IGF-II play a significant role in remodelling and repair of the uterus after calving and its return to the condition from before pregnancy. A varied expression of proteins binding insulin-dependent growth factors such as IGFBP-3 in endometrial cells, IGFBP-2, 3, 4 and 5 in its stroma, as well as IGFBP-4 and 5 in the myometrium, suggest a strict control of IGF activity by the above mentioned components (25). On the other hand, the intensive release of endotoxins and cytokines (TNFα) in cows with considerable NEB was more strongly expressed than in cows with a moderate negative energy balance, leading to disturbances in LH release, and consequently to the formation of post-parturition ovarian cysts and the induction of premature luteolysis (14, 15, 18).

Energy deficit and quality of oocytes and embryos

There is a limited number of studies analyzing the potential effect connected with negative energy balance (NEB), i.e. reduced glucose concentration and elevated levels of β-hydroxybutyric acid (B-OHB) as well as polyunsaturated fatty acids (PUFAs), on oocyte quality. Apart from the indirect effect of hypoglycemia at the early post-parturition period (through the effect on LH secretion and response of ovaries on gonadotropins), the hypoglycemic condition (i.e. clinical ketosis) affects the microenvironment of preovulation oocytes and may hinder their development capacity, since glucose is a substance necessary for the final maturation of oocytes. This assumption is confirmed by the results of studies in which a relatively low number of embryos fit for transfer were collected from cows subjected to superovulation and burdened with high production, along with the simultaneous high percentage of degenerated embryos. In cows characterised by low productivity and donors of beef breeds the percentage of degenerated embryos was significantly lower. At the same time the proportion of embryos with dark-coloured cytoplasm, indicating their inferior quality, was lower (22). The negative effect of energy deficit on oocytes seems more complex. A deterioration of embryo quality is similarly affected by an increased blood lipid level, characteristic of the post-parturition period in cows with an excessive condition before calving, accompanying the fatty liver syndrome and disturbed energy metabolism. During *in vitro* embryo production it was found that the presence of saturated long-chain fatty acids in the culture environment results in a reduction of the percentage of maturing oocytes, their fertilization capacity, ability to divide and form the blastocyst (21, 27). One of the consequences of insufficient energy uptake and rapid protein catabolism may also be connected with elevated urea and ammonia levels in blood and follicle fluid. The presence of these metabolites in ovarian follicles has a toxic effect on oocytes (26). This fact is confirmed by the observations indicating that cows with milk urea nitrogen concentration (MUN) below 13 mg/dl had a lower calving rate between day 55 and day 70 after insemination in comparison to cows in which the MUN level was elevated (35).

Summing up, in a general common opinion an energy deficit in the transition period has a considerable effect on reproduction in cows. The point of departure of these complications is connected with metabolic disorders leading to a disturbed secretion of sex hormones. This results in different forms of disturbed post-parturition dynamics of ovarian follicles, deterioration of oocyte quality, as well as disturbances in endometrium transformation and local defence mechanisms.

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