High milk production and good fertility in modern dairy cows: the results of some recent research items

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HIGH MILK PRODUCTION AND GOOD FERTILITY IN MODERN DAIRY COWS: THE RESULTS OF SOME RECENT RESEARCH ITEMS

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Summary: The present manuscript summarizes the research, which is currently going on in our department regarding the interaction between negative energy balance (NEB) and fertility in modern high yielding dairy cows. In this work, it has been clearly demonstrated that nearly 50% of the recently calved dairy cows suffer from one or another ovarian dysfunction during the preservice postpartum period. Both clinical signs of a significant negative energy balance as well as the occurrence of puerperal disorders were main risk factors to suffer from these ovarian problems.

In the following research using repeated ovum pick sessions it was demonstrated that homeorhetic changes of metabolites known as typical indicators of the negative energy balance, were reflected in the follicular fluid of the dominant follicle during the immediate postpartum period. Based on in vitro experiments it was furthermore shown that these metabolites were able to affect bovine granulosa cells at concentrations, which were found in the in vivo study. The latter opens perspectives in elucidating the question why high yielding dairy cows express less heat symptoms and why modern dairy cows are at an increased risk to suffer from these ovarian dysfunctions.

Besides the effect on granulosa cells, studies were also carried out to investigate the effect of elevated levels of non esterified fatty acids (NEFAs) on the reproductive competence of the oocyte. In these studies it was demonstrated that NEFAs at levels, which could be found in vitro within the follicular fluid of the dominant follicle during NEB, may influence fertility of high yielding dairy cows by hampering the oocyte maturation as expressed in lower fertilization rates and subsequently lower cleavage and blastocyst development. The latter opens possibilities to explain the worldwide mentioned decreasing fertility results seen in modern high yielding dairy cows.

Key words: Cattle diseases - etiology; fertility; energy metabolism; follicular fluid - chemistry; fatty acid, nonestrified - analysis; ovarian follicle; postpartum period; cattle - female

Introduction

Currently farmers, veterinarians, consultants and even researchers are very demanding for our dairy cows. Besides the demand to produce lots of milk containing high levels of protein, we want them to calve each year. The latter implies that we like every dairy cow to be pregnant or to be recovering from pregnancy and preparing for a new pregnancy at all times, which makes fertility a full time job for her. Getting pregnant during the first 85 days after calving demands, however, a supreme co-operation of the involuting uterus, the hypothalamus, the pituitary, and the ovaries, leading to an undisturbed involution of the uterus, to the resumption of normal ovarian cyclicity, to the expression of heat symptoms and finally to conception. It is clear that the most important events concerning reproduction occur while the cow is at its peak production and experiences severe metabolic stress. At the Ghent University research is going on to reveal the effects of a high level of production on the fertility of the modern dairy cow, and to detect the underlying causes of fertility disturbances. The current review summarizes some of the results of this research.
Decreasing fertility?

Many studies report a decrease in the fertility of modern dairy cows. Since these studies originate from regions all over the globe, the situation seems to be widespread and universally accepted. In the United States, for example, the conception rate has been reported to decrease by 0.45% per year over a twenty year period (1). In the UK this decrease has been in the order of 1% per year (2,3). As comparable results from several other countries continue to appear, these reports have provoked an alarm response that goes on unabated. While over the same time period, significant increases in the level of milk production were reported, people got tempted to blame the increase in milk production to cause the decrease in fertility. In Flanders, average milk production of Holstein Fresian cows increased from 7,496 liters in 1995 towards 8,440 liters in 2000. During the same time period, the calving interval increased from 399 towards 407 days, while the 56-day non-return rate remained relatively stable (69.7 in 1995 and 69.9 in 2000). Analyses of fertility data of the local AI center revealed that the prolongation of the calving interval was mainly due to a prolongation of the interval from parturition to first insemination, while the data expressing the ability of the cows to conceive (i.e. pregnancy rate after first and second insemination, number of inseminations per pregnancy) were not associated with the prolongation of the interval between two consecutive calvings. Hence, we came to the conclusion that at least in Flanders the decrease in fertility of dairy cows as expressed by a prolongation of the calving interval, was mainly due to the inability of the farmers to see their cows in heat at the moment they should inseminate them.

Anoestrus main problem

Based on the aforementioned knowledge, more research was set up to further elucidate the anoestrus problem in Flemish high-yielding dairy herds. In a detailed study examining fertility data of 3,108 lactations (4), in 1,291 (42%) of all studied lactations no heat was observed within 60 days after calving. Cows not seen in heat within 60 days after calving had an average increase in days open with 26 days (days open: 111 vs. 85). Of the 1,817 cows which had been seen in heat during the first 60 days after calving, 622 (34%) had to be examined later on because they had not been seen in heat at the time they should be inseminated. The latter cows were described as suffering from ‘a cessation of observed heat symptoms’, and had an average increase of 24 days in the interval from calving to conception (days open: 109 vs. 85). Hence in total, 1,913 (62%) off all lactations were identified as having suffered from one or another kind of preservice postpartum anoestrus. Both cows not seen in heat within 60 days after calving and cows suffering from ‘cessation of observed heat symptoms’ had a significantly increased risk of being culled in the current lactation.

Abnormal ovarian activity post partum

The aforementioned results led to the question whether the anoestrus problem is merely due to shortcomings in the management (e.g. failure to detect estrus) or whether it is peculiar to the modern high-yielding dairy cow herself. Furthermore, when problems could indeed be designated as being inherent to the high-yielding dairy cow, the next question arises as whether the anoestrus problems are caused by a lack of expressing heat symptoms by the cow, or by ovarian/uterine disorders leading to the symptom of anoestrus. In order to investigate this into more detail, further research was carried out based on the analysis of milk progesterone profiles (5). Although it is nearly impossible to compare the results of different studies because of different sampling protocols and the use of different definitions for both normal and abnormal progesterone profiles, authors nowadays come to very comparable conclusions. The first significant rise in progesterone is stated to occur on the average at 37 days after calving (5), indicating that the first postpartum ovulation in the modern-day dairy cow occurs around day 30 after calving. The very wide range and standard deviation mentioned, however, suggested the presence of many cows with ovarian abnormalities. The latter was confirmed by the same study, in which 47% of the 448 examined progesterone profiles showed an abnormal pattern during the preservice postpartum period. The two most frequently recognized abnormalities were delayed cyclicity or anovulation (= no significant progesterone rise during the first 50 days after calving), and prolonged luteal phase (= a period of at least 20 days of positive progesterone levels without a preceding insemination). In comparison with moderate yielding Friesians, modern high yielding Holsteins showed an increased incidence of postpartum abnormal ovarian cycles (Table 1).

By means of regular rectal palpations, we
found that small, inactive ovaries and not cysts were the most important reason of delayed cyclicity. Searching for the causes of prolonged luteal phases, in almost half (48%) of these cows an abnormal uterine content could be palpated, in 3% a cyst-like structure on one of the ovaries was discernable while on 49% no specific reasons for this ovarian abnormality could be found (5).

Table 1: A comparison of postpartum reproduction parameters based on measurement of progesterone in milk twice weekly in two different studies using moderate yielding Friesians (6) or modern high yielding Holsteins (5)

<table>
<thead>
<tr>
<th>Ovarian activity based on progesterone profiles</th>
<th>Traditional herds (Fagan and Roche, 1986)</th>
<th>Modern high yielding dairy herds (Opsomer et al., 1998)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>448</td>
<td>448</td>
</tr>
<tr>
<td>Number of cyclical patterns (%)</td>
<td>78</td>
<td>53</td>
</tr>
<tr>
<td>Delayed cyclicity (%)</td>
<td>7</td>
<td>20.5</td>
</tr>
<tr>
<td>Temp. cessation of cyclicity (%)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Prolonged luteal phase (%)</td>
<td>3</td>
<td>20.5</td>
</tr>
<tr>
<td>Short cycles (%)</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>Other irregular patterns (%)</td>
<td>4</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Based on a multivariate analysis at farm level, taking into account a number of relevant factors, we demonstrated that calving during the stable period, an extended length of the previous dry period, health problems during the first month of lactation and clinical parameters illustrating the appearance of a severe negative energy balance (NEB), significantly increase the risk for delayed cyclicity before service. Parity, problem calvings, health problems during the first month of lactation and (too) early resumption of ovarian cyclicity after calving significantly increase the risk for prolonged luteal cycles before service (7). Hence, these field studies clearly confirmed previously described clinical trials, in which the health status and the NEB of the animals shortly after calving were demonstrated to be the most important risk factors leading to delayed cyclicity and anovulation, while the occurrence of prolonged luteal cycles is not directly dependent on the energy balance of the animals, but is mainly caused by puerperal disturbances. The latter is furthermore enhanced by the fact that cows in NEB do suffer from a reduced immunity by means of a decreased killing activity of the neutrophils, which renders them more susceptible towards different kinds of puerperal infections (8).

Causal relationship between negative energy balance and fertility problems

Although it is nowadays generally accepted that the NEB during early lactation is associated with many ovarian disturbances, there remain a lot of questions about the possible mechanisms lying behind this association. Hence, much research is currently going on to investigate further the relationship between NEB and fertility in the dairy cow. As the typical homeorhetic changes of several hormones and metabolites are known to act as specific markers for the adaptation of the cows to the metabolic challenge they face during the first weeks after calving, investigations have been done to see whether elevated or lowered levels of these metabolites may be seen as the link between the NEB and the fertility decrease we currently notice in the modern-day dairy cow. As elevated serum concentrations of non esterified fatty acids (NEFAs) are an important characteristic of the cow in NEB, NEFAs have been tested to see whether they may have a negative impact on fertility. As studies until now failed to show a clear relationship between LH pulsatility characteristics, and the concentrations of several energy metabolites in the immediate postpartum period (9), researchers started to focus more and more on the effect of these metabolites at the ovarian level. In vivo, increased NEFA concentrations are correlated with lowered progesterone concentrations and a decrease in the weight of the corpus luteum (CL). Furthermore, other studies found lower CL weights in animals during NEB. Hence, while it is currently accepted that elevated NEFA concentrations are indeed good indicators of the NEB in the recently calved dairy cow, and while there are serious indications that those elevated NEFA levels have a detrimental effect on the ovary, it is not known to which degree the plasma NEFA concentrations are reflected in the follicular fluid at different time points after calving and what kind of effect these elevated NEFA concentrations may have on the different cell types of the ovary.
**Recent research**

Currently, at our department research is going on to investigate to what extent metabolic changes that occur in early postpartum high-yielding dairy cows are reflected in the follicular fluid (FF) of the dominant follicle (>8mm) (10). Nine blood samples were taken per cow from nine high-yielding dairy cows between 7 days before and 46 days after parturition. From day 14 post partum on and together with blood sampling, FF samples of the largest follicle were collected from the same cows by means of transvaginal follicle aspiration. Serum and FF samples were analyzed using commercial clinical and photometric chemistry assays for glucose, \( \beta \)-OH butyrate (\( \beta \)-OHB), urea, total protein (TP), triglycerids (TG), NEFA and total cholesterol (TC). All cows lost body condition during the experimental period, illustrating a NEB during the experimental period. In FF, glucose concentrations were significantly higher and the TP, TG, NEFA and TC concentrations were significantly lower than in serum. The concentrations of glucose, \( \beta \)-OHB, urea and TC in serum and in FF changed significantly over time (P<0.05). Throughout the study, changes of all metabolites in serum were reflected by similar changes in FF (Figures 1-3). Especially for glucose, \( \beta \)-OHB and urea, the correlations were remarkably high. The results of that study confirm that the typical metabolic adaptations which can be found in serum of high-yielding dairy cows shortly post partum, are reflected in FF (Table 1) and, therefore, may affect the quality of both the oocyte and the granulosa cells (10).

Based on the study of Leroy et al. (10), we knew the concentration of several metabolites like NEFA in the FF. In the next study (11) we tested the effect of the most abundant NEFA (oleic- (OA), C18:1; stearic- (SA) C18:0 and palmitic acid (PA) C16:0) on granulosa cell proliferation using the concentrations which were measured in vivo by Leroy et al. (10). Granulosa cells were harvested through repeated aspiration of follicular fluid from large follicles (>8mm) on slaughterhouse ovaries. Cells were cultured for 48h under serum free conditions with 1 ng/ml FSH and 10 ng/ml insulin. Cells were treated with 0, 150, 300 or 500 \( \mu \)M of the individual fatty acid or 450 \( \mu \)M of a 1:1:1 combination of all three fatty acids. At the end of the culture, granulosa cell numbers were determined spectrophotometrically. Both PA and SA had a significant inhibitory effect on granulosa cell proliferation at the three concentrations tested (P<0.01). This effect was not dose dependent for PA (P>0.05) since all three concentrations reduced cell numbers evenly (52.9 to 60% reduction). Stearic acid, on the other hand, had a more severe negative effect on cell proliferation at 300 \( \mu \)M and 500 \( \mu \)M than at 150 \( \mu \)M (P<0.01). Oleic acid only inhibited cell proliferation significantly (P<0.01) at the highest concentration of 500 \( \mu \)M (66.5% reduction). The combination treatment also reduced cell numbers significantly (P<001) in comparison to controls (34.7% reduction). It could be concluded that in vitro, NEFAs reduce cell proliferation and/or survival of bovine granulosa cells. The latter indicates that elevated NEFA concentrations may affect ovarian cells and hence ovarian functioning contributing to the decrease in fertility, which is currently mentioned in high yielding dairy cows (11).

In a more recent study, we furthermore investigated the effect of elevated NEFA levels on the oocyte quality, testing their effect on fertilisation rate, cleavage and subsequent blastocyst formation using an in vitro model (12) (Tables 3 and 4). It was concluded that high levels of NEFAs during a period of NEB might influence fertility of high yielding dairy cows by hampering the oocyte maturation as expressed in lower fertilization rates and subsequent lower cleavage and blastocyst development. Using a new lipid analysis technique to evaluate the lipid content of single bovine oocytes and embryos we were furthermore able to demonstrate a significant increase of the lipid content of in vitro produced embryos, after culture in the presence of serum (13).

### Table 2: Correlation coefficients (r’s) between metabolite concentrations in follicular fluid and serum per experimental session in nine dairy cows

<table>
<thead>
<tr>
<th>Correlations (r)</th>
<th>Glucose</th>
<th>( \beta )-OHB</th>
<th>Urea</th>
<th>Total Protein</th>
<th>Triglycerides</th>
<th>NEFA</th>
<th>Total Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 days pp</td>
<td>0.834*</td>
<td>0.996**</td>
<td>NS</td>
<td>NS</td>
<td>0.892**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>20 days pp</td>
<td>0.788*</td>
<td>0.972**</td>
<td>0.929**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.787*</td>
</tr>
<tr>
<td>26 days pp</td>
<td>0.733*</td>
<td>0.992**</td>
<td>0.987**</td>
<td>NS</td>
<td>0.872**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>33 days pp</td>
<td>0.925**</td>
<td>0.976**</td>
<td>0.990**</td>
<td>NS</td>
<td>0.710*</td>
<td>0.845**</td>
<td>0.918**</td>
</tr>
<tr>
<td>40 days pp</td>
<td>0.916**</td>
<td>0.971**</td>
<td>0.973**</td>
<td>0.860**</td>
<td>NS</td>
<td>NS</td>
<td>0.862**</td>
</tr>
<tr>
<td>46 days pp</td>
<td>0.901*</td>
<td>1.00**</td>
<td>0.782*</td>
<td>NS</td>
<td>NS</td>
<td>0.908*</td>
<td>0.948*</td>
</tr>
</tbody>
</table>

Values are presented for significant correlations (* P < 0.05; ** P < 0.01; NS: not significant).
Figure 1: Average (± SEM) β-hydroxybutyrate concentrations (mM) in serum (full line) and follicular fluid (dotted line) of 9 high yielding dairy cows during the experimental period.

Figure 2: Average (± SEM) Nefa concentrations (mM) in serum (full line) and follicular fluid (dotted line) of 9 high yielding dairy cows during the experimental period.

Figure 3: Average (± SEM) Urea concentrations (mM) in serum (full line) and follicular fluid (dotted line) of 9 high yielding dairy cows during the experimental period.
Figure 4: Effects of different concentrations of palmitic (A), stearic (B) and oleic (C) acid alone or combined (D) on granulosa cell proliferation (cells per well; mean – SEM) after 48 h of culture. The NEFA-combination (D) contains 150μM of each of the fatty acids. Data are expressed as percentage of controls. Means with different superscripts differ significantly (P<0.05).
How to translate this knowledge towards practice?

The biggest challenge for practitioners is to 'translate' this knowledge into practice and use it to help the herds they have in their herd health control program to reach an acceptable level of reproduction. As modern herd health control programs should focus on taking preventive measures rather than on increasing curative treatments (14), not only modern cows but also their 'coaches' have to adapt to the current levels of milk production. This adaptation has to do with an optimization of the management! While reproduction is a full time job for the dairy cow, coaching her to reproduce well takes no less time.
Based on the above it is clear that implementing a dairy herd fertility control program should definitely be more than putting our arms in cows’ rectums to examine cows with problems. Giving advice upon the management of the dairy ‘top athletes’ to prevent fertility problems for sure needs at least the same amount of energy. The challenge is to integrate the current knowledge into nutritional management, production medicine, and reproductive management procedures taking into account the specific obstacles each individual herd has to face, to finally optimize fertility of the herd (15). In the absence of such a holistic approach, the response to traditional veterinary therapies and herd health programmes may become increasingly diminished.

References

VISOKA PROIZVODNOST IN DOBRA REPRODUKTIVNA SPOSOBNOST PRI SODOBNIH KRAVAH MOLZNICAH: REZULTATI NEKATERIH NOVIH RAZISKAV

G. Opsomer, J. Leroy, T. Vanholder, P. Bossaert, A. de Kruif


Ključne besede: govedo, bolezni - etiologija; plodnost; energetski metabolizem; folikularna tekočina - kemija; maščobne kisline neestrificirane - analize; ovarski folikel; poporodno obdobje; krave