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NEGATIVE ENERGY BALANCE AND REPRODUCTION: A REVIEW

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ABSTRACT

The peripartum period is of critical importance to subsequent health and fertility. Most cows enter a state of negative energy balance (NEB) associated with many metabolic changes which have carry over effects on the resumption and normality of estrous cyclicity and the success of subsequent inseminations. High producing dairy cows need to mobilize body reserve to be able to sustain their milk production. In early lactation, until energy intake assures the requirements, dairy cows, especially high producing breeds, enter a state of negative energy balance (NEB), losing high amounts of body condition. The NEB is detectable by measurement of body condition score (BCS), which has been useful in the past and by more sophisticated ways to measure the relationship between adipose tissue and fertility, like metabolic hormones: IGF-I, leptin. The aim of this study is to summarize the effect of negative energy balance and the risk for reproductive disorders in dairy cows.

Key words : Reproduction, Uterus, Dairy cows, Negative energy balance.

Over the past 30 years, fertility in dairy cows has been declining as milk production has increased (Butler, 1998). Majority of dairy cows enter a period of negative energy balance (NEB) in the early post-partum period which may last for up to 15 weeks (Beever *et al.*, 2001, Chandra, 2009). Tissue energy balance is a function of energy intake, body maintenance and milk energy yield. Although selection for yield has involved an increase in appetite, high producing cows in early lactation are still unable to consume sufficient feed to fulfil their needs. This shortfall in energy is met by mobilisation of lipids from adipose tissue and from skeletal muscle protein which is achieved by both endocrine and metabolic adaptations (Bauman and Currie, 1980; Chilliard, 1999). GH secretion increases and insulin secretion decreases after calving, thus increasing lipolysis and resulting in raised levels of non-esterified fatty acids (NEFAs). Liver metabolism is altered and in severe cases hepatic lipidosis occurs, accompanied by the formation of ketone bodies (Kruip *et al.*, 1998). The body reserves available for mobilisation during early lactation depend on the nutritional management during transition period. However, cows that were over conditioned during transition subsequently had a lower feed intake and a higher incidence of metabolic diseases (Huyler *et al.*, 1999). In late gestation and early lactation the nutrient requirements for fetal growth and milk synthesis increase dramatically and the cow is unable to meet these energetic demands from her feed intake. Most cows, therefore, enter a period of negative energy balance (NEB) in the peripartum period from which it may take them many weeks to

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recover (Pryce *et al*., 2001). NEB is thus a metabolic disorder affecting high yielding cows that can impair health and have carry over effects on fertility some months later.

As the demand for milk synthesis increases, reproductive functions may be depressed when no compensatory intake of nutrients is achieved. Numerous recent studies have reported that reproductive performance is compromised by the nutrient demands associated with high levels of production. Milk yield increases at a faster rate in the first 4 to 6 wk after parturition than energy intake, consequently high yielding cows will experience some degree of negative balance of energy and other nutrients during the early postpartum period. When cows experience a period of NEB, the blood concentrations of nonesterified fatty acids (NEFA) increase, at the same time that insulin-like growth factor-I (IGF-I), glucose, and insulin are low. These shifts in blood metabolites and hormones might compromise ovarian function and fertility. It has also been reported that energy balance and dry matter intake (DMI) might affect plasma concentrations of progesterone (Vasconcelos *et al*., 2003; Villa-Godoy *et al*., 1988), which may interfere with follicle development and maintenance of pregnancy. During the last decades, genetic selection and improved management of herds have dramatically increased milk production of dairy cows, at the same time their fertility has decreased (Butler, 2000). Selection for higher milk production in dairy cattle has changed endocrine profiles of cows so that blood concentrations of bovine somatotropin and prolactin have increased; whereas insulin has decreased (Bonczeck *et al*., 1988). These hormonal changes and the increased nutrient demands for production might negatively impact reproduction of dairy cows. However, adequate nutrition and sound management have been shown to offset depression of fertility in herds with average milk production exceeding 12,000 kg/cow/yr (Nebel and McGilliard, 1993; Jordan and Fourdraine, 1993).

Negative energy balance in dairy cows

Dairy cattle are at increased risk for many diseases and disorders during early lactation. At this time, usually during the first third of lactation, there is an increasing milk production, but a lags in feed intake. This combination creates a negative energy balance (Leslie *et al*., 2003, Chawla, 2001).

High yielding cows have been selected and bred to produce more milk, mostly through their ability to mobilize fat and muscle to support milk production in early lactation. This results in a loss of body condition and is associated with alterations in blood metabolite and hormone profiles which in turn, influence fertility (Lesile *et al*., 2003, Taylor *et al*., 2003). In this situation, the cows rely on the mobilization of adipose reserves and they often lose 60% or more of their body fat in the first weeks after parturition.

Patton *et al*. (2006) showed that genetic selection for increased milk yield has amplified the difference between feed intake potential and milk yield potential in early lactation, resulting in cows that are genetically predisposed to a greater risk of NEB.

It has been documented that the severity and duration of NEB are positively associated with the interval to first postpartum ovulation. In addition, NEB in early lactation exerts latent negative effects on the quality of oocytes ovulated 80-100 days later, reducing conception rates in the first weeks of the breeding season. Minimizing the extent and duration of NEB in early lactation is an important factor for achieving optimum reproductive performance (Patton *et al*., 2006).

de Vries and Veerkamp (2000) have shown in their study that a low nadir of EB is correlated with a delay in the postpartum start of luteal activity. Cows experiencing stronger negative EB postpartum average fat percentage later in lactation. Therefore, the decrease in fat percentage in the first weeks postpartum can be used as an indicator of energy deficits during early lactation and of EB-related problems of a cow, such as delayed resumption of ovarian activity (de Vries and Veerkamp, 2000).

Management of prepartum period is also very important to control because high body condition score (BCS) at calving leads to a greater loss after calving and thus a lower postpartum BCS (Chandra, 2009). This excessively rapid mobilization of fat early in the postpartum period is a major risk factor for prolonged anovulatory periods. The delay to the beginning of energy balance recovery after parturition is positively correlated with the delay from parturition to first ovulation (Kadokawa and Martin, 2006).

Metabolic and endocrine changes in the periparturient cow

The major adaptive changes occur around the time of parturition. The late dry period coincides with the last phase of fetal growth when nutrient requirements for gravid uterus increase (Bauman and Currie, 1980). Within a few days before calving, a further dramatic rise in the demand for glucose, amino acids and fatty acids for milk synthesis occurs as lactation becomes established. Nonesterified fatty acids (NEFA) released from lipid stores are taken up by the liver, where they may be oxidized to carbon dioxide to provide energy, or partially oxidized to produce ketone bodies or acetate. These are transported for use elsewhere in the body. Alternatively NEFAs may be esterified to triacylglycerols (TAGs) which accumulate in the liver, peaking in concentration at 7-13 days after calving, then declining gradually (Bauman and Currie, 1980; Chandra, 2009). Beta-hydroxybutyrate (BHB) is the predominant form of ketone body in blood and its concentration is an index of fatty acid oxidation. At least 50% of all dairy cows are thought to go through a temporary period of subclinical ketosis in the first month of lactation. This adaptive strategy is for maintaining blood glucose; despite the large increase in demand, circulating concentrations of glucose generally only show a brief fall for around 1-2 weeks at calving.

Blood urea level often fluctuates around calving, influenced by a variety of factors. Glucose availability may be supplemented by increased catabolism of amino acids stored in skeletal muscle and other tissue proteins, thus increasing urea production (Bell, 1995). The concentrations of rumen degradable and undegradable protein (RDP and UDP), the ratio of energy to protein in the diet and the impact of these dietary factors on protein synthesis by rumen microorganisms are all important (Bell, 1995; Moore and Varga, 1996). Degradation of RDP causes a rise in circulating ammonia concentrations, particularly during energy deficit, and urea production by the liver also requires energy, and may exacerbate NEB. Impaired liver function, as commonly occurs after calving, and reduces the metabolic clearance of urea (O'Callahan *et al.*, 2001).

Many metabolic hormones and their receptors also alter in concentration over this critical peripartum period. In particular, interdependent changes occur in the GH-insulin-IGF-I-glucose signaling pathway (Lucy *et al.*, 2001). IGF-I is believed to be the main mediator of GH (growth hormone) on milk production, regulating milk synthesis by the mammary gland. Most IGF-I in the circulation is released from the liver in response to growth hormone (GH) coupling to GH receptors, with systemic IGF-I negatively feeding back to the pituitary to regulate GH release. During NEB, however, the GH-IGF axis uncoupled due to a down-regulation in liver GH-R, associated with a reduction in IGF-I and elevated GH concentrations. This, coupled with the low prevailing insulin, provides an endocrine environment which promotes direct action of GH on lipolysis and gluconeogenesis in early lactation. At the same time, the indirect actions of GH on growth, which are supported by IGF-I in peripheral tissues, are attenuated. This uncoupling means that the relationship between IGF-I concentrations and yield is initially negative in early lactation, whereas later in lactation, an increase in liver GH-R restores the positive relationship between GH and milk production (Taylor *et al.*, 2003).

Changes in blood metabolites associated with lipid mobilization may affect oocyte quality directly, or indirectly, via alterations in the follicular environment as presented in Fig.1 (Taylor *et al.*, 2003). Profound changes in the liver lead to reductions in the concentration of GH receptor, IGF-I, several of IGF binding proteins and the acid labile subunit whilst IGFBP-2 is increased. This results in a marked decline in the circulating concentration and half-life of IGF-I which can also impair follicular maturation and steroidogenesis. The uterine repair mechanisms, after calving, are so delayed although it remains uncertain how this effect is mediated.

Leptin is another metabolic hormone of interest in relation to EB as its circulating concentrations is strongly correlated to BCS and falls in late pregnancy (Taylor *et al.*, 2003). However, leptin concentrations remain low postpartum even when the EB status has improved (Ingvartsen and Boisclair, 2001; Wathes *et al.*, 2006). Leptin can influence voluntary feed intake and may also contribute to the peripheral insulin resistance which occurs in peripartum ruminants (Ingvartsen and Boisclair, 2001; Wathes *et al.*, 2006).

Leptin related to reproduction

Leptin is a cytokine-hormone secreted mainly by adipose tissue that has been proposed to act as a direct metabolic signal to the sites in central nervous system that control pulsatile LH release. It seems very likely that leptin accompanies IGF-I in the control of the resumption of ovulation. Kadokawa and Martin (2006) made a study on the relationship between plasma leptin concentrations and the timing of the first ovulation postpartum in Holstein dairy cows. Leptin concentrations declined after parturition and, after reaching nadir, it increased

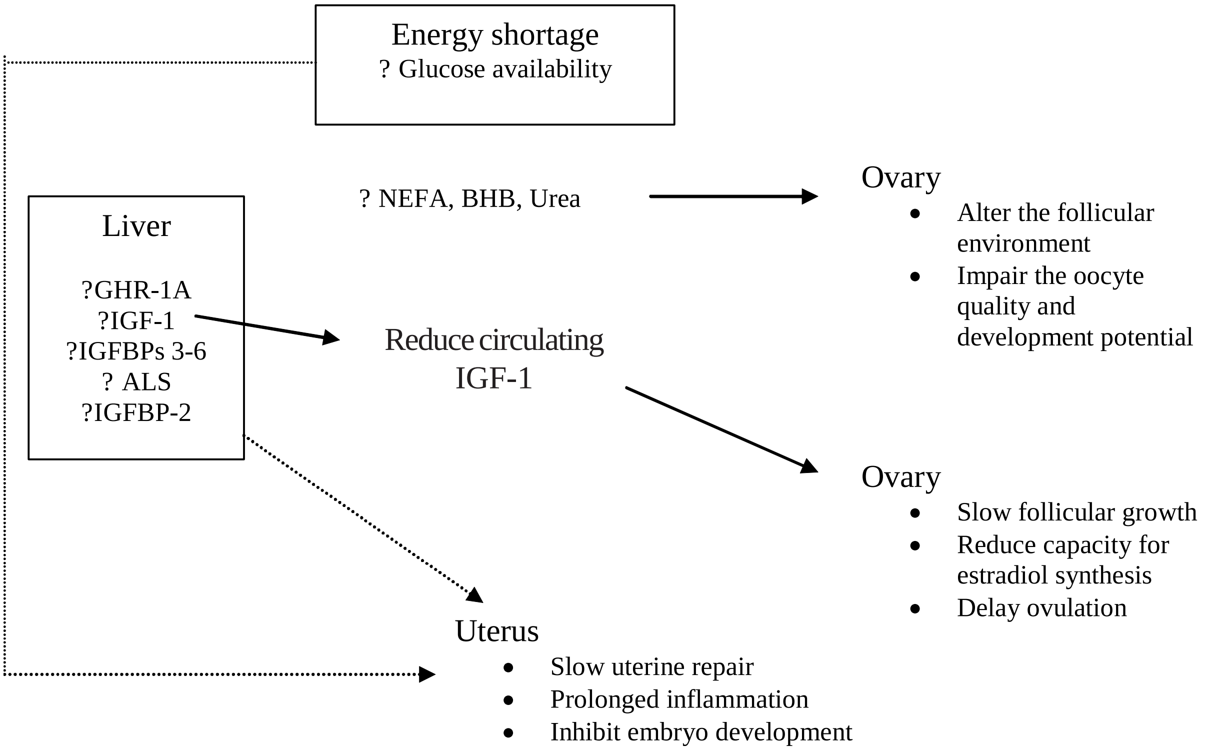

Fig. 1 : Summary diagram showing how negative energy balance may influence fertility through effects on the liver, ovary and uterus.

and became stable near the first time of first ovulation. The delay to first ovulation was correlated with the interval from parturition to the leptin nadir, suggesting that a delay in the recovery of leptin secretion increases the delay to the first ovulation. Other studies found that leptin levels are low during the early postpartum period, when LH pulses are likely to be inhibited because GnRH neuronal activity is suppressed by various neurotransmitters, such as opioids. The decline in leptin production is likely to be due in part to the negative energy (Aggarwal, 2010). These observations suggest that there should be a direct relationship between pulsatile LH release and leptin concentrations in dairy cows during the postpartum period before the first ovulation (Aggarwal *et al.*, 2009).

Kadokawa and Martin (2006) have tested this hypothesis, and found that LH pulse frequency was positively correlated with energy balance and the plasma concentrations of leptin, and also that LH pulse amplitude was correlated only with leptin values. Between leptin production and the reproductive axis seems to be a strong physiological link, thus, the most obvious possibility is that leptin is being involved in the control of pulsatile LH secretion as a major factor in the ovulatory process. Leptin concentrations remain low postpartum even when the EB status has improved. Like this, leptin can influence voluntary feed intake and may also contribute to the peripheral insulin resistance which occurs in peripartum ruminants. In ruminants, circulating leptin concentrations are positively correlated with body fatness, but this relationship only explains about 10-30% of variation in plasma leptin concentration. This means that other factors must be playing more important roles. Kadokawa and Martin (2006) consider that among those factors is also the feed intake. In their study they observed postprandial increases in leptin concentration; glucose did not affect leptin secretion from ruminant adipocytes, but it is possible that there are indirect effects induced by glucose infusion or by volatile fatty acids (VFA) that may stimulate the leptin secretion. Therefore, leptin secretion is regulated in the short, mid- and long term by feed intake, nutrients and hormones by feeding level, energy balance, physiological stage, body fatness and the reflection of the nutritional history of the animals (Aggarwal *et al.*, 2009).

Mechanisms by which NEB status may affect fertility

This review will focus on activity at the level of the ovaries and reproductive tract though it is recognized that signaling of EB status to the brain may also be an important mediator of fertility.

Effects on the ovary

Development of a preovulatory follicle in the dairy cow takes at least 40 days from the early antral stage to ovulation. There is extensive evidence that NEBAL can affect follicular and luteal development and also the quality of the oocyte. In an ideal situation a cow will ovulate for the first time around 20–30 days after calving, thus allowing time to have 1–2 ‘‘normal’’ (19– 24 day) estrous cycles before first insemination after a voluntary wait period of around 60 days. This means that follicles that are due to ovulate at the start of the service period undergo their earlier stages of development during the NEBAL nadir. Many studies which have monitored commercial dairy herds using milk progesterone profiles have shown that NEB is associated with a greater incidence of irregular cycles that can both increase the interval to first service and reduce conception rates (Taylor *et al.*, 2003; Wathes *et al.*, 2003). Problems include a delay to the first ovulation (DOV1), cycles which are longer than the normal range (prolonged corpus luteum, PCL) and long intervals between successive luteal phases, when cows fail to ovulate again at an appropriate time (DOV2). DOV2 may occur when the cow suffers from stress, illness and/or reduced feed intake just before the expected time of ovulation following luteolysis (Wathes *et al.*, 2003). The condition of PCL has traditionally been associated with uterine infection. The incidence of such problems has increased over the past 40 years as milk yields have raised, and now typically around half of the cows in a high yielding dairy herd experience obvious cycle irregularities (Taylor *et al.*, 2003). The initiation of follicle waves after calving occurs regardless of NEBAL status, but the development to a preovulatory size and likelihood of ovulation is decreased when BCS is poor (Beam and Butler, 1999). Effects of IGF-I and insulin on the ovary were reviewed by Wathes *et al.* (2003). In the cow, the concentration of IGF-I in the follicular fluid reflects systemic IGF-I concentrations, whereas IGF-II is derived locally from theca cells. Both IGF-I and IGF-II enhances the ovarian action of gonadotropins and stimulate follicle cell proliferation and steroidogenesis. As outlined above, circulating IGF-I concentrations fall sharply in the first week after calving and the extent of the fall influences both the interval to first ovulation and to conception (Taylor *et al.*, 2004; Wathes *et al.*, 2007). Insulin also acts directly on bovine antral follicles, appearing less potent than IGF-I at stimulating proliferation but equipotent in relation to estradiol production (Wathes *et al.*, 2003). There is conflicting evidence as to whether circulating insulin concentrations are related to the time of re-initiation of ovarian cyclicity after parturition. This may be because most cows remain within a normal physiological range. Severe undernutrition can cause insulin levels to fall below this, whereas in grossly overweight cows hyperinsulinaemia may be related to insulin resistance. Either extreme could potentially have adverse effects on fertility. The alterations in blood metabolites associated with NEB are reflected in follicular fluid (Leroy *et al.*, 2004) and higher NEFA concentrations were shown to hamper oocyte maturation and developmental competence in vitro (Leroy *et al.*, 2005). An adverse effect of urea on oocytes was suggested by Sinclair *et al.* (2000), who showed that in vitro blastocyst production was adversely affected when oocytes were derived from heifers fed a diet which generated high ammonia concentrations in follicular fluid.

Effects on the uterus

In addition to direct effects on the ovary, NEBAL may also affect conception rates adversely through actions on the reproductive tract. Uterine involution is a critical component of postpartum reproduction, which involves endometrial tissue repair, myometrial contraction and bacterial clearance. We are investigating how NEBAL can affect uterine recovery, using the NEB model involving differential feeding and milking frequency described above in relation to the liver. Cows in mild or severe EB status were culled in week 2 of lactation, RNA was isolated from the previously gravid horn for screening with a 23K bovine microarray (Affymetrix) and data were analyzed as outlined above. Of the many immune related genes identified as being differentially expressed, 77/79 were significantly up-regulated in SNEB relative to MNEB uteri. Examples included toll like receptor 4 (1.7-fold increase), interleukin (IL)-1b (2.6-fold increase), IL-8 (8.1-fold increase) and the natural antimicrobials beta-defensin-1 (up 2.3-fold) and beta-defensin-5 (up 1.7-fold). The other main category of genes differentially regulated by EB concerned those involved in protein modification and collagen catabolism, such as matrix metalloproteinases (MMPs). For example, MMP-I was the most highly elevated gene in SNEB (>28-fold relative to MNEB). These results suggested that NEB may impede uterine recovery due to a delay in the clearance of puerperal pathogens, so histological sections taken from both uterine horns were also examined for the presence of immune cells. Cows in the SNEB group had more segmented inflammatory cells but fewer mononuclear cells in both the stratum compactum and the luminal epithelium. These differences suggest that a poor EB status is associated with a greater degree of uterine inflammation following calving and a slowing of the repair process. Bonnett and Martin (1995) previously reported that similar histological findings were associated with poor reproductive performance. Retained placenta, metritis and endometritis all negatively affect fertility. For example, a meta-analysis by Fourchon *et al.* (2000) found that endometritis increased the calving to conception interval by 15 days on average and also reduced the relative risk of conceiving at all in <150 days by 31%. We have shown previously that MP cows that failed to conceive had lower IGF-I concentrations, both before and after calving (Taylor *et al.*, 2004). It is likely that these two aspects are linked, such that uterine recovery is more protracted in cows in poor EB status. Lower circulating IGF-I concentrations may also impair the early stages of embryo development more directly by altering the oviductal and uterine environment as reviewed previously Wathes *et al.* (2003). Butler (2001) suggested that the deleterious effect of elevated urea may be mediated through increased uterine pH that is then hostile to both gametes and embryos. On the other hand, several studies reviewed by Laven *et al.* (2007) found no effect of a high nitrate intake on embryo development. This perhaps suggests that high nitrogen diets do not necessarily have an adverse effect on the uterine environment if the EB status is adequate.

Dietary energy source and reproduction

The products of metabolism supply intermediate signals, which may have an effect on energy balance (EB), health and reproduction in dairy cows in early lactation. Carbohydrates and protein in the diet provide substrates for rumen fermentation, which results in the production of volatile fatty acids (VFA). The main VFA produced are acetate, propionate and butyrate. Acetate and butyrate split into fragments containing two carbon atoms (C2) and are considered lipogenic nutrients. Propionate is a fragment containing three carbon atoms (C3) and is considered a glucogenic nutrient. Dietary ingredients that are resistant to rumen degradation can be digested and absorbed in the intestine and provide either lipogenic or glucogenic nutrients. Dairy cows with metabolic and reproductive disorders in early lactation may suffer from an imbalance in availability of C2 and C3 compounds induced by NEB. Lipogenic nutrients are expected to increase the C2-C3 compound ratio, while glucogenic nutrients decrease the C2-C3 compound ratio. Van Knegsel *et al.* (2007) made a summary of three recent studies, with the objective to see the effect of dietary energy source on the EB and the risk for metabolic and reproductive disorders in dairy cows, in the period of early lactation. The study showed that multiparous cows fed the glucogenic diet tended to have less days postpartum to the rise of P4 (progesterone concentration in ng/ml milk) than cows fed the mixed diet or lipogenic diet. Mean P4 during the first luteal phase tended to be greater for multiparous cows fed the mixed diet or lipogenic diet compared with the glucogenic diet. In conclusion, glucogenic diets fed during the transition period and early lactation decreased milk fat and milk energy output and tended to stimulate the partitioning of energy to body reserves and improve the EB in early lactation compared with lipogenic diets. As indicated by lower plasma BHBA, NEFA and liver TAG concentration, multiparous cows fed a mainly glucogenic diet seemed to have a decreased risk for metabolic disorders, like ketosis and fatty liver. Multiparous cows fed a glucogenic diet tended to have fewer days to first postpartum ovulation (Van Knegsel *et al.*, 2007).

CONCLUSIONS

There is clear evidence that a period of severe NEB around calving adversely affects the future fertility of the cow, in some cases resulting in an animal which fails to conceive at all and must therefore be culled. Many factors influence the extent of the EB deficit. In cows calving for he first time, the age, state of maturity and BCS before calving are all important. In mature cows with a higher potential for milk production, an adequate BCS (BCS, 3) at the start of the service period is essential and this is affected by the genetic potential of the cow to mobilize tissue to achieve a high peak yield in early lactation. Once the cow has entered a state of severe NEB, this is very difficult to rectify by remedial veterinary management during the lactation and the process of recovery may take many weeks. The nutritional management of the cow in the prepartum period is, therefore, absolutely crucial to prevent metabolic diseases and a difficult calving. Recent suggestions that current feeding practices for dry cows may promote deposition of fat in the liver and viscera, thus predisposing the animal to later health problems, make this issue worth revisiting in an attempt to improve fertility. It is also more difficult to manage high genetic merit animals appropriately. Considerations therefore need to be given on an individual farm basis as to the optimum genotype to select for that environment, to provide animals in the herd which can achieve reasonable yields whilst maintaining health and fertility. The metabolic changes associated with tissue mobilization can probably cause damage to oocytes either directly or via alterations of the follicular environment. Adaptive alterations in the GH-IGF axis reduce the bioavailability of circulating IGF-I. This is likely to be a key mediator of EB status on reproductive function, delaying the time to first ovulation and reducing conception rates, possibly through actions on the oviductal and uterine environments. Elevated urea concentrations at the start of the service period are also associated with reduced fertility, but it remains uncertain if this is a direct effect or another symptom of the poor EB status (Kadokawa and Martin, 2006).

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