Reprod Dom Anim **43**, 623–632 (2008); doi: 10.1111/j.1439-0531.2007.00961.x
ISSN 0936-6768

# Reduced Fertility in High-yielding Dairy Cows: Are the Oocyte and Embryo in Danger? Part II

## Mechanisms Linking Nutrition and Reduced Oocyte and Embryo Quality in High-yielding Dairy Cows*

**JLMR Leroy[1], A Van Soom[2], G Opsomer[2], IGF Goovaerts[1] and PEJ Bols[1]**

[1]*Laboratory for Veterinary Physiology, Department of Veterinary Sciences, Faculty of Biomedical, Pharmaceutical and Veterinary Sciences, University of Antwerp, Wilrijk;* [2]*Department of Reproduction, Fertility and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*

**Contents**

Dairy cow fertility has been declining during since the mid-80s and this has given rise to numerous scientific studies in which important parts of the pathogenesis are elucidated. Reduced oocyte and embryo quality are acknowledged as major factors in the widely described low conception rates and in the high prevalence of early embryonic mortality. Apart from the importance of the negative energy balance (NEB) and the associated endocrine and metabolic consequences, there is a growing attention towards the effect of the milk yield promoting diets which are rich in energy and protein. Starch-rich diets can improve the energy status and thus the ovarian activity in the early postpartum period but the oocyte and embryo quality can suffer from such insulinogenic diets. Supplementation of dietary fat has a similar dual effect with a beneficial stimulation of the ovarian steroid production while the oocyte and the embryo display an altered energy metabolism and excessive lipid accumulation. High-protein diets can elevate the ammonia and urea concentrations in the blood, leading to changed intrafollicular, oviductal and uterine environments. Oocytes and embryos are highly sensitive to such changes in their microenvironment, possibly leading to a disturbed maturation, fertilization or early cleavage. 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 proposed and comprehensively reviewed in the present report.

## Introduction

Studies over the last two decades clearly established extensive knowledge about the link between nutrition and ruminant fertility (for review: Robinson et al. 2006). Dairy cow fertility has been declining, and in addition to the negative energy balance (NEB), high-protein and high-energy diets have also been proposed to adversely affect the finely tuned process of becoming pregnant (Butler 2003). Dietary changes can cause an immediate and rapid alteration in a range of humoral factors that can profoundly alter endocrine and metabolic signalling pathways known to be crucial for the function of reproductive mechanisms (O'Callaghan et al. 2000; Boland et al. 2001; Diskin et al. 2003). Modern dairy cows are typically fed starch-, or fat- and protein-rich diets, to maximize milk production. In the present report, we will overview some of the possible causes through which such rations may interfere with oocyte and/or embryo quality. After all, it has been shown that the competence of an oocyte or embryo to establish a full-term pregnancy is reduced in lactating animals, compared to their non-lactating herd mates (Wiltbank et al. 2001; Sartori et al. 2002). Furthermore, results of our previous work suggested that, even without taking NEB into account, the metabolic adaptations sustaining high milk production, and most probably the typical milk-stimulating diet, may contribute to inferior embryos with a significantly darker appearance and higher lipid content (Leroy et al. 2005a). Most studies linking nutrition and fertility describe the influence of short-term changes in feed intake on various types of fertility parameters. A large portion of these studies try to establish an optimal diet to stimulate superovulatory responses to increase the yield of viable oocytes or embryos. The results of these studies are, therefore, of limited value in elucidating the specific situation of high-yielding dairy cows, because in their case, typical milk-stimulating diets are fed for several months, and superovulation treatments are generally not applied. Furthermore, postpartum dairy cows are rarely used in such experimental designs, because milk production and the accompanying NEB during the first 5–10 weeks postpartum are known to confound the possible effects of nutrition on fertility. Literature focusing on the long-term effects of milk-driving diets on fertility, and more specifically, on oocyte or embryo quality in the early postpartum period, is therefore very limited.

## Starch-rich Diets to Augment Dietary Energy Intake

In an attempt to reduce the extent and the duration of the NEB, dairy cows are fed high-energy diets (usually based on high starch content). These high-energy diets (or insulinogenic or glucogenic diets) can attenuate a severe drop in insulin and glucose concentrations or can avoid excessively high non-esterified fatty acid (NEFA) concentrations during the period of NEB (Van Knegsel et al. 2007a). Glucogenic diets have clearly been shown to be beneficial for fertility as they stimulate the resumption of normal endocrine signalling leading to

*This manuscript is the second part of a twin review paper. The title of the companion manuscript is: Reduced Fertility in High-yielding Dairy Cows: Are the Oocyte and Embryo in Danger? Part I: The Importance of Negative Energy Balance and Altered Corpus Luteum Function to the Reduction of Oocyte and Embryo Quality in High-yielding Dairy Cows.

the early onset of ovarian activity (Gong 2002; Van Knegsel et al. 2005). In this way, the possible direct harmful effects of elevated NEFA and reduced glucose concentrations on oocyte and granulosa cell quality (Leroy et al. 2005b; Vanholder et al. 2005; Leroy et al. 2006) may be avoided. An in-depth overview on the effects of a NEB on oocyte and embryo quality is presented in the companying review paper (Leroy et al. 2008).

Several weeks after calving, when cows resume a positive energy balance, the amount of energy intake can affect follicular dynamics and circulating concentrations of steroids and growth factors (for reviews see: O'Callaghan and Boland 1999; Webb et al. 2004) that can affect oocyte and embryo quality in a direct or indirect way (Fig. 1). In addition to the favourable effects on energy balance, high feeding levels increase liver blood flow and upregulate the steroid catabolism in the liver which results in reduced circulating progesterone concentrations, on the one hand (Vasconcelos et al. 2003) but, on the other hand, may result in increased progesterone production by the corpus luteum (CL; Armstrong et al. 2001). Reports in the literature about a possible causal link between energy intake, peripheral progesterone concentrations and embryo viability are contradictory (Abecia et al. 1997; Dunne et al. 1999; McEvoy et al. 2001; Lozano et al. 2003) which necessitates further research.

Wrenzycki et al. (2000) demonstrated more specifically that both energy source and quantity can have significant effects on the expression of developmentally important genes in embryos, such as: Cu/Zn super oxide dismutase (SOD; prevention of oxidative stress), and on pyruvate utilization in day 6 bovine embryos. *Ad libitum* intake of barley-based concentrates, under experimental circumstances, reduced pyruvate utilization and significantly enhanced the expression of Cu/Zn SOD in embryos. Yaakub et al. (1999a) found similar adverse effects on embryo quality of a barley-based high concentrate low fibre diet, fed before superovulation and embryo recovery. It is possible that a diet induced shift in the volatile fatty acid profiles in the rumen (proprionate vs acetate), affects embryo quality indirectly, via an altered energy metabolism [insulin and/or insulin-like growth factor I (IGF-I) concentrations] or directly, via compositional changes in follicular, tubal or uterine fluids (Wrenzycki et al. 2000). This is an interesting finding because our modern dairy cows typically receive a high concentrate and low fibre diet.

There is an increasing evidence that possible adverse effects of high-energy diets on early embryonic development may be programmed even before fertilization during the acquisition of oocyte developmental competence in the follicle (O'Callaghan and Boland 1999; McEvoy et al. 2001; Lozano et al. 2003). Oocytes collected from heifers (McEvoy et al. 1997a; Nolan et al. 1998; Yaakub et al. 1999b) or ewes (Lozano et al. 2003) that were fed a low-energy diet showed an improved developmental competence *in vitro*. Whether altered pre- and/or post-ovulatory progesterone concentrations, because of such dietary changes, may explain differences in oocyte quality remains a point of discussion (McEvoy et al. 1995; Yaakub et al. 1999b). It is clear from the above that the interaction between altered steroid secretions in the ovary and steroid metabolism in the liver are hard to elucidate and therefore the distinct effect of each of these factors, induced by nutrition, on oocyte quality is hardly impossible to investigate in an *in vivo* approach.

As explained above, adequate insulin and IGF-I concentrations are beneficial for follicular growth (Landau et al. 2000; Armstrong et al. 2002a). However, the dietary induced higher bioavailability of intrafollicular IGF-I, through reduced IGF-binding protein (BP) concentrations, has the potential to interact directly with the oocyte (Armstrong et al. 2001; Comin et al. 2002). However, an over stimulation of the oocyte, by dietary-induced high IGF-I, and probably an associated

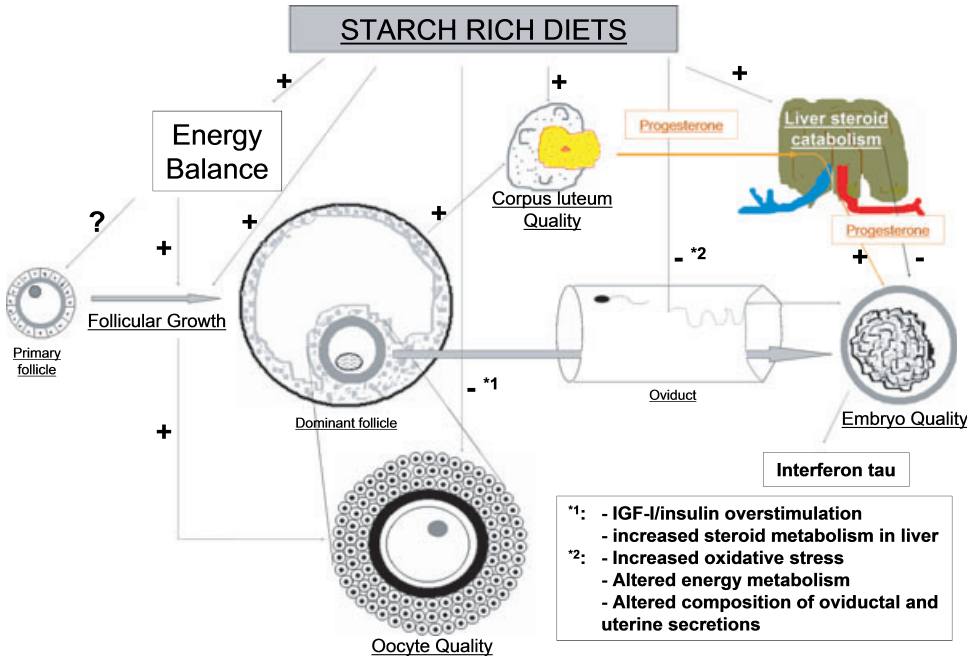


Fig. 1. Diagrammatic presentation of the major mechanisms through which starch-rich diets can affect oocyte and embryo quality in high-producing dairy cows. Starch-rich diets have a glucogenic effect on the energy balance which promotes follicular growth and steroid production which has a positive effect on oocyte quality. A sound follicle gives rise to a healthy corpus luteum, producing adequate amounts of progesterone, capable of supporting early pregnancy

increased insulin concentration, may have a negative effect on the oocyte's developmental competence (Armstrong et al. 2002b). It is important to mention that the level of energy intake stimulates oestrogen secretion by granulosa cells, resulting in beneficial effects on oocyte quality (Armstrong et al. 2002c; Comin et al. 2002). All mechanisms described above are schematically represented in Fig. 1.

Finally, a recent study of Adamiak et al. (2005a) demonstrated that the effect of nutrition on oocyte quality is dependent on the body condition of the animal. A high plane of energy intake proved to be beneficial to oocytes from lean animals, while it was detrimental to oocytes from animals of moderately high body condition. Therefore, it can be concluded that the level of nutrition (energy content) an animal receives has the potential to influence their oocytes' developmental competence, and subsequent embryo viability in a direct and/or indirect way. The mechanisms described above, include changes in progesterone, oestrogen, IGF and insulin concentrations, and alterations in the follicular and uterine microenvironment. Concerning the specific reproductive physiology of high-producing dairy cows, our main assumption is that high-energy diets were revealed to be beneficial for oocyte quality in the early postpartum period, most likely by reducing the depth and duration of the NEB (Kendrick et al. 1999; Gwazdauskas et al. 2000). Later postpartum, however, when dairy cows regained a positive energy balance, a (excessively) high intake of nutritional energy may result in the production of over stimulated, and thus inferior, oocytes (Armstrong et al. 2001) and significantly reduced embryo quality. This may ultimately lead to decreasing conception rates, and to a higher incidence of embryo mortality. Hence, diets shown to enhance follicular growth may not necessarily be advantageous for ovum quality!

## Dietary Fat Supplementation and the Effect on Oocyte and Embryo Quality

Modern dairy rations are often supplemented with rumen protected fat to increase the energy intake in the early postpartum period and to increase fertility (Beam and Butler 1997; Thatcher et al. 2006). There are several hypotheses as to how dietary fat could influence reproductive performance (reviewed by Staples et al. 1998; Fig. 2), but once again, reports of the effects on fertility are contradictory (Staples et al. 1998; McNamara et al. 2003; Van Knegsel et al. 2005). Attempting to improve the energy balance (DeFrain et al. 2005), fat supplementation increases the dietary energy content which stimulates milk production and thus energy loss, ultimately resulting in even higher NEFA and beta-hydroxibutaric acid ($\beta$-OHB) and lower glucose and insulin concentrations (McNamara et al. 2003; Van Knegsel et al. 2005; Moallem et al. 2007). Even in a very recent study with isocaloric diets, Van Knegsel et al. (2007b) found out that lipogenic diets resulted in a higher energy partitioning to milk. Supplemental dietary fat increases the size as well as the estradiol production of the pre-ovulatory follicle (Lucy et al. 1991; Beam and Butler 1997; Moallem et al. 2007), most likely via the induction of high cholesterol concentrations in follicular fluid and plasma. This increased follicle size may have beneficial effects on both oocyte quality and CL function, as has been discussed above (Vasconcelos et al. 2001). The resulting hypercholesterolaemia also enhances progesterone secretion, thus, supporting early embryo developmental competence (Ryan et al. 1992; Lammoglia et al. 1996;

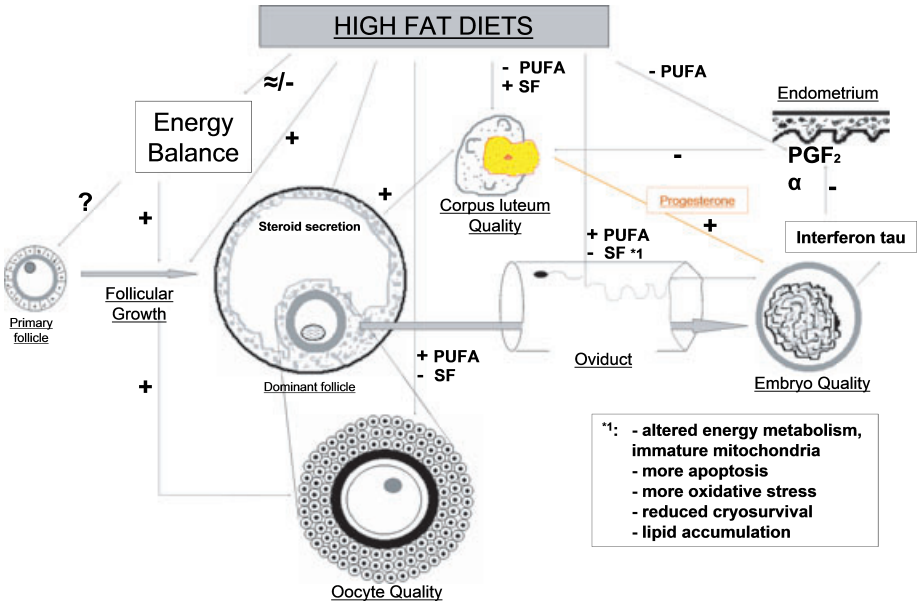


Fig. 2. Diagrammatic presentation of the major mechanisms through which high fat diets can affect oocyte and embryo quality in high-producing dairy cows. Fat-supplemented diets have no positive effect on energy balance. However, they promote follicular growth and steroid secretion (higher availability of the substrate cholesterol). Polyunsaturated fatty acids are said to have a positive effect on oocyte quality and can support early embryonic survival via reduction of the prostaglandin F2$\alpha$ secretion in the endometrium. Saturated fatty acids may have opposite effects. PUFA: polyunsaturated fatty acids; SF: saturated or monounsaturated fatty acids; PGF2$\alpha$: prostaglandin F2$\alpha$

McNamara et al. 2003). Apart from rations supplemented merely with saturated or monounsaturated fatty acids (to increase energy intake), also polyunsaturated fatty acids are becoming increasingly popular; particularly as a way to increase milk concentrations of omega-3 (n-3) fatty acids. Supplementation with these polyunsaturated fatty acids can reduce prostaglandin secretion by the endometrium, and hence support the lifespan of the CL (Staples et al. 1998; Cheng et al. 2001; Thatcher et al. 2006), an effect which would be beneficial for embryo survival. It was only recently that Bilby et al. (2006) illustrated the mechanism behind this observation, showing that diets rich in fish oil (n-3 polyunsaturated) have the potential to reduce the expression of endometrial cyclo-oxygenase-2, an essential enzyme for prostaglandin biosynthesis (Bilby et al. 2006; Thatcher et al. 2006). Hinckley et al. (1996), on the other hand, demonstrated that fish oil is also capable of inhibiting progesterone production by luteal cells cultured *in vitro*. The latter was confirmed *in vivo* by a study in which cows were fed a linseed-rich diet (linolenic acid, C18:3, n-3) leading to significantly reduced plasma progesterone concentrations (Robinson et al. 2002). Mattos et al. (2002) were not able to corroborate this negative effect on progesterone production in synchronised cows fed either eicosapentaenoic acid (EPA; C20:5, n-3) or docosahexaenoic acid (DHA; C22:6, n-3). In other words, it is important to consider the exact type of the supplemented fat (length of the carbon chain and degree of saturation) when estimating the effect on fertility. Clearly, a conclusive result of the effects of fat supplementation in dairy rations on the reproductive outcome, awaits further investigation (Fig. 2).

It is generally accepted that the quality of an oocyte and embryo is closely related to their fatty acid composition, in particular the composition of the phospholipid fraction (McEvoy et al. 2000). Focussing on possible direct effects of fat-rich diets on oocyte and embryo quality, it has recently been demonstrated that addition of 6% protected fat (saturated and monounsaturated) can alter the fatty acid profile, both in serum and in follicular fluid (FF) (Adamiak et al. 2004). Whether this will also hold true for the tubal and/or uterine microenvironment is still unknown. Furthermore, the change in FF fatty acid composition is also reflected in the fatty acid content and profile of the cumulus–oocyte complex (Adamiak et al. 2005b; Rooke et al. 2006). Fatty acid composition, and thus membrane fluidity, of an oocyte or embryo can be affected by the microenvironment (Zeron et al. 2001). The triglycerides, stored in lipid droplets, are proposed to be an important energy store (Kim et al. 2001). During pre-maturation and maturation *in vivo*, there seems to be a physiological lipid accumulation in the oocyte (Fair 2003). Sata et al. (1999) and Kim et al. (2001) demonstrated that oocytes and embryos *in vitro* are able to accumulate fatty acids from their environment. Such an excessive lipid accumulation is, however, known to impair the quality of the embryos by increasing their sensitivity to oxidative stress, chilling and cryopreservation (Abe et al. 1999; Reis et al. 2003). In addition, an increased lipid accumulation has been associated with suboptimal mitochondrial function and a deviation in the relative abundance of developmentally important gene transcripts from stress responsive genes, thus hampering the quality and hence the viability of the embryo (Abe et al. 2002; Rizos et al. 2003; Fig. 2). As mentioned earlier, we recently described extremely high lipid concentrations in embryos collected from high-producing dairy cows, when compared to the lipid content of maiden dairy heifer or beef cow embryos (Leroy et al. 2005a). The high-lipid content coloured the dairy cow embryos dark, and they appeared very similar to *in vitro* produced embryos when cultured in the presence of serum (Leroy et al. 2005a). The causal reason for this observation is as not yet known, and at present this interesting finding is under further investigation. It is hypothesized that physiological adaptations sustaining high milk production alter the microenvironment of the oocyte and/or embryo, ultimately resulting in a changed lipid metabolism and/or an excessive lipid uptake.

The consequences of feeding polyunsaturated fatty acid-rich diets on the subsequent quality of the oocyte or embryo have been scarcely considered. Bilby et al. (2006) found a negative effect on the oocyte's developmental capacity when dairy cows were fed linoleic acid (C18:2, n-6)-rich diets. Studies in sheep, on the other hand, revealed a favourable effect on oocyte quality of dietary supplementation with rumen protected fish oil (Zeron et al., 2002). Furthermore, Zeron et al. (2002) were able to demonstrate changes in the fatty acid composition of the oocyte, resulting in a positive effect on oocyte membrane integrity following chilling. Only recently, Pereira et al. (2007) were able to reduce lipid accumulation during *in vitro* culture and to improve the cryosurvival of bovine blastocysts by supplementing the serum containing culture medium with conjugated linoleic acid (C18:2, *trans*-10 *cis*-12). Further research is certainly needed.

## High Milk Production via an Elevated Crude Protein Intake and Possible Consequences on Oocyte and Embryo Quality

One of the strategies to sustain a high milk production in early lactation is increasing dietary crude protein levels (up to 19% or higher on a dry matter basis; Butler 1998). A great deal of attention has been paid to the portion of the protein that is quickly degradable by the rumen bacteria and protozoa. An excessive intake of such degradable protein and a relative shortage of energy (carbohydrates) to synthesize bacterial proteins will result in an accumulation of excessive ammonia in the rumen (Sinclair et al. 2000a). This is absorbed through the ruminal wall and will be converted into urea in the liver. This detoxification process consumes energy and thus may exacerbate NEB early postpartum, thereby reducing fertility (Butler 1998; Fig. 3). A second source of urea produced by the liver is the deamination and catabolism of amino acids.

In spite of the milk-stimulating features, high dietary protein levels have been associated with inferior reproductive performance in most, but not all, studies (reviewed by Butler 2003; Melendez et al. 2003). Furthermore, the possibility of confounding effects of protein intake and lactation-induced NEB on reproduc-

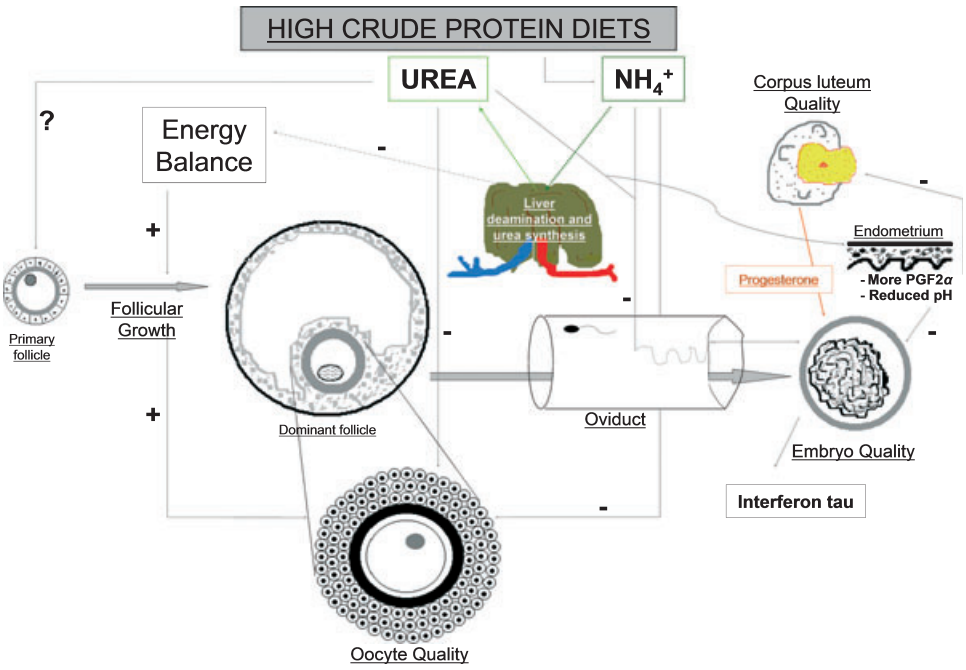


Fig. 3. Diagrammatic presentation of the major mechanisms through which rations with a high crude protein content can affect oocyte and embryo quality in high-producing dairy cows. Diets rich in crude protein can result in elevate blood ammonia and urea concentrations which are paralleled in the follicular fluid and which can harm the oocyte proper. Also, an altered oviductal and uterine environment has been demonstrated to have a direct toxic on the embryo. The deamination process and urea synthesis in the liver needs energy and therefore can exacerbate the negative energy balance

tive performance can make the correct interpretation of some study results difficult (Butler 1998; Gath et al. 1999; Kenny et al. 2001, 2002). High crude protein levels in the diet do not appear to have a deleterious impact on the reinitiation of ovarian cyclicity in the postpartum dairy cow. However, reduced conception rates (up to 30% and 20% decrease in lactating cows and heifers, respectively) in animals with serum urea nitrogen concentrations exceeding 20 mg/dl (or milk urea nitrogen concentrations >19 mg/dl) have frequently been reported (Butler et al. 1996; Westwood et al. 1998; Sinclair et al. 2000b; Melendez et al. 2003). The major pathogenesis suggested for this conception failure (or early embryonic mortality) is the potential toxicity of the direct by-products of protein catabolism (ammonia and urea) for the oocyte and the embryo (Fig. 3). For example, murine embryos cultured in the presence of high $NH_4^+$ concentrations displayed morphological, metabolic and genetic abnormalities (Gardner and Lane 1993; Lane and Gardner 2003). However, it is known that the lactating dairy cow can metabolically adapt to a prolonged high intake of quickly degradable protein, by opposing possible adverse effects of long-term high urea concentrations on embryo growth (Dawuda et al. 2002; Laven et al. 2004). This, however, could not be confirmed in ewes (McEvoy et al. 1997a,b). In one Finnish study, a long-term very moderate increase in dietary crude protein content from 14% to 18% turned out to be advantageous to the quality of Ayrshire heifer embryos despite the concomitant elevated blood urea concentrations (Mikkola et al. 2005).

High systemic urea concentrations have been associated with a reduction in uterine pH (7.1 to 6.8) and an alteration in the ionic composition of uterine fluid, both of which create a hostile environment for the developing embryo (Jordan et al. 1983; Elrod and Butler 1993). This has recently been confirmed *in vitro* by Ocon and Hansen (2003). Additionally, endometrial cell cultures incubated with urea secreted significantly higher amounts of $PGF_2\alpha$ compared to controls (Butler 1998). Finally, it has been suggested that such uterine environments are also hostile for the viability and motility of spermatozoa (Westwood et al. 1998). Interestingly, Fahey et al. (2001) found reduced embryo quality in donor ewes fed high-protein diets, but the diet of the embryo recipients had no effect on survival of transferred embryos. Hence, they suggested that the adverse effects of urea on embryo quality are likely to be due to deleterious alterations 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). This finding was very recently confirmed in a study of lactating dairy cows (Rhoads et al. 2006). Transfer of embryos, collected from dairy cows with elevated plasma urea nitrogen concentrations (24 mg/dl) for several weeks, resulted in a significantly reduced pregnancy rate. It is important to mention that this was irrespective of the urea nitrogen status of the recipient (Rhoads et al. 2006). Oocytes recovered from beef heifers that experienced elevated ammonia concentrations, both in serum and in FF, indeed showed a compromised developmental competence *in vitro* (Sinclair et al. 2000b). Hammon et al. (2000a) demonstrated that effects of ammonia on bovine oocytes *in vitro* depend on timing and duration of exposure (Hammon et al. 2000b). Furthermore, as ammonia is also toxic for

granulosa cells *in vitro*, they lose their ability to support *in vitro* oocyte maturation (Rooke et al. 2004). Leroy et al. (2004) and also Hammon et al. (2005) found a very good correlation between urea concentrations in plasma and follicular or uterine fluid in high-producing dairy cows in the early postpartum period. De Wit et al. (2001) reported a retarded nuclear maturation and reduced fertilization and cleavage rates in bovine oocytes matured in the presence of 6 mM urea, probably through inhibition of the polymerization of tubulin into microtubules (De Wit et al. 2001). Similar toxic effects on oocyte maturation have been documented by Ocon and Hansen (2003).

In conclusion, it can be said that notwithstanding the wide variation in experimental design, and the sometimes conflicting results, there is enough evidence to assume that diets inducing excessively high urea and ammonia concentrations in blood can have detrimental effects on oocyte and embryo quality. This adverse effect can act at the level of both the embryo (especially through ammonia) and oocyte (particularly through urea). The duration of exposure to such high-protein diets is, however, also important because cows are able to compensate for negative effects when such diets are fed over a period of weeks.

## Other Possible Factors Affecting Oocyte and Embryo Quality in Modern Dairy Cows

For decades, dairy cows have primarily been selected for high milk yield. Some studies suggest that this genetic selection itself could have an adverse effect on oocyte quality (Fig. 4). Snijders et al. (2000) collected oocytes from high and low genetic merit cows and observed a significantly lower developmental competence *in vitro* for oocytes originating from high merit cows irrespective of milk yield. Moreover, a greater number of high than of medium genetic merit cows were not pregnant at the end of the breeding season. Surprisingly, there were no obvious differences in NEFA concentrations in these two groups indicating no significant differences in EB (Snijders et al. 2001). Also, in the pregnancy study, no differences were found in postpartum follicular development, suggesting therefore, that the quality of the oocyte itself may be impaired in cows of high genetic merit. In contrast with the findings of Snijders et al. (2001), Veerkamp et al. (2003) and Horan et al. (2005) suggested that high genetic merit for milk production is also associated with a more severe NEB. This higher metabolic stress may explain poorer oocyte quality and unsatisfactory reproductive performance. Silke et al.

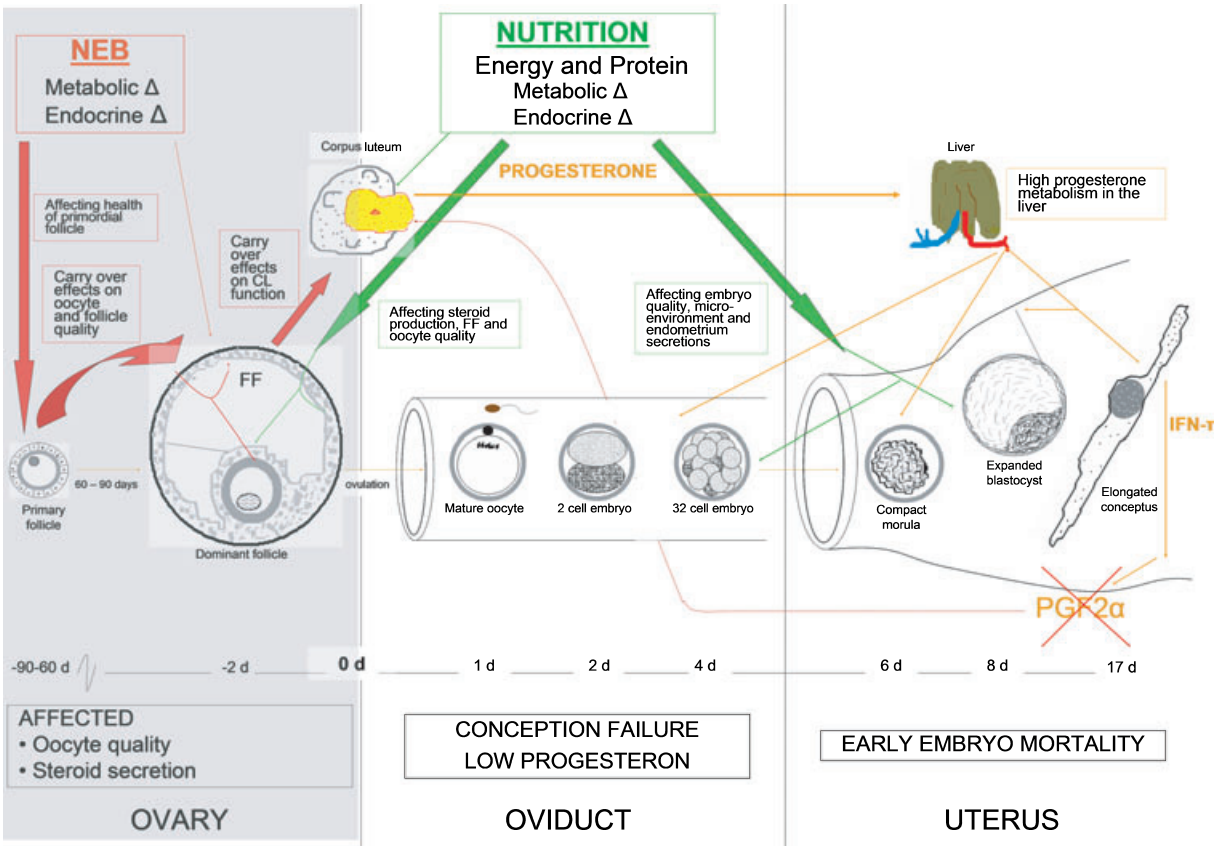


Fig. 4. Diagrammatic presentation of the major mechanisms through which negative energy balance, corpus luteum function or nutrition can directly influence oocyte and/or embryo quality. The grey zone represents the mechanisms, associated with negative energy balance, which are explained in the first part of companion review paper (Leroy et al. 2008). As all these mechanisms are inescapably linked to each other and to provide the reader with a broad overview, the entire scheme is depicted. *Also, other important factors such as a high genetic merit for milk production, heat stress or infectious diseases can affect oocyte and embryo quality in a direct or indirect way. However, for clarity reasons the interactions of these factors with the depicted mechanisms are not shown. NEB: negative energy balance; Δ: 'changes'; IFN-T: interferon-tau; PGF2α: prostaglandin F2α

(2002) did not find any significant association between the extent or pattern of late embryonic loss and genetic merit for milk production. Possible influences of genetic selection are, therefore, thought more likely to operate on the oocyte or on the early embryo (within 2 weeks of fertilization).

Along with genetic selection towards higher milk production, modern dairy cows became more sensitive to heat stress, as their metabolism and thus internal heat production has also significantly increased (reviewed by Kadzere et al. 2002). It has been observed that heat stress is injurious to reproduction (reviewed by De Rensis and Scaramuzzi 2003). In addition to its detrimental effects on energy balance, follicular dynamics, and hypothalamus–pituitary–ovarian axis, it has also been suggested that high body temperatures can be directly toxic to the oocyte and the pre-implantation embryo (Edwards and Hansen 1997; Rocha et al. 1998; Hansen 2007). Heat stress occurring 20–50 days prior to artificial insemination (AI) can result in drastically reduced conception rates (Chebel et al., 2004). This indicates that there is a long-term effect of heat stress that impacts the ultimate quality of the oocyte to be ovulated several weeks later. Heat stress is also able to induce degeneration of granulosa and theca cells, and thus, to compromise steroidogenesis (Chebel et al. 2004). An excellent overview on the effect of heat stress on embryo quality is presented by Hansen (2007).

It is generally accepted that high-yielding dairy cows are more vulnerable to attack from metabolic and infectious diseases. Postpartum diseases are even suggested to be a more important risk factor for reproductive failure compared to NEB (Loeffler et al. 1999). Both the incidence and the severity of clinical mastitis are significantly increased in modern dairy cows, probably because of a depressed immune system early after parturition (Ingvartsen et al. 2003; Burvenich et al. 2003). Mastitis in the early postpartum period, but also intramammary infections occurring at the time of AI, are significantly associated with reduced conception rates (Loeffler et al. 1999), and more specifically with higher risks of abortion within the first 90 days (Risco et al. 1999). The possible mechanisms involved in the link between infectious diseases and embryonic mortality have been extensively reviewed by Hansen et al. (2004) and are beyond the scope of this review. A summarization of all the mechanisms discussed herein, is depicted in Fig. 4.

## Conclusions

Milk yield maximization per cow remains preferable from an economical and environmental point of view. However, fertility in modern dairy cows has been declining, and one wonders whether we have reached the boundaries of what is physiologically achievable. Currently, there is increasing scientific evidence that suggests that diminished oocyte and embryo quality are two major factors in the complex pathogenesis of reproductive failure. The oocyte and embryo are vulnerable to various types of endocrine and metabolic changes in their microenvironment. It is only recently that an increasing number of studies are being performed in an attempt to expand our understanding of the specific composition of the microenvironments in the follicle, oviduct and uterus.

Several mechanisms, through which oocyte and/or embryo quality can be affected in modern dairy cows, well after the period of NEB, have been proposed and are comprehensively reviewed in the present report. The standard milk-stimulating diet can be associated with significant endocrine and metabolic alterations that probably have the capacity to endanger the quality of the female gamete or embryo in either a direct or an indirect way. Dietary recommendations for optimal follicular growth appear to be different from those that promote ovum quality. Therefore, complex and intricate formulations are required and practically applicable management advices for the dairy industry and the dairy farmer are a real challenge to develop. In addition to the dietary concerns, also genetic factors, infectious diseases and heat stress are among the factors that can endanger oocyte and embryo quality. It is obvious that extensive further research is indispensable to elucidate the various clues, and answer the questions which have been raised.

## References

Abe H, Yamashita S, Itoh T, Satoh T, Hoshi H, 1999: Ultrastructure of bovine embryos developed from *in vitro*-matured and -fertilized oocytes: comparative morphological evaluation of embryos cultured either in serum-free medium or in serum-supplemented medium. Mol Reprod Dev **53,** 325–335.

Abe H, Yamashita S, Satoh T, Hoshi H, 2002: Accumulation of cytoplasmic lipid droplets in bovine embryos and cryotolerance of embryos developed in different culture systems using serum-free or serum-containing media. Mol Reprod Dev **61,** 57–66.

Abecia JA, Lozano JM, Forcada F, Zarazaga L, 1997: Effect of level of dietary energy and protein on embryo survival and progesterone production on day eight of pregnancy in Rasa Aragonesa ewes. Anim Reprod Sci **48,** 209–218.

Adamiak SJ, Mackie K, Ewen M, Powell KA, Watt RG, Rooke JA, Webb R, Sinclair KD, 2004: Dietary carbohydrates and lipids affect in vitro embryo production following OPU in heifers. Reprod Fertil Dev **16,** 193–194 (abstract).

Adamiak SJ, Mackie K, Watt RG, Webb R, Sinclair KD, 2005a: Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biol Reprod **73,** 918–926.

Adamiak S, Ewen M, Rooke J, Webb R, Sinclair K 2005b: Diet and fatty acid composition of bovine plasma, granulosa cells, and cumulus-oocyte complexes. Reprod Fertil Dev **17,** 200–201 (abstract).

Armstrong DG, McEvoy TG, Baxter G, Robinson JJ, Hogg CO, Woad KJ, Webb R, Sinclair KD, 2001: Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: associations with the ovarian insuline-like growth factor system. Biol Reprod **64,** 1624–1632.

Armstrong DG, Baxter G, Hogg CO, Woad KJ, 2002a: Insulin-like growth factor (IGF) system in the oocyte and somatic cells of bovine preantral follicles. Reproduction **123,** 789–797.

Armstrong DG, Gong JG, Webb R, 2002b: Interactions between nutrition and ovarian activity in cattle: physiological, cellular and molecular mechanisms. Reproduction (Suppl. ) **61,** 403–414.

Armstrong DG, Gong JG, Gardner JO, Baxter G, Hogg CO, Webb R, 2002c: Steroidegenesis in bovine granulose cells: the effect of short-term changes in dietary intake. Reproduction **123,** 371–378.

Beam SW, Butler WR, 1997: Energy balance and ovarian follicle development prior to first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod **56,** 133–142.

Bilby TR, Guzeloglu A, MacLaren LA, Staples CR, Thatcher WW, 2006: Pregnancy, bovine somatotropin, and dietary n-3 fatty acids in lactating dairy cows: II. Endometrial gene expression related to maintance of pregnancy. J Dairy Sci **89,** 3360–3375.

Boland MP, Lonergan P, O'Callaghan D, 2001: Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology **55,** 1323–1340.

Burvenich C, Van Merris V, Mehrzad J, Diez-Fraile A, Duchateau L, 2003: Severity of *E. coli* mastitis is mainly determined by cow factors. Vet Res **34,** 521–564.

Butler WR, 1998: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J Dairy Sci **81,** 2533–2539.

Butler WR, 2003: Energy balance realtionships with follicular development, ovulation and fertility in pp dairy cows. Livest Prod Sci **83,** 211–218.

Butler WR, Calaman JJ, Beam SW, 1996: Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J Anim Sci **74,** 858–865.

Chebel RC, Santos JEP, Reynolds JP, Cerri RLA, Juchem SO, Overton M, 2004: Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim Reprod Sci **84,** 239–255.

Cheng Z, Robinson RS, Pushpakumara PGA, Mansbridge RJ, 2001: Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. J Endocrin **171,** 463–473.

Comin A, Gerin D, Cappa A, Marchi V, Renaville R, Motta M, Fazzini U, Prandi A, 2002: The effect of an acute energy deficit on the hormone profile of dominant follicles in dairy cows. Theriogenology **58,** 899–910.

Dawuda PM, Scaramuzzi RJ, Leese HJ, Hall CJ, Peters AR, Drew SB, Wathes DC, 2002: Effect of timing of urea feeding on the yield and quality of embryos in lactating dairy cows. Theriogenology **58,** 1443–1455.

De Rensis F, Scaramuzzi RJ, 2003: Heat stress and seasonal effects on reproduction in the dairy cow – a review. Theriogenology **60,** 1139–1151.

De Wit AAC, Cesar MLF, Kruip TAM, 2001: Effect of urea during in vitro maturation on nuclear maturation and embryo development of bovine cumulus-oocyte-complexes. J Dairy Sci **84,** 1800–1804.

DeFrain JM, Hippen AR, Kalscheur KF, Patton RS, 2005: Effects of feeding propionate and calcium salts of long-chain fatty acids on transition dairy cow performance. J Dairy Sci **88,** 983–993.

Diskin MG, Mackey DR, Roche JF, Sreenan JM, 2003: Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim Reprod Sci **78,** 345–370.

Dunne LD, Diskin MG, Boland MP, O'Farrell KJ, Sreenan JM, 1999: The effect of pre- and post-insemination plane of nutrition on embryo survival in beef heifers. Anim Sci **69,** 411–417.

Edwards JL, Hansen PJ, 1997: Differential responses of bovine oocyte and preimplantation embryos to heat shock. Mol Reprod Dev **46,** 138–145.

Elrod CC, Butler WR, 1993: Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. J Anim Sci **71,** 694–701.

Fahey J, Boland MP, O'Callaghan D, 2001: The effects of dietary urea on embryo development in superovulated donor ewes and on early embryo survival and development in recipient ewes. Anim Sci **72,** 395–400.

Fair T, 2003: Follicular oocyte growth and acquisition of developmental competence. Anim Reprod Sci **78,** 203–216.

Gardner DK, Lane M, 1993: Amino acids and ammonium regulate mouse embryo development in culture. Biol Reprod **48,** 377–385.

Gath V, Lonergan P, Boland MP, O'Callaghan D, 1999: Effects of diet type on establishment of pregnanacy and embryo development in beef heifers. Theriogenology **51,** 224 (abstract).

Gong JG, 2002: Influence of metabolic hormones and nutrition on ovarian follicle development in cattle: practical implications. Domest Anim Endocrinol **23,** 229–241.

Gwazdauskas FC, Kendrick KW, Pryor AW, Bailey TL, 2000: Impact of follicular aspiration on folliculogenesis as influenced by dietary energy and stage of lactation. J Dairy Sci **83,** 1625–1634.

Hammon DS, Wang S, Holyoak GR, 2000a: Ammonia concentration in bovine follicular fluid and its effect during in vitro maturation on subsequent embryo development. Anim Reprod Sci **58,** 1–8.

Hammon DS, Wang S, Holyoak GR, 2000b: Effects of ammonia during different stages of culture on development of in vitro produced bovine embryos. Anim Reprod Sci **59,** 23–30.

Hammon DS, Holyoak GR, Dhiman TR, 2005: Association between blood plasma urea nitrogen levels and reproductive fluid urea nitrogen and ammonia concentrations in early lactation dairy cows. Anim Reprod Sci **86,** 195–204.

Hansen PJ, 2007: To be er not to be – determinants of embryonic survival following heat shock. Theriogenology, doi: 10.1016/j.theriogenology.2007.03.013.

Hansen PJ, Soto P, Natzke RP, 2004: Mastitis and fertility in cattle – possible involvement of inflammation or immune activation in embryonic mortality. Am J Reprod Immun **51,** 294–301.

Hinckley T, Clark RM, Bushmich SL, Milvae RA, 1996: Long chain polyunsaturated fatty acids and bovine luteal cell function. Biol Reprod **55,** 445–449.

Horan B, Mee JF, O'connor P, Rath M, Dillon P, 2005: The effect of strain of Holstein-Friesian cow and feeding system on postpartum ovarian function, animal production and conception rate to first service. Theriogenology **63,** 950–971.

Ingvartsen KL, Dewhurst RJ, Friggens NC, 2003: On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production disease in dairy cattle? A position paper. Livest Prod Sci **82,** 277–308.

Jordan ER, Chapman TE, Holtan DW, Swanson LV, 1983: Relantionship of dietary crude protein to composition of uterine secretions and blood in high-producing postpartum dariy cows. J Dairy Sci **66,** 1854–1862.

Kadzere CT, Murphy MR, Silanikove N, Maltz E, 2002: Heat stress in lactating dairy cows: a review. Livest Prod Sci **77,** 59–91.

Kendrick KW, Bailey TL, Garst AS, Pryor AW, Ahmadzadeh A, Akers RM, Eyestone WE, Pearson RE, Gwazdauskas FC, 1999: Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. J Dairy Sci **82,** 1731–1740.

Kenny DA, Boland MP, Diskin MG, Sreenan JM, 2001: Effect of pasture crude protein and fermentable energy supplementation on blood metabolite and progesterone concentrations and on embryo survival in heifers. Anim Sci **73,** 501–511.

Kenny DA, Boland MP, Diskin MG, Sreenan JM, 2002: Effect of rumen degradable protein with or without fermentable

carbohydrate supplementation on blood metabolites and embryo survival. Anim Sci **74,** 529–537.
Kim JY, Kinoshita M, Ohnishi M, Fukui Y, 2001: Lipid and fatty acid analysis of fresh and frozen-thawed immature and in vitro matured bovine oocytes. Reproduction **122,** 131–138.
Lammoglia MA, Willard ST, Oldham JR, Randel RD, 1996: Effects of dietary fat and season on steroid hormonal profiles before parturition and on hormonal, cholesterol, triglycerides, follicular patterns, and postpartum reproduction in Brahman cows. J Anim Sci **74,** 2253–2262.
Landau S, Braw-Tal R, Kaim M, Bor A, Bruckental I, 2000: Preovulatory follicular status and diet affect the insulin and glucose content of follicles in high-yielding dairy cows. Anim Reprod Sci **64,** 181–197.
Lane M, Gardner DK, 2003: Ammonium induces aberrant blastocyst differentiation, metabolism, pH regulation, gene expression and subsequently alters fetal development in the mouse. Biol Reprod **69,** 1109–1117.
Laven RA, Dawuda PM, Scaramuzzi RJ, Wathes DC, Biggadike HJ, Peters AR, 2004: The effect of feeding diets high in quickly degradable nitrogen on follicular development and embryo growth in lactating Holstein dairy cows. Anim Reprod Sci **84,** 41–52.
Leroy JLMR, Opsomer G, Van Soom A, Goovaerts IGF, Bols PEJ, 2008: Reduced fertility in high-yielding dairy cows: are the oocyte and embryo in danger? part i: the importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high-yielding dairy cows. Reprod Dom Anim, doi: 10.1111/j.1439-0531.2007.00960.x.
Leroy JLMR, Vanholder T, Delanghe JR, Opsomer G, Van Soom A, Bols PEJ, de Kruif A, 2004: Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows. Anim Reprod Sci **80,** 201–211.
Leroy JLMR, Opsomer G, De Vliegher S, Vanholder T, Goossens L, Geldhof A, Bols PEJ, de Kruif A, Van Soom A, 2005a: Comparison of embryo quality in high-yielding dairy cows, in dairy heifers and in beef cows. Theriogenology **64,** 2022–2036.
Leroy JLMR, Vanholder T, Mateusen B, Christophe A, Opsomer G, de Kruif A, Genicot G, Van Soom A, 2005b: Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. Reproduction **130,** 485–495.
Leroy JLMR, Vanholder T, Opsomer G, Van Soom A, de Kruif A, 2006: The in vitro development of bovine oocytes after maturation in glucose and beta-hydroxyburyrate concentrations associated with negative energie balance in dairy cows. Reprod Domest Anim **41,** 119–123.
Loeffler SH, de Vries MJ, Schukken YH, 1999: The effects of timing of disease occurance, milk yield, and body condition on fertility of dairy cows. J Dairy Sci **82,** 2589–2604.
Lozano JM, Lonergan P, Boland MP, O'Callaghan D, 2003: Influence of nutrition on the effectiveness of superovulation programmes in ewes: effect on oocyte quality and post-fertilization development. Reproduction **125,** 543–553.
Lucy MC, Staples CR, Michel FM, Thatcher WW, 1991: Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J Dairy Sci **74,** 473–482.
Mattos R, Staples CR, Williams J, Amorocho A, McGuire MA, Thatcher WW, 2002: Uterine, ovarian and production responses of lactating dairy cows to increasing dietary concentrations of Menhaden fich meal. J Dairy Sci **85,** 755–764.
McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Palmer RM, Robertson IS, 1995: Dietary-induced suppression of pre-ovulatory progesterone concentrations in superovulated ewes impairs the subsequent in vivo and in vitro development of their ova. Anim Reprod Sci **39,** 89–107.
McEvoy TG, Sinclair KD, Staines ME, Robinson JJ, Armstrong DG, Webb R, 1997a: In vitro blastocyst production in relation to energy and protein intake prior to collection. J Reprod Fertil Abstr Series **19** (abstract 132).
McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Robertson IS, 1997b: Dietary excesses of urea influence the viability and metabolism of preimplantation sheep embryos and may affect fetal growth among survivors. Anim Reprod Sci **47,** 71–90.
McEvoy TG, Coull GD, Broadbent PJ, Hutchinson JS, Speake BK, 2000: Fatty acid composition of lipids in immature cattle, pig and sheep oocytes with intact zona pellucida. J Reprod Fertil **118,** 163–170.
McEvoy TG, Robinson JJ, Ashworth CJ, Rooke JA, Sinclair KD, 2001: Feed and forage toxicants affecting embryo survival and fetal development. Theriogenology **55,** 113–129.
McNamara S, Butler T, Ryan DP, Mee JF, Dillon P, O'Mara FP, Butler ST, Anglesey D, Rath M, Murphy JJ, 2003: Effect of offering rumen-protected fat supplements on fertility and performance in spring-calving Holstein-Friesian cows. Anim Reprod Sci **79,** 45–56.
Melendez P, Donovan A, Hernandez J, Bartolome J, Risco CA, Staples C, Thatcher WW, 2003: Milk, plasma, and blood urea nitrogen concentrations, dietary protein, and fertility in dairy cattle. J Am Vet Med Assoc **223,** 628–634.
Mikkola M, Mäntysaari P, Tammiranta N, Peippo J, Taponen J, 2005: Effect of dietary protein on embryo recovery rate and quality in superovulated heifers. Anim Reprod Sci **87,** 193–202.
Moallem U, Katz M, Lehrer H, Livshitz L, Yakoby S, 2007: Role of peripartum dietary propylene glycol or protected fats on metabolism and early postpartum ovarian follicles. J Dairy Sci **90,** 1243–1254.
Nolan R, Duffy P, Wade M, O'Callaghan D, Boland MP, 1998: Effect of quantity and type of diet and frequency of trans-vaginal ovum aspiration on in-vitro embryo development in heifers. Theriogenology **49,** 402 (abstract).
O'Callaghan D, Boland MP, 1999: Nutritional effects on ovulation. Anim Sci **68,** 299–314.
O'Callaghan D, Yaakub H, Hyttel P, Spicer LJ, Boland MP, 2000: Effect of nutrition and superovulation on oocyte morphology, follicular fluid composition and systemic hormone concentrations in ewes. J Reprod Fertil **118,** 303–313.
Ocon OM, Hansen PJ, 2003: Disruption of bovine oocytes and preimplantation embryos by urea and acidic pH. J Dairy Sci **86,** 1194–2000.
Papadopoulos S, Lonergan P, Gath V, Quinn KM, Evans AC, O'Callaghan D, Boland MP, 2001: Effect of diet quantity and urea supplementation on oocyte and embryo quality in sheep. Theriogenology **55,** 1059–1069.
Pereira RM, Baptista MC, Vasques MI, Horta AE, Portugal PV, Bessa RJ, Silva JC, Pereira MS, Marques CC, 2007: Cryosuvrvival of bovine blastocysts is enhanced by culture with trans-10cis-12 conjugated lineolic acid (10t, 12c CLA). Anim Reprod Sci **98,** 293–301.
Reis A, Rooke JA, McCallum GJ, Ewen M, Staines ME, Lomax MA, McEvoy TG, 2003: Fatty acid content of polar and neutral lipids from bovine blastocysts produced in vitro in the presence or absence of serum. Reprod Abstr Series **30,** 57–58 (abstract).
Rhoads ML, Rhoads RP, Gilbert RO, Toole R, Butler WR, 2006: Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. Anim Reprod Sci **91,** 1–10.
Risco CA, Donovan GA, Hernandez J, 1999: Clinical mastitis associated with abortion in dairy cows. J Dairy Sci **82,** 1684–1689.

Rizos D, Gutierrez-Adan A, Perez-Garnelo S, De La Fuente J, Boland MP, Lonergan P, 2003: Bovine embryo culture in the presence or absence of serum: implications for blastocyst development, cryotolerance, and messenger RNA expression. Biol Reprod **68,** 236–243.

Robinson RS, Pushpakumara PGA, Cheng Z, Peters AR, Abayasekara DRE, Wathes DC, 2002: Effects of dietary poluunsaturated fatty acids on ovarian and uterine function in lactating dairy cows. Reproduction **124,** 119–131.

Robinson JJ, Ashworth CJ, Rooke JA, Mitchell LM, McEvoy TG, 2006: Nutrition and fertility in ruminant lifestock. Anim Feed Sci Technol **126,** 259–276.

Rocha A, Randel RD, Broussard JR, Lim JM, Blair RM, Roussel JD, Godke RA, Hansel W, 1998: High environmental temperatures and humidity decrease oocyte quality in *Bos taurus* but not in *Bos indicus* cows. Theriogenology **49,** 657–665.

Rooke JA, Ewen M, Mackie K, Staines ME, McEvoy TG, Sinclair KD, 2004: Effect of ammonium chloride on the growth and metabolism of bovine ovarian granulosa cells and the development of ovine oocytes matured in the presence of bovine granulosa cells previously exposed to ammonium chloride. Anim Reprod Sci **84,** 53–71.

Rooke JA, Ewen M, Reis A, McEvoy TG, 2006: Comparison of the fatty acid contents of bovine follicular fluid, granulose cells and cumulus enclosed or denuded oocytes. Reprod Domest Anim **41,** 306 (abstract).

Ryan DP, Spoon RA, Williams GL, 1992: Ovarian follicular characteristics, embryo recovery, and embryo viability in heifers fed high-fat diets and treated with follicle-stimulating hormone. J Anim Sci **70,** 3505–3513.

Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC, 2002: Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J Dairy Sci **85,** 2803–2812.

Sata R, Tsujii H, Abe H, Yamashita S, Hoshi H, 1999: Fatty acid composition of bovine embryos cultured in serum-free and serum containing medium during early embryonic development. J Reprod Dev **45,** 97–103.

Silke V, Diskin MG, Kenny DA, Boland MP, Dillon P, Mee JF, Sreenan JM, 2002: Extent, pattern and factors associated with late embryonic loss in dairy cows. Anim Reprod Sci **71,** 1–12.

Sinclair KD, Sinclair LA, Robinson JJ, 2000a: Nitrogen metabolism and fertility in cattle: I. Adaptive changes in intake and metabolism to diets differing in their rate of energy and nitrogen release in the rumen. J Anim Sci **78,** 2659–2669.

Sinclair KD, Kuran M, Gebbie FE, Webb R, McEvoy TG, 2000b: Nitrogen metabolism and fertility in cattle: II. Development of oocytes recovered from heifers offered diets differing in their rate of nitrogen release in the rumen. J Anim Sci **78,** 2670–2680.

Snijders SE, Dillon P, O'Callaghan D, Boland MP, 2000: Effect of genetic merit, milk yield, body condition and lactation number on *in vitro* oocyte development in dairy cows. Theriogenology **53,** 981–989.

Snijders SEM, Dillon PG, O'Farrell KJ, Diskin M, Wylie ARG, O'Callaghan D, Rath M, Boland MP, 2001: Genetic merit for milk production and reproductive success in dairy cows. Anim Reprod Sci **65,** 17–31.

Staples CR, Burke JM, Thatcher WW, 1998: Influence of supplemental fats on reproductive tissue and performance of lactating cows. J Dairy Sci **81,** 856–871.

Thatcher WW, Bilby TR, Bartolome JA, Silvestre F, Staples CR, Santos JEP, 2006: Strategies for improving fertility in the modern dairy cow. Theriogenology **65,** 30–44.

Van Knegsel ATM, Van de Brand H, Dijkstra J, Tamminga S, Kemp B, 2005: Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cows. Reprod Nutr Dev **45,** 665–688.

Van Knegsel ATM, Van den Brand H, Graat EAM, Dijkstra J, Jorritsma R, Decuypere E, Tamminga S, Kemp B, 2007a: Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. J Dairy Sci **90,** 1477–1485.

Van Knegsel ATM, Van den Brand H, Dijkstra J, van Straalen WM, Heetkamp MJW, Tamminga S, Kemp B, 2007b: Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. J Dairy Sci **90,** 1467–1476.

Vanholder T, Leroy JLMR, Van Soom A, Opsomer G, Maes D, Coryn M, de Kruif A, 2005: Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation *in vitro*. Anim Reprod Sci **87,** 33–44.

Vasconcelos JLM, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC, 2001: Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology **56,** 307–314.

Vasconcelos JLM, Sangsritavong S, Tsai SJ, Wiltbank MC, 2003: Acute reduction in serum progesterone concentrations after feed intake in dairy cows. Theriogenology **60,** 795–807.

Veerkamp RF, Beerda B, van der Lende T, 2003: Effects of genetic selection for milk yield on energy balance, levels of hormones, and metabolites in lactating cattle, and possible links to reduced fertility. Livest Prod Sci **83,** 257–275.

Webb R, Garnsworthy PC, Gong JG, Armstrong DG, 2004: Control of follicular growth: local interactions and nutritional influences. J Anim Sci **83** (Suppl. E)**,** E63–E74.

Westwood CT, Lean IJ, Kellaway RC, 1998: Indications and implications for testing of milk urea in dairy cattle: a quantitative review: Part 2. Effect of dietary protein on reproductive performance. N Z Vet J **46,** 123–130.

Wiltbank MC, Sartori R, Sangsritavong S, Lopez H, Haughian JM, Fricke PM, Gumen A, 2001: Novel effects of nutrition on reproduction in lactating dairy cows. J Dairy Sci **84** (Suppl. )**,** 32 (abstract).

Wrenzycki C, De Sousa P, Overström EW, Duby RT, zHerrmann D, Watson AJ, Niemann H, O'Callaghan D, Boland MP, 2000: Effects of superovulated heifer diet type and quantity on relative mRNA abundances and pyruvate metabolism in recovered embryos. J Reprod Fertil **118,** 69–78.

Yaakub H, O'Callaghan D, Boland MP, 1999a: Effect of type and quantity of concentrates on superovulation and embryo yield in beef heifers. Theriogenology **51,** 1259–1266.

Yaakub H, O'Callaghan D, Boland MP, 1999b: Effect of roughage type and concentrate supplementation on follicle numbers and in vitro fertilisation on follicle numbers and in vitro fertilisation and development of oocytes recovered from beef heifers. Anim Reprod Sci **44,** 1–12.

Zeron Y, Ocheretny A, Kedar O, Borochov A, Sklan D, Arav A, 2001: Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. Reproduction **121,** 447–454.

Zeron Y, Sklan D, Arav A, 2002: Effect of polyunsaturated fatty acid supplementation on biophysical parameters and chilling sensitivity of ewe oocytes. Mol Reprod Dev **61,** 271–278.

Submitted: 14 Jun 2007

Author's address (for correspondence): JLMR Leroy, Laboratory for Veterinary Physiology, Department of Veterinary Sciences, Faculty of Biomedical, Pharmaceutical and Veterinary Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium. E-mail: jo.leroy@ua.ac.be