Effect of Energy Balance on Oocyte and Embryo Quality in Modern Dairy Cows

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Take Home Messages

- Reduced oocyte and embryo quality are recognized as major factors in the problem of disappointing fertility.
- Changes in the growth patterns of the ovarian follicles during a period of negative energy balance (NEB) can indirectly affect oocyte quality.
- Endocrine and biochemical changes associated with NEB are reflected in the microenvironment of the growing and maturing female gamete. In vitro models showed that these alterations can be toxic for the oocyte.
- Inadequate corpus luteum function, associated with reduced progesterone, and probably also low insulin-like growth factor concentrations, can cause a suboptimal microenvironment in the uterus that is incapable of sustaining early embryonic life.
- Several nutrition-linked mechanisms, through which oocyte and/or embryo quality can be affected in modern dairy cows well after the period of NEB, are recognized, and should be taken into account when proposing solutions for this subfertility problem.

Introduction

Before the second half of the 20th century, cattle breeds were mainly selected for dual purposes: milk and meat. However, during the last fifty years, breeding focused on one single purpose, leading to specialized breeds such as the Holstein Friesian, which can yield up to 12,000 litres of milk per year, and the Belgian Blue beef breed. These are the two predominant cattle breeds in Belgium. The excessive specialization which occurred in Holstein Friesian and Belgian Blue cattle has led to major fertility problems. In particular, the disappointing fertility outcome in high yielding dairy cattle is a cause of concern, since it is a major threat for the farmer's income in the first place and an important impediment for sustainability and longevity in the dairy industry.

How did these fertility problems originate in the first place? Ruminants are under general circumstances quite fertile animals. An extreme example of ruminant fertility is the reindeer, in which 99.5% of matings lead to viable fetuses and under ideal conditions, fawn crops may reach 85-95%. Even in modern dairy cows showing normal oestrus cycles, more than 90% of the optimally timed AI's lead to fertilization of the oocyte. However, oocyte and early embryo quality seems to be jeopardized as more than 40% of these early embryos die during the first 2 weeks after conception. And also late embryonic losses (after day 28 post insemination) can count for 20% of the pregnancy losses. Finally, 5% of cows lose their fetus during later pregnancy (for review, see Wathes et al., 2008). However, describing the variety of reasons for late embryonic and fetal losses and the causes of perinatal mortality are not within the scope of this paper.

What is the underlying cause for these early pregnancy losses? In fact, fertilization and establishment of a sound pregnancy are such complicated and finely tuned processes that there are numerous causes for fertility problems, and if you come to think of it, it is a miracle that offspring are being produced in the first place!

In order for a live calf to be born, a number of well-orchestrated events have to take place in timely order, and any error in this sequence of events leads to prevention of fertilization or to the untimely death of the conceptus. In this series of events I will not consider the importance of estrus detection by the farmer or of timing of insemination. First of all, a healthy oocyte has to develop in a dominant follicle, followed by the ovulation of this oocyte. The oocyte surrounded by the cumulus cells, and the follicle, form one functional unit in which the somatic cells support the oocyte's nutrition, growth and maturation. At the time of ovulation, a limited number of capacitated sperm at the site of fertilization in the fallopian tube is sufficient to allow for fertilization of the oocyte. The fertilizing sperm has to bind to the zona pellucida, traverse the zona, merge with the oolemma and trigger oocyte activation and further embryo development. The resulting zygote will then go through a series of cleavage divisions whilst residing in the oviduct, and after less than one week, it will arrive in the uterus. After compaction, which occurs at about day 6 after insemination, bovine embryos can be flushed from the uterus and transferred into another recipient cow either fresh or frozen, or used for research purposes. Under normal conditions, the embryo remains in the uterus, goes through the blastocyst stage, and hatches from the zona pellucida. At this stage, the embryo becomes implantation competent, but in cattle, implantation is not completed until 30 days after fertilization. After implantation, a host of causes may induce embryonic or fetal mortality such as chromosomal, placental, or ovarian/uterine abnormalities and infections.

These finely tuned processes seem to be hampered in our modern dairy cows. Years of intensive research revealed that oocyte and embryo quality indeed are of questionable quality in our high producing dairy cow, being a major cause of the subfertility syndrome.

Is the Oocyte Dwelling in the Ovarian Follicle Vulnerable to Negative Energy Balance?

In vivo, conditions in the antral follicle determine oocyte quality. During the follicular growth phase of the oocyte, maternal genes are transcribed and the resulting mRNA and protein molecules are synthesized and accumulated in the oocyte (van den Hurk and Zhao, 2005). The latter is crucial to guarantee the survival of the early embryo prior to embryonic genome activation. During this process, the embryo starts using its own, newly formed DNA for making transcription factors. This is a highly sensitive step in pre-implantation embryo development and occurs at the 8-16 cell stage. In other words, although a perfect fertilization took place, adverse follicular conditions during oocyte growth and maturation may have detrimental consequences for the viability of the embryo. This early embryonic mortality stays unobserved by the farmer and the cow will come in heat again. This potential carry-over effect of adverse conditions during oocyte growth and maturation on further fertility outcomes was first proposed by Britt in 1992. Britt hypothesized that the developmental competence of the oocyte and the steroidogenic capacity of the follicle in high yielding dairy cowsare determined by their biochemical environment during the long period (up to 80 days) of follicular growth prior to ovulation. 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 (after ovulation). Moreover, such follicles would be doomed to contain an inferior oocyte, which will then ovulate approximately 60-80 days postpartum.

Furthermore, it has been said that the environment experienced by the oocyte and the very young embryo not only affects the chances for full term pregnancy and live offspring, but has also effects that persist into adulthood and subsequent generations. This vulnerability of the embryo to its early environments has also become obvious to those scientists who have been transferring embryos from in vitro fertilization and cloning, and whose efforts have been plagued by early embryonic losses and by the "Large Offspring Syndrome". Due to the typical metabolic pressure during the first weeks postpartum, dairy cows form an intriguing model to study, for example, the consequences of epigenetic effects in the oocyte and embryo on the developmental origin of health and diseases.

In the previous review paper it is described in detail what is going on in the dairy cow's metabolism early postpartum. This particular NEB and concurrent weight loss can hamper the well orchestrated process of follicular growth at the level of the hypothalamus-pituitary-ovary axis. Many studies demonstrated that oocytes originating from a disabled follicle or from a follicle with a deviant growth pattern will be of inferior quality. Furthermore, deviant concentrations of estrogen, leptin, growth hormone, insulin-like growth factor-I (IGF-I), insulin and progesterone as described above may all directly affect to a certain extent the viability of the oocyte. They are implicated in various important processes in oocyte maturation all of which have been extensively reviewed earlier (Leroy et al., 2008a). Only a few studies have examined possible effects of NEB associated low glucose, elevated β-hydroxybutyrate (β-OHB) or non-esterified fatty acid (NEFA) concentrations on oocyte quality. Apart from indirect effects of hypoglycaemia in early postpartum dairy cows (through an effect on LH secretion or ovarian responsiveness to gonadotrophins), hypoglycaemic conditions (e.g. clinical ketosis) are reflected in the microenvironment of the pre-ovulatory oocyte, and can compromise the oocyte's developmental capacity, because glucose is an indispensable molecule for proper oocyte maturation. Kruip and Kemp (1999) suggested possible direct toxic effects of high NEFA concentrations at the ovarian level. Indeed, in an in vitro maturation model, saturated long chain fatty acids reduced rates of maturation, fertilization, cleavage, and blastocyst formation. Apoptosis, and even cumulus cell necrosis during maturation could explain these observations (Leroy et al. 2005b). Finally, elevated ammonia and urea concentrations in the follicular fluid, due to an unbalanced diet and protein catabolism were toxic for the oocyte (Sinclair et al. 2000).

From the above it is obvious that the oocyte is a very important player in the process of fertilization and embryo development: there are sufficient data to suspect that oocyte quality is indeed impaired in lactating dairy cattle. Also the oocyte suffers from metabolic stresses imposed on dairy cows early postpartum.

▪ Does Energy Balance Affect Embryo Quality in Dairy Cows?

As stated above, early embryonic death is a major cause of reproductive failure in dairy cows. There are four major factors impinging on embryo quality in the specific case of high producing dairy cows: gamete quality, corpus luteum quality combined with the circulating progesterone concentration, uterine involution, and nutrition. However, only those that are related to NEB will be discussed in this chapter.

Adverse pre-ovulatory conditions, such as NEB, may have carry-over effects on embryo metabolism and viability resulting in early embryonic mortality. Distinguishing oocyte effects on embryo quality from post-fertilization influences is extremely difficult. Embryos earlier than 6 days of age cannot be transferred to a recipient to assess the impact of uterine environment on embryo quality. Lucy (2007) supports the concept that fertility could be improved in dairy cows by using embryo transfer and thus circumventing the period of oocyte and early embryonic development.

Well-timed and balanced post-mating progesterone concentrations are vital for zygote viability as progesterone modulates the endometrial secretions, and thus optimal uterine receptivity. It has been suggested that disappointing pregnancy results in modern dairying are partially caused by the retarded onset of the progesterone rise and suboptimal progesterone concentrations during the luteal phase (Mann and Lamming, 2001). The progesterone-pregnancy interaction is a self-enforcing mechanism. The high progesterone concentrations and the subsequent optimal uterine condition will induce the embryo to produce adequate amounts of interferon-*tau*. This interferon-*tau* is crucial to prevent upregulation of the estrogen and oxytocin receptor in the endometrium and thus to avoid PGF2α synthesis (Mann and Lamming, 2001). Furthermore, the typical NEB observed early postpartum can reduce the number of ovulatory oestrous cycles preceding AI which may hamper adequate uterus preparation. Villa-Godoy et al. (1988) showed that cows in NEB postpartum had lower progesterone concentrations during the first three ovarian cycles following calving. Despite larger volumes of luteal tissue, compared to non-lactating heifers, maximal progesterone concentrations in lactating cows are lower, possibly due to a higher rate of degradation in the liver.

Also other hormones related to the energy status of the animal, like insulin and IGF-I are implicated in the regulation of progesterone production by the luteal cells and in promoting embryonic growth by altering the micro-environment in the oviduct and uterus. IGF-I and IGF-II and their binding proteins are important in regulating the interactions between conceptus and endometrium for maintaining pregnancy. Only recently it was shown that negative energy balance may alter IGF and IGF-binding protein expression in the oviduct.

Good postpartum uterine involution, comprised of endometrium repair and evacuation of bacterially contaminated contents, is of critical importance for acceptable reproductive performance (for review: Roche et al., 2006). Due to a reduced immune response, negative energy status can dramatically delay this process, jeopardizing future fertility (Wathes et al., 2007).

▪ Nutrition

Apart from the interest in the consequences of NEB and associated endocrine and metabolic imbalances, there is a growing focus toward the effect of diet on general fertility and more specifically on oocyte and embryo quality (for review: Leroy et al. 2008c). From these studies we can learn that optimum nutritional conditions in terms of energy supplementation for follicle growth and ovulation are not compatible with oocyte quality, embryo survival and maintenance of pregnancy (O'Callaghan and Boland 1999). Energy rich diets fed to animals in positive energy balance seem to be harmful for the oocyte and embryo. Also diets rich in crude protein are said to have direct toxic effects at the oocyte level through the elevated ammonia and urea concentrations in the blood of the animals. Finally, fat feeding became common practice in the dairy industry, depending on the country or the region where the farm is located. Different goals are set, and depending on the goal, a different fat source can be applied. Saturated fatty acids like palm oil can increase milk yield but may aggravate NEB and thus fertility when fed during the first weeks postpartum. Unsaturated fats are given to reduce the de novo fat synthesis in the udder and thus the milk fat content, which may be slightly beneficial for the energy balance. The n-6 and n-9 polyunsaturated fatty acids have furthermore, the potency to alter steroid

Table 2. Survey of studies focusing on the effect of different types of fatty acids on oocyte and embryo quality.

Author	Findings
Zeron et al., 2002	positive effects of fish oil supplemented diets on oocyte quality
Adamiak et al., 2006	altered lipid intake is reflected in changed fatty acid composition in follicular fluid and cumulus oocyte complex
Bilby et al., 2006	negative effects of n-6 rich diets on oocyte quality
Fouladi-Nashta et al., 2007	positive effect of 800g Megalac® supplementation for 14d on oocyte quality
Pereira et al., 2007	positive effect of polyunsaturated diets during in vitro embryo culture on embryo quality
Marei et al., 2008	positive effect of linolenic acid on oocyte maturation
Leroy et al., 2008d	serum from palm oil fed heifers, added to in vitro culture system reduces embryo quality
Cerri et al., 2009	better embryos after linoleic feeding compared to palm oil in dairy cows

synthesis and prostaglandin metabolism in the ovary and endometrium, respectively. The consequences of these fat feeding strategies on oocyte and embryo quality remain an intriguing issue for debate. To date, research results are somewhat conflicting most probably due to differences in fat sources used, diet, duration of supplementation and experimental set up. Some important studies focusing on the effect of fat feeding are listed in Table 2.

▪ Conclusions

The modern dairy industry encounters a worrisome decline in fertility and there is increasing evidence to assume that reduced oocyte and embryo quality are two major players in this “disappointing fertility syndrome”. Compromised follicular growth, cause and consequence of altered steroid and growth factor concentrations, affects the quality of the female gamete. Also the typical biochemical changes during an episode of negative energy balance are reflected in the follicular fluid and may be toxic for the residing oocyte. Certainly, more studies are required to assess the intricate long-term effects of these metabolic and endocrine disturbances on fertility, oocyte quality, fetus viability and health of the living offspring.

Once the oocyte is successfully ovulated and fertilized, carry-over effects of the negative energy balance can jeopardize corpus luteum function and thus progesterone concentrations. Other growth factors may be involved in this. Furthermore, nutrition has the potency to alter the micro-environment of the oocyte and the embryo, making it more hostile to optimal fertilization and pre-implantation embryonic growth.

Well balanced diets should prevent severe negative energy balance and an excess intake of specific dietary components. More research should focus on functional diets in dairying in order to stimulate reproductive functions.

Alot of papers have been used to compose this overview. Only the key references are given. However, an extensive list of the references used can be obtained from the first author.

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