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Effect of Metabolic Stress on Ovarian Activity and Reproductive Performance of Dairy Cattle: A Review

Review Article

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ABSTRACT

Reproductive efficiency in dairy cattle has been decreased over the last 5 decades despite significant gains in genetic selection, improved reproductive technologies and nutritional care. In high-yielding dairy cattle, the negative energy balance (NEB) during the first week post-partum adversely affects ovarian activity, mainly follicle growth and steroidogenesis. Circulating levels of non-esterified fatty acids (NEFAs) and urea are elevated during NEB and are known to be accumulated in the follicular fluid of dominant follicle where they exert adverse effect on oocyte maturation, leading to low fertilization rate and early embryonic development. This study aimed to review the literatures on the interaction between NEB, metabolic stress and reproductive performance of dairy cattle. In this review, the adverse effects of high circulating NEFAs, a biomarker of NEB and urea on oocyte maturation, embryonic quality and developmental and pregnancy outcomes is highlighted.

KEY WORDS dairy cattle, fertility, metabolic stress, negative energy balance, non-esterified fatty acids.

INTRODUCTION

Reproductive efficiency of dairy cattle has decreased over the past 50 years, despite significant gains in genetic selection and nutritional care for increased milk production. One possible reason for this decline has been attributed to change in the nutritional intake to meet the increased energy and protein demands. The links between nutrition, milk production and reproductive performance constitute important issues in dairy cattle. Nutritional impact on reproductive performance is very complex involving many interactions between food components and hormonal signals. Understanding of mechanisms involved in nutrition-reproduction interactions can be better exploited to improve the reproductive performance of dairy cattle. Intensive genetic selection for increased milk production, coupled with improvement of reproductive technologies and livestock nutrition, has led to significant increases in milk yield in cows in recent decades (Fouladi Nashta *et al*. 2007). However, this increase in milk output has been accompanied by a worldwide decline in cattle fertility (Macmillan *et al*. 1996). The pregnancy rate to a single artificial insemination has been reported to be declining approximately 0.45-1% per year in dairy cattle (Butler *et al*. 2003). High-productivity dairy cows are usually in a state of negative energy balance (NEB) post-partum because the amount of nutrients and energy required for metabolic requirement and milk production, as well as maintenance of animal health, exceeds the amount of energy intake (Butler and Smith, 1989). Embryo mortality during the pre-implantation period is a key contributor to the reduced fertility in the cattle, with up to 40% of total embryo losses

occurring between days 8-17 of pregnancy (Thatcher *et al*. 2000; Bell, 1995). Insufficient energy supply results in poor reproductive performance, which includes a delay in the onset of oestrous cycles during the post-partum period (Butler and Smith, 1989) and a reduction in oocyte quality (Snijders *et al*. 2000). These reproductive alterations result in early embryonic loss and low conception rates (Lucy *et al*. 2001). A prerequisite for good lactation performance, during a cow's life span, is the production of offspring at regular intervals. Therefore, concerns for reproductive efficiency are worldwide in the modern dairy industry, once fertility influences average daily milk production, average days in milk, number of calves born the generational interval and ultimately the farmer's livelihood (Kruif *et al.* 2008). Thisstudy aimed to review the literature on the interaction between NEB, metabolic stress and reproductive performance of dairy cattle. A discussion is devoted to the adverse effects of high circulating non-esterified fatty acids (NEFAs), a biomarker of NEB, and urea on oocyte maturation, embryonic quality, and developmental and pregnancy outcomes.

Metabolic stress in dairy cattle

Pronounced metabolic change takes place in cows during the transition state from non-pregnant to pregnant, non-lactating to lactating. During this time span, major changes take place in the energy partitioning over the different body tissues. Although the energy needs for the pregnant uterus are modest compared to those of the mammary gland at that specific moment in time, the late gestation period is important as it imposes a catabolic state marked by decreasing peripheral insulin responsiveness period. After parturition, a massive demand for glucose and, to a lesser extent, fat and protein is established as milk production starts. During early lactation, cows are unable to compensate for such increased energy demands by feed intake, resulting in NEB. A reduction in basal and glucose-stimulated insulin release reduces glucose uptake by insulin-dependent tissues, maximizing glucose availability for the insulin independent udder for lactose synthesis period. Hypo-insulinemia further promotes gluconeogenesis in the liver and acts as a massive "body reserve mobilization trigger". This results in mobilization of body reserves to overcome the energy deficit, which in turn leads to weight loss. This loss of body condition is associated with alterations in blood metabolite and hormone profiles that, in turn, influence fertility (Leroy *et al*. 2008a; Leroy *et al*. 2008b; Leroy *et al*. 2008c). Thus, in a metabolically stressed animal there will be elevated levels of metabolites such as NEFAS, beta-hydroxybutyrate (β-OHB) in blood that has direct negative effect on ovary and uterus, which subsequently may result in a poor fertility. Thus, the metabolic stress caused by the side products of this heavy energy traffic from the digestive tract and body reserves towards the udder, is responsible for the deteriorating reproductive functions in high yield dairy animals (Leroy *et al*. 2008a; Leroy *et al*. 2008b; Leroy *et al*. 2008c). Non-esterified fatty acids and urea are risk factors for reproductive performance in dairy cattle. For example, the peri-partum period of dairy cattle is characterized by high lipid mobilization from adipose tissue leading to increased blood concentrations of NEFAs.

Mechanism behind NEFAs production

Non-esterified fatty acids are the major component of triglycerides (the fat stores in the body), which consist of three fatty acids linked to a glycerol backbone. Hydrolysis of stored triglycerides in adipose tissue by hormone sensitive lipase (HSL) liberates NEFAs and glycerol. Hormone sensitive lipase (which is found within the cytosol of adipocytes) is stimulated by various hormones, including glucagon (which is released from α-cells in pancreatic islets in response to low glucose). Lipolysis of fat stored as triglycerides in adipose tissue occurs in response to increasing energy demands that cannot be adequately supplied by glucose. Hormones, such as glucagon, catecholamines, adrenocorticotropichormone (ACTH), corticosteroids and growth hormone, stimulate HSL, whereas insulin inhibits this enzyme. Lipolysis of triglycerides releases NEFAs (which are usually long-chain fatty acids) and glycerol. Glycerol is taken up by cells and used for glucose production or can be used to re-form triglycerides. Non-esterified fatty acids are water-insoluble and are transported bound to albumin.

Once taken up by hepatocytes, NEFAs are esterified. The esterified fatty acids then have several fates. They can recombine with glycerol to form triglycerides, which are packaged into very low-density lipoprotein (VLDL). The VLDL are exported from the liver or (if produced in excess) are stored as fat within the hepatocyte (eventually causing lipidosis). They can enter the mitochondria (in a reaction that requires carnitine) and be used for energy production (through the Kreb's cycle) or ketone formation within the mitochondria; esterified fatty acids undergo β-oxidation to acetyl CoA. Acetyl CoA combines with oxaloacetate in the Kreb's cycle (tricarboxylic acid cycle) to form citrate. Continued oxidation in this cycle leads to energy (ATP) production. If oxaloacetate supplies are low (oxaloacetate is used as a substrate for gluconeogenesis in states of NEB, acetyl CoA is then used to form ketones. Low concentrations of NEFAs are found in the blood of healthy animals. Increased concentrations indicate breakdown of fat (lipolysis), which occurs in response to increased energy demand. Thus, NEFAs are considered a biomarker of NEB, where the supply of glucose is insuffi-

cient to meet energy needs. Negative energy balance can be detrimental because it predisposes animals to hepatic lipidosis (excess NEFAs are stored as triglyceride within hepatocytes) and ketosis (http://eclinpath.com; http://eliasnutri.files.wordpress.com).

Metabolic stress, negative energy balance and fertility

During follicular growth, maternal genes are transcribed and the resulting mRNA and protein molecules are synthesized and accumulated in the oocyte (Leroy *et al*. 2006; Lonergan *et al*. 2003; Vassena *et al*. 2003) that are crucial for the survival of the early embryo prior to embryonic genome activation. Once genome activation has occurred, at the 8-16 cell stage in the cow (Van Den Hurk and Zhao, 2005), the embryo starts using its own, newly formed DNA to produce transcription factors. This is a highly sensitive step in pre-implantation embryo development. In other words, even when a perfect fertilization has taken place, adverse follicular conditions during oocyte growth and maturation can impact on the viability of the embryo later on. Farm animal breeders suffer major economic losses through high early embryonic mortality; in cattle, 35-50% of the fertilized oocytes fail to develop (Sreenan, 1986). The potential effect of adverse conditions during oocyte growth and maturation on further fertility outcome was first proposed by Britt *et al*. (1992). The developmental competence of the oocyte and the steroidogenic capacity of the follicle, in high yielding dairy cows, are determined by their biochemical environment during the long period (up to 80 days) of follicular growth prior to ovulation (Britt *et al*. 1992). Thus, primary follicles exposed to adverse conditions associated with the metabolically challenging period of NEB early postpartum may be less capable of producing adequate amounts of estrogens and progesterone post-ovulation (Britt *et al*. 1992). Moreover, such follicles are likely to contain poor quality oocytes.

Metabolic stress and ovarian activity

The high circulating NEFAs levels, associated with NEB, are indeed reflected in the follicular fluid of dominant follicles in early postpartum dairy cows (Leroy *et al*. 2005; Roth *et al*. 2001). Furthermore, this knowledge was applied in an *in vitro* model, in which the oocyte maturation medium was supplemented with the predominant fatty acids at concentrations observed in follicular fluid of dairy cows during an episode of NEB. Results revealed that the saturated long chain fatty acids- in particular palmitic and stearic acid- provoked an inhibition of maturation rate, leading to relatively low fertilization, cleavage and blastocyst formation rates. Furthermore, it has been shown that stearic acid and palmitic acid induce apoptotic changes in the cumulus cells (Leroy *et al*. 2005; Roth *et al*. 2001), which in turn influence oocyte maturation and probably also embryo development in a negative way. The metabolic imbalance of high NEFAs concentrations during oocyte maturation increased the cryosensititvity of the resulting embryos. Increased cryosensitivity is due to increased fatty acid concentrations in the oocytes' microenvironment during maturation may lead to the accumulation of these fatty acids in the oocyte that may alter their lipid content and composition (Leroy *et al*. 2008a; Leroy *et al*. 2008c; Leroy *et al*. 2005; Kim *et al*. 2001; Adamiak *et al*. 2005). High NEFAs (>0.4 mmol/L) in the last 7 to 10 days before expected calving is associated with 2 to 4 times increased risk of left displaced abomasum (Rooke *et al*. 2006) increased risk of retained placenta (LeBlanc *et al*. 2005) and 1.1 kg/day less milk production in the first 4 months of lactation (Leroy *et al*. 2008a; Quiroz-Rocha *et al*. 2009).

Exposing immune cells *in vitro* to NEFAs at concentrations compatible with those observed in high producing postpartum dairy cows (0.12 to 1 m*M*) has been shown to reduce function and viability. Increasing the concentration of NEFAS in the culture media prevented the synthesis of interferon-γ and IgM by peripheral blood mononuclear cells (Leroy *et al*. 2005; Leroy *et al*. 2008a; Carson *et al*. 2008). Furthermore, NEFAs reduced phagocytosis dependent oxidative burst in polymorphonuclear leucocytes (Lacetera *et al*. 2004). When concentrations of NEFAs in the culture medium were further increased to 2 m*M*, more leukocytes underwent necrosis (Leroy *et al*. 2005; Leroy *et al*. 2008a; Lacetera *et al*. 2004), thereby impairing immune system function and indirectly increasing the susceptibility to reproductive diseases by the way of interfering in the proper functioning of immune system and decreasing the fertility. According to Adewuyi *et al*. (2005) high level of NEFAs in plasma leads to several diseases including milk fever, retained fetal membranes, abomasal displacement mastitis along with these reduced milk and loss of body weight has been reported (Adewuyi *et al*. 2005).

One way for increasing productivity was achieved by increasing dietary crude protein levels (Leroy *et al*. 2008a; Butler, 1998). High intake of degradable protein and low energy will result in an accumulation of excessive ammonia in the rumen (Leroy *et al*. 2008a; Sinclair *et al*. 2000a), which may cause NEB (NEB) early postpartum, thereby reducing fertility (Leroy *et al*. 2008a; Butler, 1998). High dietary protein levels have been reported to be associated with inferior reproductive performance (Sinclair *et al*. 2000b; Butler *et al*. 2003; Melendez *et al*. 2003; Gath *et al*. 1999; Kenny *et al*. 2001; Kenny *et al*. 2002). High crude protein levels in the diet do not have negative impact on the reinitiating of ovarian cyclicity in the postpartum dairy cow. However, reduced conception rates in animals with serum urea nitrogen concentrations exceeding 20 mg/dL (or

milk urea nitrogen concentrations >19 mg⁄dL) have been reported (Leroy *et al*. 2008a; Melendez *et al*. 2003; Butler *et al*. 1996; Westwood *et al*. 1998; Sinclair *et al*. 2000a). The conception failure is due to the toxicity of the direct by-products of protein catabolism (ammonia and urea) for the oocyte and the embryo. Murine embryos cultured in the presence of high NH_4^+ concentrations displayed morphological, metabolic and genetic abnormalities (Leroy *et al*. 2008a; Gardner *et al*. 1993; Lane *et al*. 2003). Lactating dairy cow can also metabolically adapt to aprolonged high intake of quickly degradable protein, byopposing possible adverse effects of long-term high urea concentrations on embryo growth (Leroy *et al*. 2008a; Dawuda *et al*. 2002; Laven *et al*. 2004) which could not be confirmed in ewes (Leroy *et al*. 2008a; McEvoy *et al*. 1997a; McEvoy *et al*. 1997b). Long-term very moderate increase indietary crude protein content from 14% to 18% turned out to be advantageous to the quality of heifer embryos despite the concomitant elevated blood urea concentrations (Mikkola *et al*. 2005). High blood urea concentrations have been associated with a reduction in uterine pH (7.1 to 6.8) and with an alteration in the ionic composition of uterine fluid, and therefore both can have deleterious effect for the developing embryo (Jordan *et al*. 1983; Elrod *et al*. 1993) which is confirmed *in vitro* by Ocon and Hansen (2003). Adverse effects of urea on embryo quality are likely to be due to deleterious change in the environment of the follicle and/or oviduct, rather than due to a changed uterine environment (Fahey *et al*. 2001; Papadopoulos *et al*. 2001), which is confirmed in a study of lactating dairy cows (Rhoads *et al*. 2006). Embryo transfer (donors with elevated plasma urea nitrogen concentrations) resulted in a significantly reduced pregnancy rate (Rhoads *et al*. 2006).

Oocytes recovered from donor with elevated serum and follicular fluid ammonia concentrations showed a declined developmental competence *in vitro* (Leroy *et al*. 2008a; Sinclair *et al*. 2000b; Hammon *et al*. 2000a), however the effect depend on timing and duration of exposure (Leroy *et al*. 2008a; Hammon *et al*. 2000b). Granulosa cells exposed with high level of ammonia lose their ability to support *in vitro* oocyte maturation (Rooke *et al*. 2004). In high producing cows in the early postpartum period exists a very good correlation between urea concentrations in plasma and follicular or uterine fluid (Leroy *et al*. 2004; Leroy *et al*. 2008a).

Low nuclear maturation and reduced fertilization and cleavage rates in bovine oocytes matured in the presence of 6 m*M* urea was reported (Leroy *et al*. 2008a; De Wit *et al*. 2001).

High levels of blood urea have a toxic effect not only on the ovum and developing embryo but also on the spermatozoa. There was an increase in the urea levels under the effect of non-protein nitrogen feeding, either in the blood (Khadr *et al*. 1995) or in the seminal plasma (El-Azab *et al*. 1998).

A significant negative correlation between pH and sperm cell concentration and sperm motility has been reported (El-Chahida *et al*. 1977). Negative energy balance and rate of mobilization of body reserves appear directly related to the postpartum interval to first ovulation and lower conception rate.

Negative energy balance may cause delayed ovarian activity by impinging on pulsatile secretion of LH (Butler and Smith, 1989). Lower availability of glucose and insulin may also decrease LH pulsatility or limit ovarian responsiveness to gonadotropins.

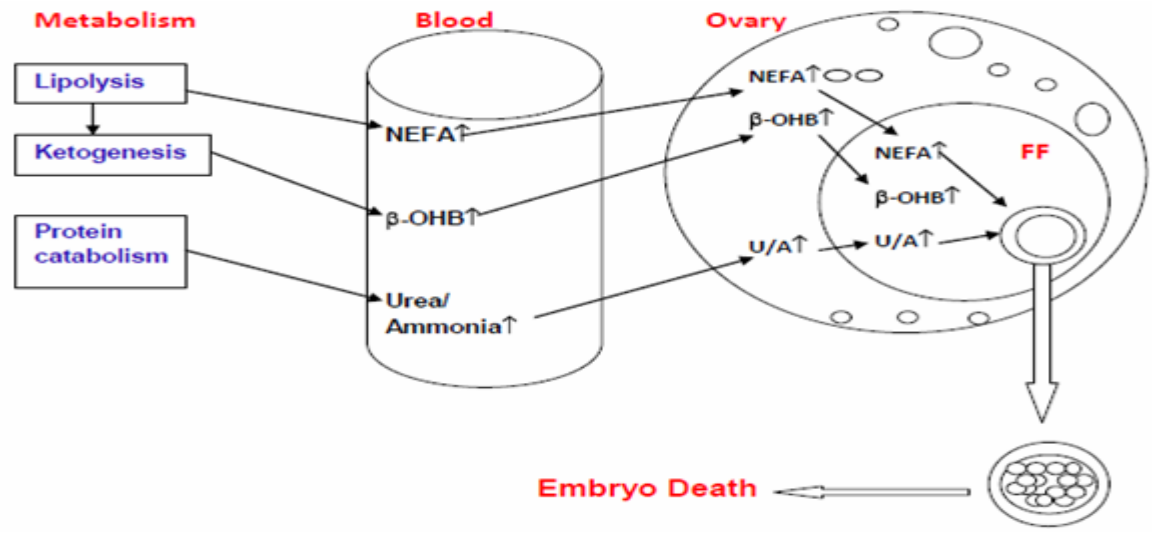

Figure 1 Possible mechanisms by which embryo can impair quality by metabolic stress (FF: follicular fluid; U/A: urea/ammonia; NEFAs: non esterified fatty acids and β-OHB: beta-hydroxybutyrate

Alternatively, release of endogenous opioids in association with increasing feed intake or other lactational hormone responses may provide neural or pituitary inhibition of the pulsatile LH production that is requisite for ovarian follicular development (Butler and Smith, 1989). In low milk producing primiparous cows, no significant differences in body weight loss, milk yield, energy balance, leptin and NEFAs plasma levels were seen between the cows with and without resumption of ovarian activity within 7 weeks post partum.

However, significantly higher IGF-I levels in the first two weeks after calving were found in cows with resumption of post partum ovarian activity, highlighting the important role of IGF-I as sensitive signal between metabolism and reproduction (Konigsson *et al*. 2008). Non-esterified fatty acids during periods of NEB negatively affected theca cell proliferation, induced inhibitory effects of saturated fatty acids on bovine granulosa cells and reduced progesterone production *in vitro* (Vanholder *et al*. 2006). Non-esterified fatty acids may also cause a decline in the proliferation of *in vitro* cultured granulosa cells and increased NEFAs concentrations as occur during NEB may cause lowered corpus lutetium (CL) weights and progesterone concentrations that are related to lowered fertility (Jorritsma *et al*. 2004). The possible mechanisms (Leroy *et al*. 2008a; Leroy *et al*. 2008b) by which embryo can impair quality by metabolic stress is depicted in Figure 1.

CONCLUSION

Metabolic stress and NEB are well known risk factors for decreased fertility in dairy cattle. Lipolysis of fat stored in the body as triglycerides occurs in response to increasing energy demands. Lipolysis of triglycerides releases NEFAs in the general circulation. In high yielding dairy cattle, the NEB during the early post-partum period increases the concentrations of NEFAs in follicular fluid of the dominant ovarian follicle and adversely affects ovarian activity mainly follicular growth, steroidogenesis and oocyte maturation. In post-partum dairy cows and under condition of metabolic stress, the presence of NEFAs and urea in blood and ovarian follicular fluid may reduce fertility due to inhibition of oocyte maturation and abnormal embryonic development.

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