MODERN RESEARCH IN THE REDUCED FERTILITY OF HIGH YIELDING DAIRY COWS: AN INNOVATIVE WAY OF THINKING

Recent wetenschappelijk onderzoek van de verminderde vruchtbaarheid bij hoogproductieve melkkoeien: een vernieuwende kijk

J.L.M.R. Leroy, A. Van Soom, A. de Kruif, G. Opsomer

Department of Reproduction, Obstetrics and Herd Health
Faculty of Veterinary Medicine, University of Ghent, Salisburylaan 133, B-9820 Merelbeke, Belgium
Jo.leroy@UGent.be

ABSTRACT

It has frequently been reported that, along with continuously increasing milk production, dairy cow fertility has been declining. Maintaining the fertility of dairy cows is of capital importance for guaranteeing optimal milk yield and profitability. The endocrine pathways leading to this subfertility have been extensively investigated. However, disappointing fertilization rates and early embryonic mortality have recently been proposed as major factors in the problem of disappointing reproductive performance. It is suggested that oocytes and embryos are highly sensitive to any disruption in their environment caused by metabolic, dietary or other factors, which can have fatal consequences for final fertility. Because knowledge of the oocyte's microenvironment and of oocyte and embryo quality in high yielding dairy cows is extremely limited, future research should concentrate on providing this missing knowledge.

SAMENVATTING

Het is algemeen bekend dat de vruchtbaarheid van hoogproductieve melkkoeien sterk is afgenomen terwijl de melkproductie almaar blijft stijgen. Een goede vruchtbaarheid is echter noodzakelijk om een optimale melkopbrengst te garanderen. De endocriene aspecten van de pathogenese van de gedaalde vruchtbaarheid werden reeds uitvoerig bestudeerd. Echter, de significant afgenomen bevruchtingskans na inseminatie en de vroeg embryonale sterfte staan een prima vruchtbaarheid in de weg. Eicellen en embryo's zijn heel gevoelig voor de minste wijziging in hun micromilieu dat veroorzaakt kan worden door metabole of diëtaire factoren. Elke reductie van de eicel- en/of embryokwaliteit heeft nefaste gevolgen voor de uiteindelijke fertiliteit. De kennis van de eicel- en embryokwaliteit en het folliculaire milieu waarin de eicel groeit en matureert, is echter heel beperkt tot onbestaande. Toekomstig onderzoek dient hierop verder in te gaan.

INTRODUCTION

Bovine milk and dairy products have always been an appreciated part of man's diet. A cow can process grass, which is useless for human consumption, into highly nutritious milk (or meat). It was due to this specific and valuable feature that cattle came to be numbered among the first domesticated animals, albeit some time after goats and sheep. Since their domestication, several cattle breeds have been developed and further selected for the production of beef, for use as draft animals or for the production of milk. During the last decades, significant genetic improvements, combined with increased nutritional management, have allowed the modern dairy industry to create highly sophisticated dairy breeds producing enormous amounts of milk. However, it has frequently been reported that, along with continuously increasing milk production, dairy cow fertility has been declining (Lean *et al.*, 1989; Royal *et al.*, 2000; Lucy, 2001; Butler, 2003; Lopez-Gatius, 2003; Bousquet *et al.*, 2004; Mee *et al.*, 2004). In order to maintain profitability, modern dairy cows should conceive within the first three to four months after parturition, which is, metabolically speaking, their most demanding period. Furthermore, disappointing reproductive performance plays a predominant role in culling decisions (Rajala-Schultz and Gröhn, 2001). Maintaining an optimal health and high fertility rates, without a decrease in milk yield is the ultimate goal of the modern dairy industry in its attempt to meet the increasing demands of a rapidly expanding human population in an economically and ecologically acceptable way.

THE NEGATIVE ENERGY BALANCE AND ITS METABOLIC CONSEQUENCE

Shortly after calving, dairy cows' milk production increases tremendously and thus they encounter huge energy losses. This gives rise to a period of negative energy balance (NEB), since it is not possible for this drain of energy to be sufficiently compensated for by energy uptake through feed (Rukkwamsuk *et al.*, 1999). Figure 1 shows how dairy cows physiologically adapt to this period of NEB. The overall function of a dairy cow's adaptation during a period of NEB is to shift the body's fuel supply away from glucose, which is necessary for milk production, and towards the use of lipid derived energy sources (Herdt, 2000; Vernon 2002). However, because of the high milk production, modern dairy cows are more and more frequently experiencing maladaptation. Their physiological feedback mechanisms fail, leading to pathological situations such as fatty liver and (sub)clinical ketosis. Especially overconditioned cows, but also cows of high genetic merit for milk or animals with suboptimal health, have difficulties adapting and are therefore extremely vulnerable during this transition period (Herdt, 2000; Jorritsma *et al.*, 2003). Typically, these cows display a reduced appetite early postpartum, leading to an even higher lipid mobilization and liver triglyceride infiltration, which may result in high plasma ketone levels (Rukkwamsuk *et al.*, 1999). The rapid mobilization of body reserves, which is reflected in the loss of body condition (up to 10% of the body weight at calving), may aggravate the already depressed dry matter intake (McMillan *et al.*, 1998; Vernon 2002). Primiparous cows typically show an even more NEB since they still need extra energy for body growth (Cavestany *et al.*, 2005). It has been shown that such metabolic stress, associated with several endocrine malfunctions, is hard to reconcile with satisfactory reproductive performance.

REDUCED FERTILITY, A COMPLEX PROBLEM

From a biological point of view, it makes sense for the dam to favor milk production over fertility, which is referred to as 'nutrient prioritization' (Lucy, 2003).

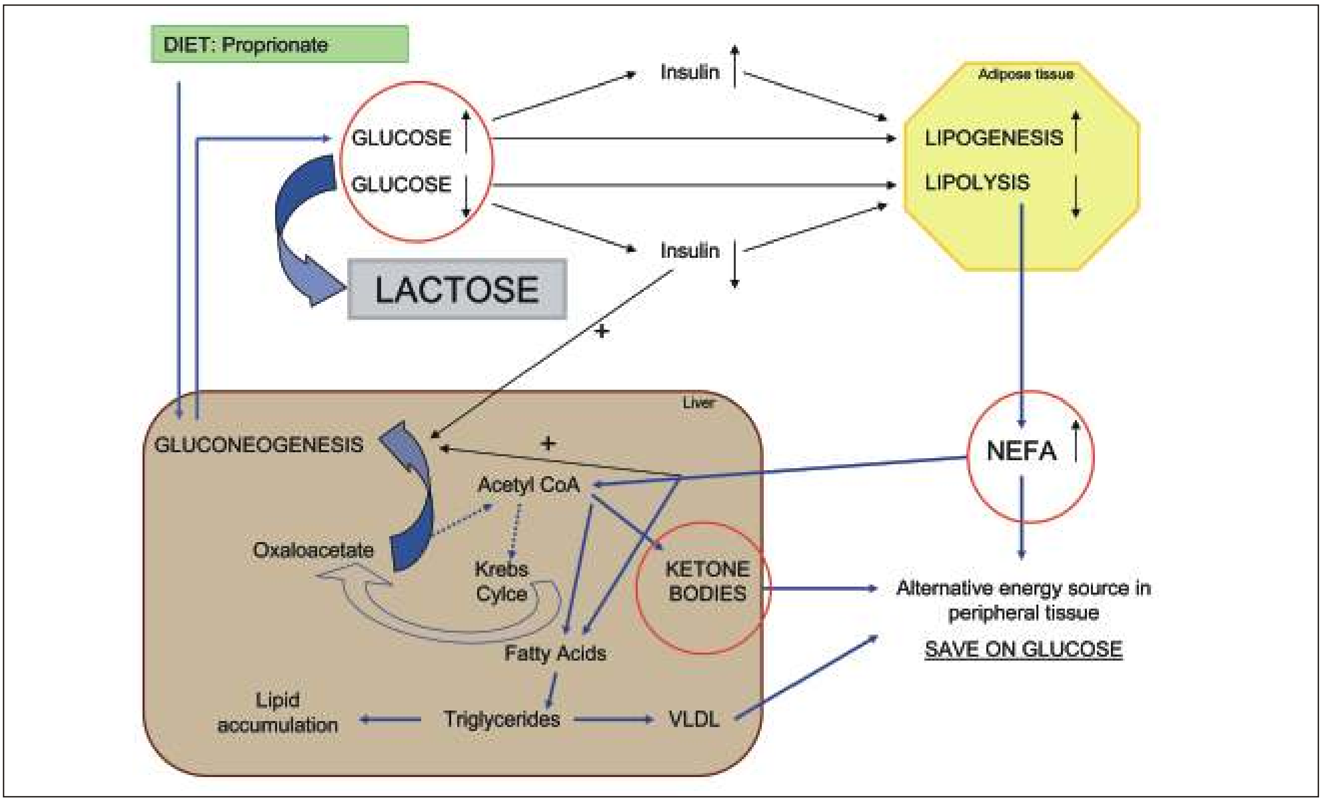

Figure 1. Feedback mechanism during a negative energy balance, resulting in reduced glucose use in peripheral tissue. The circles indicate the major metabolic 'byproducts' of the negative energy balance, which could probably hamper fertility in general and oocyte or embryo quality more specifically.

Since the available nutrients are scarce, it is more important for the dam to invest those limited nutrient resources in the survival and health of the current offspring instead of gambling on the health and survival of the oocyte that is yet to be fertilized (Silvia *et al.*, 2003). Over the past decades, the dairy industry has exploited this nutrient prioritization to maximize milk yield, creating a 'nutrient highway' from the digestive tract and body reserves directly to the udder. The energy highway metaphor is highly applicable to the specific metabolic situation of our high producing dairy cows. Other exits from this highway, providing for example energy to the reproductive system, are passed by or even closed during the first weeks postpartum. On the other hand, the energy required to grow and ovulate a follicle, to form a corpus luteum and to maintain early pregnancy is negligible compared to the energy demands for production and maintenance. So it is more rational to assume that the 'pollution' caused by the heavy energy traffic from the digestive tract and body reserves towards the udder, rather than a net energy shortage, is responsible for the hampered reproductive functions. This is a completely innovative way of thinking, however, and it needs further investigation (see below). Very recent *in vitro* findings in our lab confirmed that both granulosa and theca cell growth and function are impaired after exposure, for example, to high concentrations of non-esterified fatty acids (NEFA) (Vanholder *et al.*, 2005). These NEFA constitute one major group of 'polluting byproducts' of the NEB. The toxic effects of elevated NEFA concentrations have been demonstrated in human granulosa cells (Mu *et al.*, 2001), in Leydig cells (Lu *et al.*, 2003) and in pancreatic β-cells (Maedler *et al.*, 2001).

Reproductive failure is certainly a multifactorial problem in which the amount of milk produced, as such, plays only a minor role compared with the importance of negative energy balance, body condition and postpartum diseases (Loeffler *et al.*, 1999; de Vries and Veerkamp, 2000; Snijders *et al.*, 2000; Lucy, 2001). Daily milk yield is not an appropriate indicator of negative energy balance because feed intake and management practices both confound the association between yield and energy balance (Villa-Godoy *et al.*, 1988; McMillan *et al.*, 1998; de Vries and Veerkamp, 2000; Kruip *et al.*, 2000). Factors such as the high energy and protein-rich rations typically fed to modern dairy cows to sustain the high level of milk production, together with the increased herd size, have also been associated with the disappointing fertility outcomes (Butler, 1998; Lucy, 2001; Fahey *et al.*, 2002; Lucy, 2003). Finally, the genetic selection for high milk production as such may also be a cause of reduced fertility (Snijders *et al.*, 2000; Snijders *et al.*, 2001).

Specific pathways linking the above mentioned factors with the disturbed reproductive functions in metabolically compromised dairy cows are complex and have been intensively investigated for many years (reviewed by Butler, 2003). Much of the effort has been focused on alterations in endocrine signaling (hypothalamus-pituitary-ovary axis) and ovarian dysfunctions. The effects of NEB on follicular development and the subsequent indicators of impaired fertility such as reduced estrous symptoms or anestrus, cyst formation, delayed first ovulation, and prolonged calving to first insemination intervals have been extensively documented (Harrison *et al.*, 1990; Opsomer *et al.*, 1998; Beam and Butler, 1997; de Vries and Veerkamp, 2000; Diskin *et al.*, 2003; Vanholder *et al.*, 2002; Lopez *et al.*, 2004). However, even when a positive energy balance and correct endocrine signaling have been re-established, which ultimately results in ovulation, reproduction is not guaranteed. As reviewed by Bousquet *et al.* (2004), the success rate of artificial insemination has shown a dramatic drop in almost all countries housing high yielding dairy cows, without any obvious reduction in the quality of the sperm used. Furthermore, early embryonic mortality is proposed to be a significant cause of reproductive failure in ruminants (Dunne *et al.*, 1999; Mann and Lamming, 2001; Bilodeau-Goeseels and Kastelic, 2003). Driven by these observations, it is only recently that some studies have begun to focus on the oocyte and subsequent embryo quality as potentially important factors that , physiologically speaking, are most closely linked with conception rate and hence fertility (Boland *et al.*, 2001). O'Callaghan and Boland (1999) stated that:

"The observed decline in fertility in high producing dairy cattle is mostly a problem of bad oocyte- and hence embryo quality, rather than being an endocrine disruption."

It has been suggested that oocytes and embryos are highly sensitive to any disruption in their environment caused by metabolic, dietary or other factors, which can have fatal consequences for final fertility (McEvoy *et al.*, 2001). Knowledge of the oocyte's micro-environment and the quality of the oocyte or embryo proper in high yielding dairy cows is extremely limited. First of all, it is not known whether metabolic alterations in the peripheral circulation, such as high non-esterified fatty acid, urea or β-hydroxybutyrate concentrations or low glucose concentrations, have an impact on the follicular fluid composition. Secondly, assuming that such metabolic changes in the follicular fluid do occur, it is not known whether they have an impact on oocyte metabolism and its developmental capacity. After all, there is no scientific evidence, for example, that high NEFA concentrations are toxic for different cell types (see above). Similar adverse effects have been described for urea (Ocon and Hansen, 2003) and ketone bodies (Franklin *et al.*, 1991). Hence, it is not inconceivable that also the oocyte is vulnerable to the critical metabolites referred to above as 'polluting by-products of the NEB'. Exploration of such untrodden fields of research could reveal crucial knowledge concerning the pathogenesis of the widely reported failure of conception.

In the event an embryo of inferior quality is formed, it is not known whether this inferior quality is caused by a carry-over effect via the oocyte or by the direct effects of altered energy, protein or lipid metabolism in the modern dairy cow. On the basis of the results of ample *in vitro* studies, it is generally accepted that the post-fertilization micro-environment is determinant for embryo quality in terms of morphology, lipid content, metabolism and gene expression (Wrenzycki *et al.*, 2000; Abe *et al.*, 2002; Rizos *et al.*, 2002; Rizos *et al.*, 2003). Whether the knowledge of these *in vitro* models is also applicable to the specific *in vivo* situation in high producing dairy cows is a matter for further research.

CONCLUDING REMARKS

Finding answers to all the questions mentioned above is of capital importance for determining that in high yielding dairy cows the endocrine signaling and the ovarian activity are disturbed, but also that the oocyte and embryo can be directly affected by polluting by-products of NEB or early postpartum diets. In subsequent review articles, the possible mechanisms linking NEB or diet and oocyte (and embryo) quality in high producing dairy cows will be discussed extensively. (See coming issues of the Flemish Veterinary Journal.)

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