Anim. Reprod., v.9, n.4, p.777-788, Oct./Dec. 2012

Maternal metabolic health and oocyte quality: the role of the intrafollicular environment

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Abstract

Reduced oocyte and embryo quality are currently recognized as key factors in the problem of disappointing fertility in high producing dairy cows, but also in women undergoing assisted reproductive treatment. This review aims to highlight the importance of intrafollicular conditions in the subfertility problem, topical in both bovine and human research. Metabolic disturbances, like a negative energy balance (NEB) early postpartum in the bovine or obesity and type II diabetes in women, are associated with ovarian dysfunction. Changes in the growth pattern of the ovarian follicle, due to suboptimal metabolic conditions, can indirectly affect oocyte quality. Furthermore, maternal metabolic disorders (nutritionally induced, linked with NEB or caused by for example obesity) may alter the endocrine and biochemical composition of the follicular fluid, the microenvironment of the growing and maturing female gamete. The maturing oocyte is very sensitive to any perturbation in its direct environment and *in vitro* maturation models revealed that some of these metabolic changes have the potential to reduce the oocyte's developmental competence. Also embryo quality is significantly reduced due to maturation in adverse conditions. Well-balanced and timed oocyte metabolism and gene expression are crucial to safeguard an optimal oocyte development. In that perspective metabolic and transcriptomic parameters of the oocyte may serve to predict reproductive success rates. Importantly, there is also growing evidence that adverse conditions for oocyte growth and maturation may jeopardize the metabolism and health of the offspring.

Keywords: embryo development, fertility, follicular fluid, metabolism, oocyte quality.

Introduction

It is widely accepted that female fertility is declining rapidly, with the most pronounced effects in women and in bovine species. Multidisciplinary research is currently aimed at understanding and reversing this trend of subfertility. To establish a successful pregnancy, a number of well-orchestrated events must take place in a precise order. An 'in time' resumption of ovarian activity and cyclicity should result in the completion, selection and growth of a healthy follicle, enclosing a competent oocyte, and ultimately in ovulation, fertilization and uterine attachment of a viable embryo (Van Soom *et al.*, 2006). The concomitant development of a functional corpus luteum should safeguard, through optimal progesterone secretion, an appropriate embryo environment in which the embryo can grow and develop (Morris and Diskin, 2008). Any inaccuracy in this sequence of events may lead to fertilization failure or to the untimely loss of the conceptus.

Extensive research in the bovine has indicated that such errors may relate to dietary energy intake and diet composition, dry cow management, heat detection, housing conditions, milk production, genetic merit for milk production, disease incidence and general welfare issues (Rodriguez-Martinez *et al.*, 2008). As all factors above are significantly related to each other, reduced fertility in the bovine is truly a multifactorial problem. Two major reproductive problems can be recognized in the subfertile dairy cow. Firstly, up to half of modern dairy cows display abnormal estrous cycles postpartum leading to prolonged calving to first insemination intervals (Opsomer *et al.*, 1998). The involvement of the hypothalamus-pituitary-ovarian-uterine axis in particular has received significant attention (Lucy, 2001; Butler *et al.*, 2003; Wathes *et al.*, 2007). Concomitant reduced estrus expression or even anestrus, cyst formation and delayed first ovulation have been extensively documented, but are beyond the scope of this review. Secondly, attention has been paid to disappointing conception rates (Bousquet *et al.*, 2004; Santos *et al.*, 2009) and the increasingly high incidence of early embryonic mortality (Dunne *et al.*, 1999; Bilodeau-Goeseels and Kastelic, 2003; Mann *et al.*, 2006). Furthermore, late embryonic losses (after day 28 post insemination) can account for 20% of the pregnancy losses and 5% of cows lose their fetus later in pregnancy (for review see Wathes *et al.*, 2008).

Much like in the bovine species, women with metabolic disorders, such as type II diabetes and obesity, increasingly suffer from subfertility. This has been associated with anovulatory cycles, a longer time to conception, disturbed hormonal pathways, early pregnancy loss, decreased success rates when undergoing assisted reproductive treatment and also multiple complications during pregnancy (Sebire *et al.*, 2001; Pasquali *et al.*, 2006; Lash and Armstrong, 2009). It has become increasingly clear that many factors are involved in the failure to maintain pregnancy but a disturbed maternal metabolism may underlie this whole

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Received: August 7, 2012
Accepted: October 2, 2012

pathogenesis. A decreased oocyte and embryo quality has been proposed as one of the underlying factors in the subfertility syndrome (Leroy *et al.*, 2008c). Both the oocyte and the pre-implantation embryo are very sensitive to any perturbation of their microenvironment. It is now generally accepted that the follicular microenvironment is affected by the maternal metabolism and that the oocyte is directly influenced by this (Leroy *et al.*, 2008a). Also, after implantation, numerous causes may induce embryonic or fetal mortality such as suboptimal corpus luteum quality, chromosomal, placental, or ovarian/uterine abnormalities, and infections (Bilodeau-Goeseels and Kastelic, 2003; Sheldon *et al.*, 2006; Morris and Diskin, 2008).

In addition to conception and maintaining a pregnancy, peri- and postnatal events are also under investigation. Recently, it was shown that children, born from an obese mother, are prone to develop chronic diseases later in life (Drake and Reynolds, 2010; Ruager-Martin *et al.*, 2010). Furthermore, increasing evidence suggests that, in the bovine, offspring survival and performance are also affected by metabolic and dietary conditions of the mother around the time of conception or during early gestation (Swali and Wathes, 2006; Berry *et al.*, 2008; Micke *et al.*, 2010). Also, other animal models have clearly shown that such effects exclusively during the phase of oocyte growth and maturation (that is before conception) are sufficient to cause significant negative effects on daughter fertility outcome and offspring characteristics (Watkins *et al.*, 2008; Mitchell *et al.*, 2009; Wallace *et al.*, 2010).

In this paper we aim to review 1) the importance of microenvironmental changes of the growing and the maturing oocyte on its developmental capacity, embryonic and fetal growth and the viability of the offspring and 2) some important follicular quality parameters as predictors of oocyte quality and development.

Elevated metabolic stress affects oocyte quality

In vivo, conditions in the antral follicle play an important role in determining oocyte quality. During follicular growth, maternal genes are transcribed and the resulting mRNA and protein molecules are synthesized and accumulated in the oocyte (Lonergan *et al.*, 2003; Vassena *et al.*, 2003; van den Hurk and Zhao, 2005). These processes are crucial to guarantee 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, the 4-8 cell stage in humans or the 1-cell stage in the mouse (Niemann *et al.*, 2007), the embryo starts using its own, newly formed DNA to make transcription factors. This is a highly sensitive step in preimplantation 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. This potential effect of adverse conditions during oocyte growth and maturation on further fertility outcome was first proposed by Britt (1992). Britt (1992) hypothesized that the developmental competence of the oocyte and the steroidogenic capacity of the follicle, in high yielding dairy cows, is 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 (postovulation; Britt, 1992; Roth *et al.*, 2001a). Moreover, such follicles are likely to contain an inferior oocyte. So far, these long term carry-over effects have only been substantiated in research on heat stressed dairy cows, showing a delayed effect of heat stress during summer on oocyte quality and follicular characteristics assessed during autumn (Roth *et al.*, 2001b; Morton *et al.*, 2007; Torres-Junior *et al.*, 2008). It is important to recognize that heat stress directly affects oocyte quality as well (Gendelman *et al.*, 2010), but this is beyond the scope of the present paper. Observations of Kendrick *et al*. (1999) and Gwazdauskas (2000) support this long term effect of unfavourable intrafollicular conditions on oocyte quality by reporting that morphological oocyte quality in dairy cows seems to be better early postpartum (before day 30) compared to three to four months later. Roth *et al*. (2008), however, could not confirm that the reduction in oocyte quality is an important issue in the subfertility problem in dairy cows. Taken together, these findings suggest that the oocyte might be very sensitive to all kinds of metabolic signals and disturbances during its early stages of follicle development (for review see Fair, 2010).

What is metabolic stress?

Apparently, there is something going wrong in the dairy cow early postpartum, threatening the wellbeing of the follicle and the oocyte inside. But what is it? This question leads us to the pronounced metabolic changes that cows go through during the transition from the gestating, non-lactating to the lactating, non-gestating state. During this time span, major changes take place in the energy partitioning over the different body tissues, establishing a conflict of interest between the uterus, mammary gland and other organs. 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 (Bell, 1995). Following parturition, a massive demand for glucose and, to a lesser extent, fat and protein is established as milk production starts (for overview, see Leroy *et al*., 2008c). During early lactation, cows are

unable to compensate for such increased energy demands by feed intake, resulting in NEB. A drastic reduction in basal and glucose-stimulated insulin release (Bossaert *et al.*, 2008) reduces glucose uptake by insulin-dependent tissues, maximizing glucose availability for the insulin independent udder for lactose synthesis. Hypoinsulinemia further promotes gluconeogenesis in the liver (up to 4 kg glucose each day) and acts as a massive "body reserve mobilization trigger". The mobilized non-esterified fatty acids (NEFAs) provide alternative fuels for the whole-body metabolism in order to spare glucose. Several studies have confirmed that this prioritized partitioning of energy towards milk is genetically determined (Coffey *et al.*, 2004). Cows with high genetic merit for milk yield seem to shift additional ingested energy directly towards milk production instead of improving their body condition score, while their lower genetic merit counterparts utilize energy supplements to alleviate their NEB (Kay *et al.*, 2009; Lucy *et al.*, 2009). Recent work has suggested that genetically determined differences in insulin secretion and peripheral insulin responsiveness may underlie such differences in energy prioritization (Bossaert *et al.*, 2009).

Although tissue mobilization is a vital strategy to maintain lactogenesis during energy deficiency, excessive BCS loss during the transition period increases the risk of hepatic steatosis (Herdt, 2000) and other health and fertility disorders (Roche *et al.*, 2007) which emphasizes the importance of BCS monitoring early postpartum as a management tool (Chagas *et al.*, 2007).

Effects of metabolic stress on follicular growth

Scaramuzzi *et al*. (2011) provided the ruminant reproductionists with an excellent overview on the regulation of folliculogenesis in relation to fertility in general and oocyte quality in more detail. It is clear that the particular status of NEB can hamper the well-orchestrated process of follicular growth at different levels of the hypothalamus-pituitary-ovarian axis (Webb *et al.*, 1999; Wathes *et al.*, 2007). Firstly, a decrease in insulin and insulin-like growth factor I (IGF-I) concentrations, increased growth hormone concentrations and most probably also reduced leptin concentrations are major endocrine mechanisms through which final follicular growth and ovulation can be directly affected. Recent work has demonstrated the presence of insulin receptor protein in the granulosa cells of bovine follicles (Bossaert *et al.*, 2010), suggesting a possible direct interaction between the energy status and follicular growth. Additionally, indirect effects of NEB on follicular growth seem plausible, since associations between insulin concentration and LH pulsatility have been reported (Webb *et al.*, 1999; Lucy, 2003). Hypoglycemia is associated with an inhibited GnRH secretion in the hypothalamus leading to this failure of an adequate LH pulse mechanism. Secondly, it is known that lactating cows, compared to non-lactating heifers, have dominant follicles that are less estrogenic. These follicles require a prolonged growing phase to increase their diameters in order to trigger an adequate LH pulse frequency and surge (Lopez *et al.*, 2004; Sartori *et al.*, 2004). Apart from the reduced estrogen production, an elevated estrogen metabolism in the liver during NEB, due to a higher plane of nutrition, could also account for the lower preovulatory estrogen concentrations in lactating dairy cows compared to non-lactating heifers (Sangsritavong *et al.*, 2002). Many studies demonstrated that an oocyte originating from a compromised follicle or from a follicle with a deviant growth pattern as described above would be of inferior quality (Bilodeau-Goeseels and Panich, 2002; Lequarre *et al.*, 2005). The length of follicular dominance influences oocyte and subsequent embryo quality and thus conception rates (Cerri *et al.*, 2009; Santos *et al.*, 2010).

Additionally, the intrafollicular bioavailability of estrogen, leptin, growth hormone, IGF-I, insulin and progesterone can be affected by energy balance and nutrition (Comin *et al.*, 2002) and these changes may all have a direct influence on the viability of the oocyte. These molecules are implicated in various important processes in oocyte maturation such as spindle and microtubule formation, resumption of meiosis, down-regulation of the gap junctions between cumulus cells and oocyte, mRNA polyadenylation and apoptosis, all of which have been extensively reviewed earlier (Leroy *et al.*, 2008a). It is generally accepted that major endocrine disruptions due to NEB predominantly lead to anovulation and atresia of the dominant follicle, rather than resulting in the ovulation of an inferior oocyte.

Despite the fact that insulin promoting dietary strategies are beneficial to follicular growth, Fouladi-Nashta *et al*. (2005) and Adamiak *et al*. (2006) demonstrated that elevated insulin has detrimental effects on oocyte development *in vitro*. Supplementation of saturated fatty acids around the moment of conception improved oocyte developmental competence and it is plausible that the induced drop in insulin concentrations may explain these observations (Fouladi-Nashta *et al.*, 2007). Garnsworthy *et al*. (2009) used this knowledge to recommend a postpartum diet in two phases: the first weeks postpartum follicular growth is supported by an insulinogenic diet while immediately before breeding cows are given a saturated fat supplemented diet in order to level off insulin concentrations and to prevent overstimulation of the oocyte. Other important factors in the endocrine-linked interaction between dietary energy and oocyte quality are follicular IGF-I and II bioavailability and progesterone clearance in the liver (Leroy *et al.*, 2008a).

Maternal metabolism and the oocyte's microenvironment

Follicular fluid is derived from the blood in the

thecal capillaries. The fluid further needs to cross the thecal interstitium, the follicular basal lamina and the mural granulosa cell layer (Rodgers and Irving-Rodgers, 2010). As follicular development progresses, fluid accumulates in the antrum of the follicle; bathing the oocyte and providing the milieu for oocyte development and maturation. Only a few studies have examined possible effects of NEB-associated low glucose, elevated β-hydroxybutyrate (β-OHB) or NEFA concentrations in dairy cows on the composition of the follicular fluid and on oocyte quality. Hypoglycemia in early postpartum dairy cows has an indirect negative effect, acting through LH secretion or ovarian responsiveness to gonadotropins. However, such hypoglycemic conditions (e.g. clinical ketosis) are also reflected in the microenvironment of the preovulatory oocyte, and can compromise the oocyte's developmental capacity. Glucose is an indispensable molecule for proper oocyte maturation and the expansion of the surrounding cumulus investment (Leroy *et al.*, 2004, 2006; Bilodeau-Goeseels, 2006; Sutton-McDowall *et al.*, 2010). In particular, the relatively low glucose conditions, rather than the elevated ketone body concentrations, turned out to hamper oocyte development (Leroy *et al.*, 2006). In type II diabetes or obese patients, the opposite is true: hyperglycemic insults are reflected in the follicular fluid and may have long-term negative effects on oocyte development through a delayed nuclear maturation (Jungheim *et al.*, 2010; Sutton-McDowall *et al.*, 2010). In relation to such observations, we have shown that elevated NEFA concentrations in dairy cows during NEB are partly reflected in the follicular fluid composition. The absolute NEFA concentrations however, remain up to 60% lower and the NEFA composition of the follicular fluid in dairy cows during NEB seems to contain more unsaturated fatty acids compared to serum (Leroy *et al.*, 2005). Contreras *et al*. (2010) recently showed that during an episode of massive lipolysis due to NEB, the proportion of palmitic acid (C16:0) significantly increases in the NEFA and the phospholipid fraction. Kruip and Kemp (1999) were the first to suggest a putative direct toxic effect of elevated NEFA concentrations at the ovarian level (Kruip and Kemp, 1999). NEFA have been suggested to be a metabolic key factor in the pathogenesis of type II diabetes and associated organ dysfunctions in humans due to its cytotoxic characteristics in different cell types (Shimabukuro *et al.*, 1998; Mason *et al.*, 1999; Maedler *et al.*, 2001; Lu *et al.*, 2003; Scalia *et al.*, 2006). Especially the saturated long chain fatty acids seem to play an adverse role and they may also affect reproductive physiology. Granulosa cell viability and steroidogenic capacity are hampered when incubated with high NEFA concentrations (Vanholder *et al.*, 2005). Furthermore, in *in vitro* maturation models, 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.*, 2005). In a recent study of Aardema *et al*. (2011), these effects on the oocyte's developmental capacity were confirmed. They observed a significant drop in the amount of intracellular fat stored in lipid droplets after maturation in the high stearic or palmitic acid concentrations (Aardema *et al.*, 2011). We have also demonstrated that maturation of oocytes under high NEFA conditions can have adverse carry over effects on the blastocyst quality in terms of embryo energy metabolism, amino acid turnover, gene expression patterns, cell number and cryotolerance without altering any embryo culture conditions (Shehab-El-Deen *et al.*, 2009; Van Hoeck *et al.*, 2011). Exposure of preimplantation mouse embryos to elevated palmitic acid concentrations led to intrauterine growth retardation and a lower birth weight (Jungheim *et al.*, 2011a). As has been described for dairy cows, we have found similar correlations between serum and follicular fluid composition in women undergoing ovum pick-up (Valckx *et al.*, 2010). This strongly suggests that metabolic disorders are reflected in the microenvironment of the human preovulatory follicle (Robker *et al.*, 2009). Epidemiological research in human centres for assisted reproduction revealed a significant reduction in oocyte quality in obese patients (Pasquali *et al.*, 2003; Bellver *et al.*, 2010) and we recently expanded on this by showing that an oocyte from an overweight or obese patient had a significantly reduced chance to develop until a top quality embryo (Valckx *et al*., unpublished data). Jungheim *et al*. (2011b) associated in a similar human OPU setting high follicular fluid NEFA concentration with poor cumulus-oocyte-complex morphology (Jungheim *et al.*, 2011b). Obese murine models confirmed this showing that especially the mitochondrial function in oocytes and embryos from such animals are compromised (Igosheva *et al.*, 2010). Only by adding high NEFA concentrations during final bovine oocyte maturation were we able to induce very similar effects at the mitochondrial level (Van Hoeck *et al.*, 2011). Optimal mitochondrial function is crucial for embryo development and implantation and disabled mitochondrial activity has been associated with suboptimal fetal and placental development (Wakefield *et al.*, 2011). Based on these observations we are convinced that those elevated NEFA concentrations are an important causative link between all metabolic disorders associated with upregulated lipolysis (obesities, type II diabetes, metabolic syndrome and NEB) and reduced oocyte quality.

In addition to effects of lipid mobilization, diets rich in crude protein may alter the oocyte's environment and its quality. This may be via direct toxic effects of elevated ammonia and urea concentrations in the blood, which are reflected in the follicular fluid composition of the animals (Sinclair *et al*., 2000; De Wit *et al.*, 2001; Leroy *et al.*, 2004). However, the true

impact of this protein overfeeding on fertility remains a matter of debate. The effects of crude protein overfeeding on oocyte and embryo quality have been extensively reviewed before (Leroy *et al*., 2008b).

Finally, it is important to consider fat feeding, which became common practice in dairy industry, depending on the country or the region where the farm is located. Different goals are aimed for and depending on the desired outcome, various fat sources can be used. Saturated fatty acids such as palm oil can increase milk yield but may aggravate negative energy balance and thus fertility when fed during the first weeks postpartum (McNamara *et al*., 2003; van Knegsel *et al*., 2005). 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. Furthermore, the so called n-6 and n-9 poly unsaturated fatty acids have the potency to alter steroid synthesis and prostaglandin metabolism in the ovary and endometrium, respectively (Leroy *et al*., 2008b). The consequences of these fat feeding strategies on oocyte and embryo quality remain an intriguing matter for discussion. To date, research results are somewhat conflicting most probably due to differences in fat sources used, diet, duration of supplementation and experimental set up. We recently showed that nutritionally induced hyperlipidemic conditions are detrimental for embryo quality and significantly alter embryo gene expression (Leroy *et al*., 2010). As this review mainly focuses on the consequences for oocyte quality, Table 1 solely lists studies focusing on the effect of fat feeding on oocyte quality.

Table 1. Survey of studies focusing on the effect of different types of fatty acids on oocyte quality in ruminants.

Author	Findings
Zeron *et al*. (2002)	positive effects of fish oil supplemented diets on oocyte quality and chilling sensitivity
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 14 days on oocyte quality
Marei *et al*. (2009)	positive effect of linolenic acid on oocyte *in vitro* maturation
Marei *et al*. (2010)	Negative effects of linoleic acid on oocyte *in vitro* maturation and developmental potential
Zachut *et al*. (2010)	Better cleavage rate after *in vitro* fertilization of oocytes from linolenic acid supplemented cows
Lapa *et al*. (2011)	Improved development and embryo quality after trans-10 cis-12 CLA supplementation during bovine oocyte maturation

It is generally accepted that alterations in dietary fatty acid intake cause a similar shift in the fatty acid profile of the bovine follicular fluid, the cumulus cells and the oocyte (Wonnacott *et al*., 2010; Zachut *et al*., 2010). However, Fouladi-Nashta *et al*. (2009) concluded that the ovary can buffer the oocyte against major fluctuations in plasma n-3 and n-6 fatty acids, as they could not find significant effects on fatty acid composition in granulosa cells or in the oocyte's developmental potential. However, using a mouse model, Wakefield *et al*. (2008) found that dietary supplementation of the dam with n-3 fatty acid rich diets leads to increased production of reactive oxygen species and mitochondrial dysfunction in the oocyte. Caution is thus warranted when formulating diets during the breeding period.

From the above it is clear that the oocyte is vulnerable to disturbances in its biochemical and endocrine microenvironment. In the typical case of the dairy cow, there are sufficient data available to assume that oocyte quality is indeed impaired. The oocyte not only suffers from the metabolic stress imposed on dairy cows early postpartum but also "senses" changes induced by a shifts in feed intake and composition. Very recently, similar observations have been done in rabbit (Arias-Alvarez *et al*., 2009) and there is growing evidence that also the human oocyte suffers from metabolic disturbances in the mother. Finally, it is important to mention that the effect of diet on oocyte quality and lipid content seems furthermore to be confounded by the body condition of the oocyte donor (Adamiak *et al*., 2006; Awasthi *et al*., 2010).

Oocyte metabolism as a proxy for its quality

From *in vitro* studies, it is obvious that oocytes have a requirement for pyruvate, glucose and amino acids. For example, Rieger and Loskutoff (1994) reported that pyruvate metabolism by bovine oocytes doubled throughout the first 12 h of maturation, returning to basal levels by 24 h after maturation (Rieger and Loskutoff, 1994), a pattern also seen in human oocytes (Roberts *et al*., 2002). Follicular cells provide sufficient pyruvate to support oocyte development (Donahue and Stern, 1968), a finding confirmed by Leese and Barton (1985) who suggested that pyruvate may be formed from glucose and lactate by the cumulus cells, before being transported into the oocyte.

Glucose also plays a key role during the maturation of intact cumulus oocyte complexes *in vitro* (Leroy *et al*., 2004, 2006). In the presence of FSH, glycolytic activity is increased 6.2 fold (as determined by lactate formation) with some additional glucose oxidation (Downs *et al.*, 1996). Interestingly, glucose is relatively poorly taken up by oocytes but readily by cumulus cells which express four glucose transporters whereas oocytes express only one (Dan-Goor *et al.*, 1997). In addition to its role in energy metabolism, glucose is also important in the FSH-mediated resumption of meiosis, particularly in the mouse (Downs and Hudson, 2000). It is likely that this interaction is linked to pentose phosphate pathway (PPP) activity, since Downs *et al.* (1996) reported that, in the mouse, germinal vesicle breakdown is reduced in the presence of PPP inhibitors. PPP provides ribose-5-phosphate, a precursor for *de novo* synthesis of purines and pyrimidines. In this regard, Rose-Hellekant *et al*. (1998) and Krisher and Bavister (1999) demonstrated PPP activity in bovine oocytes during maturation where it likely plays a similar role in the regulation of meiosis. Cetica *et al.* (2002) measured the levels of phosphofructokinase (PFK) and glucose-6-phosphate dehydrogenase (G6PDH), key enzymes in glycolysis and PPP respectively, in bovine oocytes and cumulus cells. PFK was predominantly active in the cumulus whereas G6PDH activity was highest in the oocyte. The findings suggested, in agreement with Downs *et al.* (1996), that the PPP occurs mainly in the oocyte and that cumulus cells are primarily glycolytic.

Glutamine is another potential energy source for oocytes proposed by Rieger and Loskutoff (1994), who reported that bovine oocytes increased glutamine uptake 2.5 times during the first 18 h of maturation. Cumulus cells can convert glutamine into glutamate and α-ketoglutarate, which is metabolized through the tricarboxylic acid cycle (Rose-Hellekant *et al.*, 1998), a process stimulated by LH. While glutamine can provide ATP, it is unable to support complete maturation in the absence of glucose (Downs and Hudson, 2000) probably due to inadequate PPP activity (Sutton *et al.*, 2003).

This metabolic pathway overview serves to demonstrate the importance and intricacy of energy metabolism during oocyte maturation and development with regard to exogenous nutrients, which was reviewed in detail by Sutton *et al*. (2003). However, there is a growing interest in the role of endogenous energy stored in the bovine oocyte (reviewed by Sturmey *et al*., 2009). The egg is the largest cell in the female mammal and contains significant endogenous lipid concentrations, a large proportion of which can be found as triglyceride, which when metabolized through β-oxidation can generate significant ATP and can also account for a large proportion of the oxygen consumed (Sturmey and Leese, 2003). As described above, the presence of fatty acids in the diet influences the fatty acid profile of the oocyte. Thus it is vital that when studying metabolic parameters during oocyte development, adequate consideration is given to the role of endogenous energy sources (Sturmey *et al*., 2009), particularly given that dietary intervention can dramatically impact on the lipid profile of the follicular environment and the oocyte (Leroy *et al.*, 2008b).

As outlined above, the follicular environment is a reflection of the physiological status of the animal. Variance in the follicular environment is likely to have a significant effect on the metabolic activity of the oocyte and it is important to know that inappropriate metabolism of oocytes might play an important role in the reduction of oocyte and subsequent embryo quality. Hemmings *et al*. (2007) carried out a survey on amino acid metabolism of oocytes and related this to embryo viability. They reported differences in the consumption and production of neutral amino acids, relative to oocyte developmental competence, thus illustrating a link between amino acid metabolism and oocyte quality. As described above, we have shown that inappropriate supplementation of NEFA in the maturation environment has a significant negative impact on the amino acid metabolism of resulting embryos post fertilization (Van Hoeck *et al.*, 2011).

Finally, an inappropriate nutritional balance of the cow might have a direct impact on the very specific molecular regulation of oocyte development. To give one example of such a relationship, diets that raise insulin levels might mediate such an effect as has been explained above. In this regard, Uzbekova *et al*. (2009) recently reported an intriguing set of experiments, elegantly outlining a role of the enzyme Glycogen Synthase Kinase 3 (GSK-3) in oocyte development. GSK-3 is a regulator of a variety of signalling pathways in cells, particularly with regards to genes responsible for regulating gene expression. Insulin, in combination with growth factors, can inactivate GSK-3 and thus release control of the downstream signalling pathways ordinarily regulated by GSK-3. By inhibiting GSK-3, loss of appropriate regulation of key transcription factors might have severe downstream consequences. These observations require more investigation, but serve to illustrate just another mechanism by which the physiological status of the heifer/cow might influence oocyte development at a molecular level.

Changes in the oocyte's microenvironment and the consequences on offspring viability, health and performance

As explained above, the oocyte microenvironment, i.e. follicular fluid, influences oocyte metabolism, gene expression patterns, developmental competence, blastocyst quality, implantation rate and also final pregnancy results. However, there is growing evidence to suggest that the environment to which the oocyte and the early embryo are exposed to also has effects that persist into adulthood and subsequent generations

(Betteridge, 2001). The concept of the Developmental Origin of Health and Diseases refers to the programming of the offspring's metabolism and body functions in adulthood during its growth in utero. The availability of energy substrates during fetal growth determines the individual's sensitivity to metabolic and reproductive disorders (Gardner *et al.*, 2008). It is, however, noteworthy that events occurring before conception and early after can also affect the health of the offspring. We will only briefly focus here on effects at the level of the growing and maturing oocyte, before conception takes place. Different animal models have been used so far. In a rat model, maternal undernutrition through a low protein diet from the day of conception to 4.5 days after mating showed distinct negative effects on blastocyst quality and altered birth weight, postnatal growth rate, blood pressure and organ/body weight ratios (Kwong *et al.*, 2000). In mice, a low protein diet only fed before conception had no effects on gestation length, size, sex ratio or the growth rate of the offspring whereas adult offspring showed behavior disorders, cardiovascular perturbations and a lower number of brain nephrons (Watkins *et al.*, 2008). In sheep, it was recently shown that body condition of the ewe at conception is strongly related to lamb birth weight (Wallace *et al.*, 2010). It was not clear however, whether an oocyte factor is involved in this. *In vitro* maturation conditions in a mouse model had minimal effect on the long-term health of the offspring while a slight difference in cardiac functioning could be detected (Eppig *et al.*, 2009). In dairy cows, Berry *et al*. (2008) linked higher milk production around conception with reduced survival and milk yield in the progeny. Also in human assisted reproduction, *in vitro* fertilization of collected oocytes and subsequent embryo culture and transfer may affect the health of the newborn and adult (Ceelen *et al.*, 2008). Nevertheless, in a very recent study from Sakka *et al*. (2010) only a significant effect on blood pressure could be detected. Whether this is due to changes in the oocyte maturation conditions due to ovary stimulation, the *in vitro* conditions during fertilization and early cleavage or due to the significantly altered uterine milieu encountered by the embryo after transfer due to the preceding ovarian stimulation still remain a matter of debate (Boomsma *et al.*, 2010).

Many studies revealed that the periconceptual period is a vulnerable timeframe in terms of the effects of maternal metabolism and diet and the consequences for offspring health. Both the preconception and the early preimplantation period witness significant epigenetic modifications to DNA and histone proteins, modifying the expression of a number of developmentally important genes (Sinclair and Singh, 2007). Due to the typical metabolic stress during the first weeks postpartum, dairy cows form an excellent model to study for example the consequences of such epigenetic alterations in the oocyte and embryo on the developmental origin of health and diseases.

Conclusions

In conclusion, it can be said that not only ovarian activity, ovulation and maintenance of pregnancy are under pressure. The increasing incidence of embryonic and fetal losses also suggests that the quality of the female gamete at the moment of ovulation may be jeopardized.

By writing this review, we wanted to shed more light on the importance of the intrafollicular environment in the subfertility problem in general and with special attention to dairy cow and human infertility. The developmental capacity and the quality of the oocyte are crucial to establish a healthy pregnancy. However, it is clear that adverse conditions within the follicle during oocyte growth and maturation may originate from a disturbed maternal metabolism, which can lead to an incompetent oocyte and thus to impaired fertility.

Slowly, but steadily, *in vitro* and *in vivo* animal models reveal the exact mechanisms through which adverse follicular fluid conditions exert their negative effects on oocyte quality. Some of these possible pathways have been reviewed in the present paper. From a more applied point of view, generated data on the influence of metabolomic and transcriptome parameters in the follicular environment on the oocyte's development and quality may serve as a proxy to predict reproductive success rates.

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