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The consequences of metabolic changes in high-yielding dairy cows on oocyte and embryo quality*

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Unsatisfactory reproductive performance in dairy cows, such as reduced conception rates, in addition to an increased incidence of early embryonic mortality, is reported worldwide and has been associated with a period of negative energy balance (NEB) early post partum. *Typically, NEB is associated with biochemical changes such as high non-esterified fatty acid (NEFA), high β-hydroxybutyrate (β-OHB) and low glucose concentrations. The concentrations of these and other metabolites in the follicular fluid (FF) of high-yielding dairy cows during NEB were determined and extensively analyzed, and then were replicated in* in vitro *maturation models to investigate their effect on oocyte quality. The results showed that typical metabolic changes during NEB are well reflected in the FF of the dominant follicle. However, the oocyte seems to be relatively isolated from extremely elevated NEFA or very low glucose concentrations in the blood. Nevertheless, the* in vitro *maturation models revealed that NEB-associated high NEFA and low glucose levels in the FF are indeed toxic to the oocyte, resulting in deficient oocyte maturation and developmental competence. Induced apoptosis and necrosis in the cumulus cells was particularly obvious. Furthermore, maturation in saturated free fatty acid-rich media had a carry-over effect on embryo quality, leading to reduced cryotolerance of day 7 embryos. Only β-OHB showed an additive toxic effect in moderately hypoglycemic maturation conditions. These* in vitro *maturation models, based on* in vivo *observations, suggest that a period of NEB may hamper the fertility of high-yielding dairy cows through increased NEFA and decreased glucose concentrations in the FF directly affecting oocyte quality. In addition to oocyte quality, these results also demonstrate that embryo quality is reduced following an NEB episode. This important observation may be linked to the typical diet provided to stimulate milk yield, or to physiological adaptations sustaining the high milk production. Research into this phenomenon is ongoing.*

Keywords: high-yielding dairy cow, fertility, oocyte, embryo, metabolism

Introduction

Reproductive failure in high-yielding dairy cattle is a multi-faceted problem. The pathogenesis of their sub-fertility is complex. The interactions between negative energy balance (NEB), early *post partum* and the hypothalamus–pituitary–ovary–uterus axis have been studied thoroughly (Ducker *et al.*, 1985; Lucy, 2001; Butler, 2003). Energy partitioning favoring the mammary tissue makes it possible to produce large volumes of milk, putting the remainder of the body in a status of severe NEB. Disturbed endocrine signaling during NEB leads to a retarded resumption of ovarian cyclicity *post partum* that has been recognized as a major factor contributing to the reproductive failure often exhibited by high-yielding dairy cattle (Opsomer *et al.*, 1998). However, attention has recently been shifting towards the ubiquitously reported disappointingly low conception rates (Royal *et al.*, 2000; Lucy, 2001; Bousquet *et al.*, 2004) and the remarkably high incidence of early embryonic mortality (Dunne *et al.*, 1999; Mann and Lamming, 2001; Bilodeau-Goeseels and Kastelic, 2003) in these cattle. Therefore, it is critical to further investigate the quality of the oocyte and embryo, and to elucidate the direct effects of their micro-environments in order to adequately resolve the problem of sub-fertility (O'Callaghan and Boland, 1999).

Recent studies have confirmed that the quality of the female gamete and the embryo is under pressure (Kruip *et al.*, 1995; Kendrick *et al.*, 1999; Gwazdauskas *et al.*, 2000;

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Wiltbank *et al*., 2001; Walters *et al*., 2002). Oocytes retrieved from high genetic merit cows, irrespective of their milk production value, resulted in significantly lower blastocyst yields *in vitro* (Snijders *et al*., 2000). This suggests possible adverse effects on fertility of the enforced genetic selection towards milk production.

In addition to oocyte quality, embryo quality also seems to be reduced in high-producing dairy cows compared to their non-lactating counterparts (Wiltbank *et al*., 2001), and what is more, a high proportion of non-viable embryos were produced by lactating cows (Sartori *et al*., 2002). More studies are needed to obtain an informative and complete picture of average embryo quality in high-yielding dairy cows. Moreover, in the literature, many suggestions are made and much speculation offered on the causes of the reduction in quality of oocytes and embryos in dairy cattle; examples of these are provided by the following questions:

- Is oocyte growth and maturation adversely affected before ovulation due to biochemical or endocrine alterations in the intra-follicular environment (O'Callaghan and Boland, 1999; Lozano *et al*., 2003)?
- Has the micro-environment of the oviduct or uterus been changed due to dietary and metabolic changes in the modern dairy cow, thus creating a hostile environment for the early embryo (Elrod and Butler, 1993; McEvoy *et al*., 1995; Kenny *et al*., 2002)?
- Is something defective in the genetic structure of modern dairy cow oocytes due to consecutive years of rigorous genetic selection towards milk yield? Or in other words, is there a genetic correlation between merit for milk production and oocyte or embryo quality (Snijders *et al*., 2000; Hayhurst *et al*., 2007)?

In contrast with the extensive knowledge of disturbed endocrine signaling and ovarian function, clear evidence concerning the deficiencies in oocyte and/or embryo quality, and the consequent impact on the reproductive performance in high-producing dairy cows is severely lacking. In the present article, we will review a number of possible mechanisms linking NEB to oocyte quality. Furthermore, in the event an embryo is formed after fertilization, we will investigate whether the quality of early life is impaired. Accordingly, oocyte and embryo quality were investigated and compared in lactating high-yielding dairy cows, non-lactating dairy heifers and beef cows.

Follicular fluid, the missing link between blood and gamete

An NEB is characterized by a number of typical endocrine and biochemical changes in the blood of modern dairy cows (Herdt, 2000). Some studies have already inferred these metabolic changes in serum affect oocyte quality, but the physiological connection between blood and oocyte is still not well established, i.e. the follicle and the follicular fluid (FF) (Hashimoto *et al*., 2000; De Wit *et al*., 2001; Jorritsma *et al*., 2004).

The follicle is an avascular 'compartment' filled with FF in which the oocyte undergoes the fine-tuning process of oocyte growth, prematuration and final maturation (Bagavandoss *et al*., 1983; Gosden *et al*., 1988). The physiological properties of FF have been reviewed by Gosden *et al*. (1988). During the process of follicular growth, the physiochemical properties of the blood–follicle barrier transform thoroughly, suggesting that the oocyte's environment undergoes compositional changes (Edwards, 1974; Wise, 1987; Gosden *et al*., 1988). In addition, the active transport mechanisms through the follicular wall may also alter during follicular growth. Argov *et al*. (2004), for example, recently demonstrated that while lipoproteins are predominantly internalized by endocytosis in small follicles, this is not the case in large follicles, in which circulating lipoproteins contribute their cholesterol esters by selective uptake and without internalization of the lipoprotein as such. Hence, to further investigate the biochemical milieu of the oocyte prior to ovulation, the composition of FF originating in three differently sized follicles was determined and compared to the composition of serum from 30 dairy cows shortly *post mortem* (Leroy *et al*., 2004a). The data confirmed that the FF's composition changes as the follicle grows. Another important finding was that the FF's composition is correlated with that of the serum. These correlations are 'static', however, and for the confirmation of correlations in this particularly 'dynamic' system, the composition of FF of high-yielding dairy cows was established during the early *post partum* period by means of repeated trans-vaginal follicle puncture and FF aspiration.

In a subsequent study, Leroy *et al*. (2004b) concentrated on compositional changes over time in the FF of high-yielding dairy cows early *post partum*; here the data were compared with the extensively reported biochemical alterations in blood during NEB (see above). NEB typically causes some obvious changes in serum, such as high non-esterified fatty acids (NEFA) and β-hydroxybutyrate (β-OHB) concentrations, or low glucose concentrations (Baird, 1982; Chilliard *et al*., 1998; Duffield, 2000; Herdt, 2000). Urea concentrations can also be elevated, due to an increased amino acid metabolism for gluconeogenesis, or the intake of a protein-rich diet (Butler, 1998; Sinclair *et al*., 2000). Britt (1992) hypothesized that these features of the NEB can directly affect the follicle and its enclosed oocyte, leading to the ovulation of an inferior oocyte. This hypothesis is interesting because it is generally accepted that oocytes are highly vulnerable to any disruption in their environment (O'Callaghan and Boland, 1999), a point of view that has been more or less confirmed by others (Armstrong *et al*., 2001; Boland *et al*., 2001). The data of Leroy *et al*. (2004b) provided evidence to, at least partially, answer the question raised by Britt (1994): 'How does NEB directly influence the micro-environment that is most intimately linked with the oocyte?'

An adapted ovum pick-up technique (Bols *et al*., 1995) was used at six different points in time during the early *post partum* period to collect FF from the dominant follicle of high-producing dairy cows. Given that follicular size is related to FF composition (Leroy *et al*., 2004a), similar-sized follicles were aspirated throughout the entire study. Due to the reduced accessibility of the ovaries during the puerperium period,

FF was only collected from day 14 *post partum* onwards. Good correlations were found between serum and FF composition for glucose, β-OHB, urea and total cholesterol. Based on the results of repeated measurements (dynamic correlations) (Leroy *et al*., 2004b), we can state that typical *post partum* serum fluctuations are more or less reflected in the FF of the dominant follicle. For urea and β-OHB, no concentration differences between serum and FF could be detected. It is important to mention, though, that the follicle appears to be able to maintain higher glucose and lower NEFA concentrations than does serum. In other words, it can be suggested that the oocyte is isolated, possibly even protected, from excessively low glucose or high NEFA concentrations in the blood.

Despite the follicle's buffering capacities, glucose concentrations do decrease, and NEFA concentrations significantly rise, in FF during NEB. Also Jorritsma *et al*. (2003) and Comin *et al*. (2002) described an increase in NEFA concentrations in FF due to an acute dietary restriction. However, in these studies no concentration gradients between serum and FF were mentioned, there is now enough evidence to conclude that the growing and maturing oocyte is directly exposed to the typical biochemical changes that occur in high-yielding dairy cows early *post partum*. It has furthermore been established that high urea concentrations, also found in FF (Hammon *et al*., 2005), can be toxic to oocytes during maturation (Ocon and Hansen, 2003; Iwata *et al*., 2006), probably through an inhibition of the polymerization of tubulin into microtubules (De Wit *et al*., 2001). The same is true for the lowered glucose concentrations observed. Adequate glucose supplies are necessary to support normal cumulus expansion and nuclear maturation (Krisher and Bavister, 1998; Sutton-McDowall *et al*., 2004). Similarly, high NEFA and β-OHB concentrations are probably harmful to the oocyte's developmental competence, but this has, to our knowledge, never been substantiated.

Negative energy balance and its direct consequences for oocyte quality: an in vitro model

Initially, it was important to pay attention to the NEFA fraction of the FF, as not only the absolute NEFA concentration but also the NEFA composition is physiologically important. Samples were analyzed by means of a combined thin layer and gas chromatography (Folch *et al*., 1957). The results surprisingly revealed that both the NEFA concentration (see above) and the NEFA composition significantly differed between serum and FF. Differences in albumin content (on which NEFA are predominantly bound) between the two compartments could not be found, and thus did not offer any clues for explaining the observed differences in NEFA concentration and composition. Disconcertingly, the described dynamic interchange of NEFA between serum and FF (Moallem *et al*., 1999) was also not totally in agreement with these findings. The study of Chung *et al*. (1995), however, offered a possibly useful clarification. In the presence of high NEFA levels, a substantial portion of the NEFA in serum is partitioned into low-density lipoproteins (LDL). The saturated fatty acids in particular are bound on LDL, while the unsaturated ones are preferentially bound on albumin (Chung *et al*., 1995). Because LDL are absent in FF, these findings could account for the differences in concentration and composition of NEFA in FF compared to serum early *post partum* (Wehrman *et al*., 1991).

Chromatographic analyses revealed that the three most abundant free fatty acids present in FF during NEB were oleic, palmitic and stearic acid. The NEB-associated FF concentrations of these three free fatty acids were applied to an *in vitro* serum-free maturation model to evaluate their effect on oocyte quality (Leroy *et al*., 2005a). In these tests, oleic acid had no discernable effect on the oocyte's developmental capacity. However, exposing oocytes to palmitic and stearic acid, at concentrations comparable to those assessed *in vivo*, resulted in reduced maturation rates, thus leading to unsatisfactory fertilization and cleavage rates. Also, cumulus expansion was adversely affected. In these cumulus cells, a significantly higher rate of apoptosis and even necrosis could be detected after 24 h of exposure to elevated stearic or palmitic acid concentrations. Very recently, it was shown that this fatty acid exposure during maturation has carry-over effects on embryo quality in terms of open pulled straw freezeability (Shehab-El-Deen *et al*., in press). This also suggests that the fatty acid composition of the oocyte, and thus the embryo, is altered, leading to reduced freezing-warming survival rates. Rooke *et al*. (2006) demonstrated that changes in the FF fatty acid composition are also reflected in the fatty acid content and profile of the cumulus-oocyte complex.

Similar toxic effects of high NEFA concentrations on bovine or human granulosa cells *in vitro* have been shown in other studies (Mu *et al*., 2001; Jorritsma *et al*., 2004; Vanholder *et al*., 2005). Optimal granulosa and cumulus cell functioning are indispensable for oocyte maturation because these cells are responsible for endocrine and paracrine signaling (Bilodeau-Goeseels and Panich, 2002; Tanghe *et al*., 2002). Therefore, it is most likely that the toxic effect of NEFA on oocyte quality is partly an indirect effect. In contrast with the results of Leroy *et al*. (2005a), Jorritsma *et al*. (2004) did find detrimental effects of oleic acid. In their study, however, oleic acid was bound on albumin and was added in supra-physiological concentrations to an undefined *in vitro* maturation medium (contained fetal calf serum, an undefined source of fatty acids). Moreover, it is unclear whether these adverse effects were caused by the addition of BSA itself or by oleic acid. Homa and Brown (1992) showed that albumin-bound linolenic acid in *in vitro* maturation medium inhibited germinal vesicle breakdown in denuded oocytes.

Similar toxic effects of NEFA have also been described for Leydig cells, muscle cells and pancreatic β-cells, and it is the induction of apoptosis and/or insulin resistance as well as changes in membrane properties that have been specifically suggested as potential mechanisms for explaining the observed toxic effects (Shimabukuro *et al*., 1998;

Maedler *et al*., 2001; Hirabara *et al*., 2003; Lu *et al*., 2003; Jorritsma *et al*., 2004).

These indications of NEFA toxicity at the oocyte and follicle level not only are important in relation to the sub-fertility issue in modern dairy cows, but may also provide a valuable model for human research. Obesity and diabetes are characterized by increased concentrations of NEFA due to high adipose sensitivity for lipolytic triggers (Herdt, 2000; Cnop *et al*., 2001). Our data suggest that the frequently reported fertility disorders in obese or diabetic women (Pasquali *et al*., 2003) may be not only due to the toxic effects of NEFA on granulosa cells, which mainly lead to amenorrhea (Mu *et al*., 2001), but could also originate from direct detrimental effects on the cumulus-oocyte complex. The latter could explain the disappointing IVF or intracytoplasmic sperm injection (ICSI) results, and the higher risk for early pregnancy loss in obese women, as has been documented by Fedorcsak *et al*. (2000 and 2004) and Pasquali *et al*. (2003). Ongoing research on humans should confirm or refute the validity of applying this bovine model in human studies.

Not only high NEFA but also elevated ketone concentrations are a distinctive characteristic of NEB (Sato *et al*., 1999). High ketone concentrations generally go together with hypoglycemia (Herdt, 2000). Therefore, in a second *in vitro* maturation model (Leroy *et al*., 2006), the effects of combined high β-OHB and low glucose concentrations, ascertained in measurements of FF of dairy cows during NEB, were investigated. The primary conclusion of this study was that the *in vitro* model imitating sub-clinical ketosis had no effect on the oocyte's developmental capacity *in vitro*. Clinical ketosis, however, turned out to be detrimental to oocyte quality *in vitro*; this was presumed to be due to the effect of depleted glucose, rather than high β-OHB concentrations. Thus, the toxicity of β-OHB, as has been described for polymorphonuclear cells and macrophages (Hoeben *et al*., 1997; Sartorelli *et al*., 2000), could not be confirmed for cumulus-oocyte complexes. Conversely, it can be assumed, with a high degree of certainty, that inadequate glucose supplies compromise oocyte developmental competence, and this is a conclusion that is in agreement with other studies (Krisher and Bavister, 1998; Cetica *et al*., 2002; Sutton-McDowall *et al*., 2004).

When interpreting these *in vitro* results and explaining them in terms of sub-fertility in high-producing dairy cows, some prudence is warranted. In studies described above, it was hypothesized that elevated NEFA or β-OHB concentrations, together with low glucose concentrations, contributed to reducing fertility in high-yielding dairy cows by exerting detrimental effects on oocyte developmental competence. These findings more or less resemble the hypothesis of Britt (1994), who stated that a follicle grown during the period of NEB early *post partum* could be affected by unfavorable metabolic changes, and may contain a developmentally incompetent oocyte; following a growing and maturation phase of several weeks, this inferior oocyte is ovulated at the time of the initial insemination (Lucy, 2003). This hypothesis has been partially confirmed in recent *in vivo* studies (Gwazdauskas *et al*., 2000; Snijders *et al*., 2000; Sartori *et al*., 2002). It is important, however, to mention that the combined *in vitro* and *in vivo* model used in the studies of Leroy *et al*. (2005a and 2006) described above was not entirely appropriate for investigating the carry-over effect on oocyte quality as hypothesized by Britt (1994). The results only documented the FF composition in the dominant follicle during the NEB mimicked *in vitro*. Quiescent follicles, however, provide a much poorer isolation from the extrafollicular environment and blood serum for the oocytes embedded within them. As a consequence, such oocytes are probably exposed to even higher NEFA concentrations (Zamboni, 1974). Another possibility is that oocytes of primordial follicles are completely insensitive to all these metabolic disruptions. Moreover, the cumulus-oocyte complexes were exposed to elevated NEFA or β-OHB and low glucose concentrations for only 24 h, whereas *in vivo* the oocytes are exposed to these concentrations for several days or even weeks. In the ideal model, primordial follicles should be cultivated in high NEFA conditions for several weeks. However, maintaining such long-term cultures of primordial follicles presents major obstacles, and growing bovine primordial follicles up to the preovulatory stage has so far proven to be unachievable (Gutierrez *et al*., 2000); although, successes have been obtained in mice with prolonged primordial and preantral follicle culture leading to *in vitro* ovulation and, after fertilization, the birth of pups (Cortvrindt and Smitz, 2001). Current research uses this murine model to investigate the consequences of long-term exposure to elevated NEFA on follicular growth and subsequent oocyte development. Nevertheless, it is believed that the data from the present model revealed for the first time the possible toxic effects of high FF NEFA and low glucose concentrations on the developmental competence of bovine oocytes *in vitro*.

Back to the field: a closer look at embryo quality

From the data presented above, it can be concluded that the oocyte is vulnerable to at least some of the metabolic alterations associated with NEB. Clearly, obvious adverse effects on oocyte quality were observed in our *in vitro* model. Logically, the next step would be to investigate the consequences of these effects on embryo quality. It has been suggested by Rizos *et al*. (2002) that the conditions prior to fertilization are the determinants for embryo yield, while the embryo's culture environment is crucial for embryo quality. On the basis of that theory, the toxic effects of high NEFA and low glucose concentrations during oocyte maturation that have been demonstrated in high-producing dairy cows (Leroy *et al*., 2005a and 2006) should primarily lead to low fertilization (i.e. conception) rates. However, based on the reports of Royal *et al*. (2000) and Bousquet *et al*. (2004), this does seem to be the case. Also, the environment of both the oocyte and the embryo is said to be crucial to ultimate fertility. As suggested by Kenny *et al*. (2002), Elrod and Butler (1993) and McEvoy *et al*. (1995), high-energy and/or high-protein diets can alter the microenvironment of the embryo in the oviduct and uterus (for review see Leroy *et al*., 2008). Such changes are expected to

be detrimental to embryo quality (Rizos *et al*., 2002), which has been shown experimentally in heifers by Wrenzycki *et al*. (2000). However, it has never been demonstrated whether or not this is also the case in high-producing dairy cows. Accordingly, a field trial was arranged to compile data on the embryo quality of high-producing dairy cows in comparison with non-lactating dairy heifers and (non-lactating) beef cows. Through this field trial it was possible to investigate simultaneously the effects of milk production and breed (or genetic background) (Leroy *et al*., 2005b). Briefly, embryos from lactating dairy cows clearly displayed inferior quality as assessed by morphological evaluation, compared to dairy heifers or beef cows. Furthermore, it was possible to demonstrate by means of a multivariable regression model that the state of producing milk *v.* not producing milk was highly correlated with embryo quality. It is also important to mention that no differences were found in fertilization rates or in the number of transferable embryos per embryo collection among these animals.

Because the embryos of the lactating dairy cows were on average collected around day 230 *post partum*, it is very unlikely that a carry-over effect of NEB, as has been hypothesized by Britt (1992), is responsible for their poor quality. This could have been a likely explanation when embryo collection was performed on average around 2 to 3 months after calving, as was done by Sartori *et al*. (2002); he also found an obvious difference in embryo quality between lactating dairy cows and maiden heifers, suggesting that genetic merit for milk production does not account for the reduction in embryo quality observed in lactating dairy cows. In contrast with our results, Sartori *et al*. (2002) reported not only inferior embryo quality but also a lower fertilization rate, illustrated by a higher proportion of unfertilized oocytes present in the uterine flushing of lactating dairy cows. Sartori *et al*. (2002) may have described the adverse influences of a carry-over effect of NEB on oocyte quality (reduced fertilization rates), combined with the possible negative effects of lactation, management or diet on the micro-environment of the oviduct or uterus (reduced embryo quality), similar to what we found in our field trial. By way of summary, all suggested mechanisms that could potentially diminish embryo quality are diagrammatically represented in Figure 1. Further research should reveal the exact mechanism through which embryo quality is adversely affected in lactating dairy cows.

One of the major morphological characteristics evaluated in the study of Leroy *et al*. (2005b) was embryo color. By means of a new lipid evaluation technique (Leroy *et al*., 2005c) it could be demonstrated that embryo color is correlated with lipid content, as has previously been suggested by others (Sata *et al*., 1999; Abe and Hoshi, 2003). Lactating dairy cow embryos were generally dark and contained as much lipid as *in vitro* produced embryos that are known to accumulate excessive amounts of lipids (Abe *et al*., 1999). This has never been shown before. A high lipid content has undeniably been linked with impaired embryo quality (Reis *et al*., 2003; Rizos *et al*., 2003). The underlying mechanism(s) linking milk production or nutrition with embryo color and/or lipid content is unknown, and further research is necessary. A broader overview on factors influencing oocyte and embryo quality as well as corpus luteum quality was presented in earlier work (Leroy *et al*., 2008).

Perspectives for future research and some food for thought

As has been mentioned above, future research should enlighten us more about the interactions between the blood, the micro-environment in the oviduct or uterus and embryo metabolism. Furthermore, several studies have indicated that NEB is also associated with depressed immunity during the first weeks *post partum*, thus leading to an increased susceptibility to infectious diseases such as mastitis and metritis (Hoeben *et al*., 2000; Lacetera *et al*., 2005).

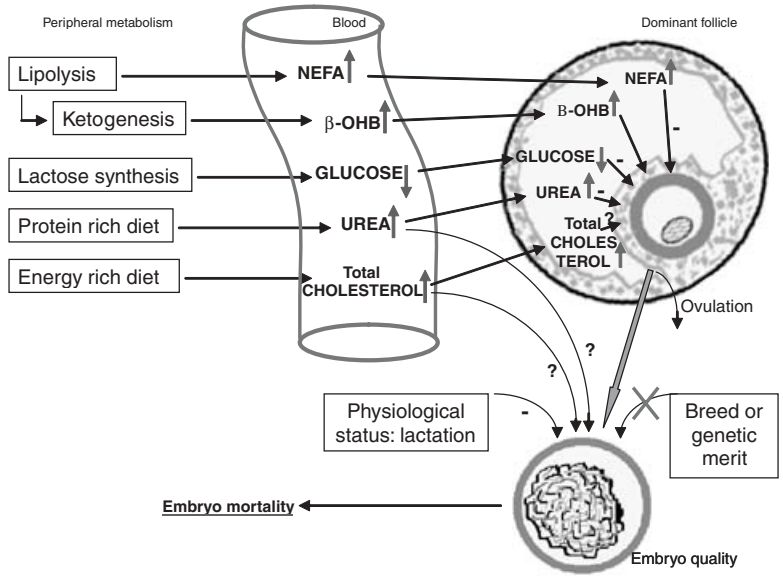

Figure 1 Diagrammatic representation of possible mechanisms by which embryo quality can be impaired in high-yielding dairy cows.

Bearing this in mind, it becomes important to consider not only the direct link between NEB and fertility but also that reproductive functions are affected indirectly by an increased incidence of infectious diseases. Mastitis, for example, that, together with low fertility, is the principal reason for the culling of dairy cows, has been shown to be directly linked to the retarded onset of ovarian activity *post partum* (Loeffler *et al.*, 1999; Rajala-Schultz and Gröhn, 2001; Huszenicza *et al.*, 2005). Whether infectious diseases can affect the oocyte and/or the embryo in a direct way has been poorly studied, and certainly requires further investigation (Hansen *et al.*, 2004). Furthermore, environmental pollution has also been associated with direct harmful effects on oocyte quality through the generation of endocrine disrupters (Brevini *et al.*, 2005), and this merits further study.

Is there still a need for high-producing dairy cows? Yield maximization per animal is indeed preferable from both an economic and environmental point of view. However, only outstanding herd management can guarantee the animal's welfare under such pressures. But even with excellent management, the demands on these animals remain high as evidenced by the fact that they are rapidly culled for reasons such as reduced fertility, metabolic disorders and infectious diseases. Now, it is becoming clear that even the oocyte and the embryo may directly suffer from high levels of productivity.

Conclusions

It can be concluded that the typical biochemical serum changes observed in dairy cattle during NEB early *post partum* are well reflected in the FF of the dominant follicle, thus exposing the granulosa cells and the maturing oocyte. *In vitro* maturation models revealed that NEB associated with elevated NEFA and lowered glucose concentrations are indeed toxic to the oocyte, resulting in both diminished oocyte maturation and embryo developmental competence.

Even after the period of NEB, and when the carry-over effects of the NEB were no longer present, high-yielding dairy cows produced statistically significantly inferior embryos in comparison with dairy heifers and beef cows. With a newly developed lipid evaluation technique, it was possible to demonstrate that high-producing dairy cow embryos contained up to 45% more lipids, compared to the embryos of non-lactating animals. These findings imply that it is not genetic merit for milk production or breed that has an adverse impact on embryo quality, but rather that a variety of factors associated with milk production as such (metabolism, nutrition, management) induce hostile conditions that prevent optimal embryo development. Further research is required to fully comprehend how a dairy cow's milk production and nutrition influences embryo health and metabolism via an altered environment in the oviduct and uterus.

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