

SUBFERTILITY IN HIGH YIELDING DAIRY COWS: HOW TO BRING SCIENCE INTO PRACTICE?

Subfertiliteit bij hoogproductieve melkkoeien: hoe onze wetenschappelijke kennis omzetten in voor de praktijk bruikbaar advies?

G. Opsomer¹, J.L.M.R. Leroy¹, T. Vanholder², P. Bossaert¹, A. de Kruif¹

¹ Department of Reproduction, Obstetrics and Herd Health,
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke

² Someren Veterinary Centre, Slievenstraat 16, 5711PK Someren, The Netherlands

Geert.Opsomer@UGent.be

ABSTRACT

The present article aims to ‘translate’ the current – mostly theoretical – knowledge on fertility disorders in modern high yielding dairy cows, towards the actual situation in the stable. While some detailed research has recently been done at our department to elucidate the association between a high level of milk production and the reproductive performance of the current dairy cow, the next challenge is to ‘translate’ this knowledge into practice and to offer possibilities and strategies to minimize the effects of the decrease in fertility. As the negative energy balance and general health status after calving are known to be paramount factors hampering fertility, it is apparent that avoiding both is among the most important preventive measures to be taken. Improvement of the energy status by achieving a high dry matter intake and the provision of optimal and well balanced nutrition during the transition period as well as during early lactation are key goals in this effort. To achieve these goals, we should not only calculate the rations on paper, but should also check in the stable to determine whether the calculated amount is really being consumed by the cows. Furthermore, veterinarians should use their ‘clinical eyes’ as well as other diagnostic tools to assess the general health status of the cows and to assess at which aspect of the process things are going wrong and need to be adjusted. Besides the control of the negative energy balance and health status, other management factors that need to be maximized include heat detection, cow comfort, insemination technique, time of insemination during estrus and sperm quality. Only if management is on a very high level can high milk production and good fertility be a feasible combination!

SAMENVATTING

Teneinde de (economische) resultaten van een melkveebedrijf te optimaliseren, dient een zo kort mogelijke tussenkalftijd te worden nagestreefd. Dit impliceert dat koeien tijdens de lactatie opnieuw drachtig moeten worden. Aan onze vakgroep wordt reeds gedurende enkele jaren onderzoek verricht naar het effect van de hoge productie op de fertiliteit van melkkoeien. Hieruit bleek dat de Negatieve Energie Balans (NEB) die de koeien gedurende de eerste weken na het afkalven doormaken en de algemene gezondheidstoestand van de dieren rond het moment van het afkalven van doorslaggevend belang zijn voor wat betreft het al dan niet bereiken van de vooropgestelde streefwaarden qua fertiliteit. Het optimaliseren van de gezondheidstoestand van de koeien in de transitieperiode en het beperken van zowel de ernst als de duur van de NEB zijn dan ook van wezenlijk belang wil men hoge economische verliezen wegens subfertiliteit minimaliseren. Dierenartsen dienen hun klanten hier niet alleen op te wijzen maar moeten hen daarenboven met raad en daad bijstaan om dit ook in de stal daadwerkelijk te kunnen verwezenlijken.

Enkel met een strikt managementsysteem dat gebaseerd is op een goed uitgebalanceerde voeding, het vermijden van stress en het optimaal verzorgen van de koeien rondom het afkalven, kunnen de vruchtbaarheidsresultaten op de hedendaagse melkveebedrijven binnen de economisch aanvaardbare grenzen worden gehouden.

INTRODUCTION

Despite the considerable expansion of our knowledge about the bovine reproductive cycle and the increasing possibilities for intervening in it, fertility in high yielding dairy herds has persistently been reported to be in decline over the past decades. Since studies reporting this fertility decrease are originating from regions all over the globe, this situation seems to be widespread and universally accepted as fact. In the United States, for example, the conception rate is reported to have decreased by 0.45% per year over a twenty year period (Beam and Butler, 1999). In the UK this decrease has been on the order of 1% per year (Royal *et al.*, 2000). As comparable results from numerous other countries continue to appear, these reports continue to provoke an unremitting alarm response (Bousquet *et al.*, 2004).

Currently, most routine contact made by veterinarians with dairy herds is via herd health visits involving fertility treatment, the principal aim of which is to assist the herd to achieve its preset targets. Reproduction management and infertility treatment have indeed become dominant focal points of dairy veterinary practice, a trend which does not appear to change in the near future (Noordhuizen, 2001). Obviously, the question arises as what veterinarians are doing wrong or why are they running behind the facts, as they are apparently unable to stop the so costly fertility decline. It is clear that veterinarians are currently failing to translate the growing body of theoretical knowledge about the pathophysiology of fertility disorders in high yielding dairy cows into practical knowledge in the field in terms of providing their clients with ready-to-use solutions.

Basically, nothing has changed about how we should get the cows in calf. We still need to bring in a sufficient amount of good quality spermatozoa at the appropriate time, in the appropriate way and at the correct place in the cow to enable fertilization and embryonic growth and development. So the basic factors that have long been recognized to be important for the fertility of a cattle population are still valid (de Kruif, 1978). What has considerably changed over time, however, is the cows themselves and the way in which they are kept. This fact is clearly illustrated by the significant increase in milk production that has been noted in recent decades. The aforementioned fertility decline seems furthermore to be associated with this marked increase in milk production. Whether this association is only a direct result of the current production levels, or whether it is confounded by factors that go along with the managerial adaptations in the modern dairy herds or by factors that can be seen as a consequence of the increase

in production, is not clear (Leroy, 2005). Probably all are important, and they may even interfere at several levels.

The present paper aims to highlight some practical points to keep in mind when advising farmers in their endeavor to reach an economically optimal calving interval. As in practice each individual herd faces its own specific threats and opportunities, it should be clear that there is no such a thing as a 'one size fits all' approach. Recent research carried out on a world-wide basis, however, continues to highlight certain basic points that continue to be valid for all high producing herds. We should focus on these basic points and try to incorporate them in the management package for each individual herd, in order to improve fertility.

DECREASED FERTILITY?

Currently, the debate is still going on about the (economically) optimal length of the calving interval, and it probably will continue for a long time to come. While the overall recommendation continues to be to strive for the shortest possible calving interval (Huirme *et al.*, 2002), we need to realize that this should be seen in perspective. While each herd has its own characteristics, as has each cow within each herd, the objectives and targets should be set to deal with specific features both at the level of the herd and of the individual cow. Thresholds, however, should be set so as to score the reproductive performances reached, based on the preset objectives of the individual herd (Sheldon *et al.*, 2006). This implies the availability of recent and accurate data based on clear animal identification, at all times.

In Flanders, the mean calving interval of Holstein cows has increased considerably in recent decades. This prolongation is mainly caused by the lengthening of the calving to first insemination interval (Moreels, 2002). Besides the obvious role of the farmer's management practice in this matter, the high yielding dairy cow herself also contributes to this problem as abnormalities in the resumption of ovarian cyclicity post partum have been demonstrated to be as high as 49% (Opsomer *et al.*, 1998). Among the abnormalities observed, delayed cyclicity (no progesterone rise during the first 50 days after calving) and prolonged luteal phases (high progesterone levels for more than 20 days without a preceding insemination) were the most prevalent. Factors that have been recognized as increasing the risk for delayed cyclicity are the appearance of puerperal diseases during the first month after calving and clinical parameters illustrating the appearance of a severe negative energy balance (NEB) after calving. Risk factors for prolonged luteal

phases include dystocia, and puerperal or other diseases during the first month of lactation (Opsomer *et al.*, 2000). However, it is not only the interval from parturition to insemination that has increased but also the average interval from first insemination to conception has extended in high producing dairy cows. Several reasons for the prolongation of this interval can be listed such as fertilization failure, an increase in embryonic and fetal death, and problems with estrus detection.

Detailed research has illustrated (Leroy, 2005; Vanholder, 2005) some of the pathophysiological pathways explaining the association between the increase in milk production and the decrease in fertility. Basically, the production levels currently being reached are being accompanied by a deeper and more prolonged NEB, as can be measured by the lowered levels of glucose, IGF1 and insulin, and the elevated levels of metabolites such as NEFAs, ketone bodies and urea. As most of these metabolites are able to reach the ovaries, they are able to affect several cell types there and hence negatively influence fertility.

Contrary to the widely accepted profile of declining fertility, Whitaker (2002) suggested that declining herd fertility has been happening only on some farms but not on all. This reflects the significant herd variation in the occurrence of risk factors for reduced fertility, and it proves that acceptable fertility performances are still feasible, even in herds with very high production levels.

HOW TO USE ALL THIS NEW KNOWLEDGE IN PRACTICE?

The biggest challenge for practitioners is to 'translate' the recent knowledge into practice and to use it to help the herds they have in their herd health control program to achieve acceptable levels of fertility. Just as modern herd health control programs should focus on taking preventive measures rather than on increasing the number of curative treatments (de Kruif and Opsomer, 2002), modern dairy managers should focus on preventive measures in adapting to the current level of milk production. Hence, not only modern cows but also their 'coaches' need to adapt to the current high level of milk production. This adaptation has to do with an optimizing of the management. For practicing veterinarians, it should be clear that implementing a dairy herd fertility control program should involve more than just putting their arms in cows' rectums to check for problems. Giving advice on the management of 'top dairy athletes' to prevent health and fertility problems most assuredly requires even more energy, knowledge and experience.

FACTORS INFLUENCING FERTILITY AND MEASURES TO BE TAKEN TO OPTIMIZE THEM

Infectious diseases affecting reproduction

The currently encountered herd expansion and intensive housing conditions increase the risk of transmission and maintenance of infectious diseases that are known to compromise reproductive efficiency (Bovine Herpes Virus (IBR), Bovine Viral Diarrhea Virus (BVDV), Salmonellosis, Leptospirosis, *Neospora caninum*). Results from clinical examinations, laboratory results and vaccination regimens need to be drawn together to get a picture of the relevance of the individual infectious agents going around in the herd. The targeting and eradication of infectious diseases must be a priority in the maintenance both of herd health and herd fertility. Details of systematic preventive herd health programs against BVDV, IBR, Leptospirosis, Johne's disease, Salmonellosis and Neosporosis should be clearly outlined and thoroughly discussed with the herd manager. Appropriate biosecurity plans should also be implemented to prevent the introduction of new agents into the herd (Sanderson and Gnad, 2002).

Negative Energy Balance

As NEB seems to be the ever-returning enemy of good fertility in high yielding dairy herds, the basic strategy to reduce the reproductive decline should definitely focus on keeping the NEB under control. While in modern dairy cows genetic progress in terms of milk yield has outstripped that for intake capacity, a certain degree of NEB is inevitable, certainly in early lactation (Thomas *et al.*, 1999). The extent of the NEB (both in depth as well as in duration) varies with the magnitude and rate of increase of milk yield compared to energy intake, however, and thus can be exacerbated if metabolic conditions, disease, housing or management practices impair nutrient intake. Hence, management strategies by which the effect of a NEB can be limited must be targeted towards increasing nutrient intakes, especially energy. Immediately after calving, the paramount goal should be to maximize energy intake without disturbing rumen fermentation. The first aim of the management of a recently calved dairy cow is to optimize her general health status. Only when optimal health – including an excellent appetite – is achieved, can the focus shift towards achieving an optimal production level. In practice, in their enthusiasm to reach top production levels, farmers often forget this basic principle.

To optimize energy intake, all the while assuring optimal rumen fermentation, the intake of high quality forages in early lactation should be maximized. Once this has

been achieved, the energy density of the ration may be increased by gradually raising the amount of concentrates. Generally, under Belgian circumstances, the maximum amount of concentrates given should not exceed 12 kg (9 kg in first lactation animals) and should only be reached at 3 weeks after calving (Opsomer *et al.*, 2004). Increasing the amount of concentrates too fast may disturb ruminal fermentation, which in turn may give rise to ruminal acidosis and an increased incidence of left abomasal displacement.

Currently a lot of research is going on to study the effect of changing the proportion of the different ingredients of the ration. Increasing the amount of fat to maximize the energy content of the ration and hence the energy intake by the animal (Mattos *et al.*, 2000), or increasing the amount of glucogenic substances to temper the steep insulin decrease around the moment of calving (Gong *et al.*, 2002) are excellent illustrations of such measures. For example, the ratio of n6:n3 fatty acids provided in the diet can influence the synthesis of the 2-series of prostaglandins, which are desirable after calving to speed up uterine involution, but undesirable after insemination as they can contribute to the breakdown of the corpus luteum of pregnancy. Hence, the practical implementation of our current knowledge needs to be a better timing of the introduction of rumen protected fats into the diet in accordance with the reproductive stage of the cow. Although primary results seem to be promising, these studies need further confirmation before definite conclusions can be drawn and the results transferred into practical recommendations.

Nutrient or dry matter intake is highly dependent on a lot of factors related both to the cow and to the environment. Among the cow factors, the general health status and body condition score are of major importance. Hence, transition cow programs should focus on maximizing general health and appetite and striving for the ideal body condition score of 3.5 (on 5-point scale) at calving. Aiming for optimal general health includes trimming of the claws at drying off, optimizing rumen health and avoiding metabolic and infectious diseases around calving. Besides this, the veterinarian should provide his herds with a specifically designed standard operating procedure for detecting ill cows as soon as possible and treating them properly.

Furthermore, efforts must be made to remove any environmental restrictions to feed intake, as the environment must be conducive to high intake. Cows need time and space for undisturbed feeding and rumination. There is clear evidence now that the design of food passages, barriers, troughs for water supply and cow traffic within the building definitely affect the intake cows will achieve

(Cook and Nordlund, 2004). Intake can vary widely between individuals in a herd with a lot of competition for feeding space. Especially the intake of heifers is easily restricted by competition with older cows. The provision of adequate feeding space reduces this kind of competition largely. The grouping of cows and social behavior also have their implications. A lot of attention should be paid to this point because during the transition period cows are transferred several times from one group to another. Each transfer or relocation implies another challenge for the cows as it brings them in contact with a new group and a new ration. All the energy that is spent in establishing a new social hierarchy is no longer available to produce or reproduce. At the same time, each change in the ration causes a serious drop in dry matter intake and should therefore also be avoided (Cook and Nordlund, 2004).

Although veterinary practitioners are currently not the only advisors on modern dairy herds, they have the advantage that they can use their "clinical eyes" to interpret what is happening in the herd (Zaaijer and Noordhuizen, 2003). Besides the use of herd production data which are usually readily available, the use of clinical scoring systems has been proven to provide the veterinarian with an extra tool to evaluate the health status of the animals in relation to their production level. Hence, these scoring systems should be used to evaluate the management system used on the herds at a regular time interval.

Furthermore, today's dairy cows may face a wide variety of environmental stressors. These may include overcrowding, infectious challenges, poor ventilation, poor footing or other forms of chronic or even acute pain, uncomfortable stables, rough handling, and frequent relocation in another group. Most of these stressors affect fertility and should therefore be avoided (Dobson and Smith, 2001). Although stress is difficult to define and to show to the herd manager, a lack in cow comfort compromising the cows' health and fertility should be noticed and discussed during the regular herd health visits. While top managers have it at their finger-tips and do not need a lot of explanation to adapt their herd to the needs of their modern top producers, others definitely need to be confronted with some eye-openers.

Heat detection

As has been noted for a long time already, under European conditions where it is not feasible to commonly use synchronization protocols, one of the most important limitations of the reproductive performance of a cattle herd is poor detection of estrus (de Kruif, 1978; Opsomer *et al.*, 2002). Besides the higher incidence of ovarian disturbances in high yielding dairy cows, the expression of heat

symptoms is also reported to be diminished. For example, less than 50% of the estrous cows “stand to be mounted” (Lopez *et al.*, 2004). Therefore some authors propose using other – or more than one – behavioral signs besides this long recognized gold standard to declare a cow in heat (Van Vliet and Van Eerdenburgh, 1996; Kerbrat and Disenhaus, 2004). The fact that the ‘stand to be mounted’ reflex is being significantly less displayed, implies that the aids for detecting estrus which are based on it are currently losing their applicability to a large extent. The use of other aids such as activity meters, however, seems to be giving promising results. Systems for automatically collecting diagnostic data may also provide a gentle solution in this respect. For example, an in-line sensor to measure progesterone and hence to detect the presence or absence of a corpus luteum may be a futuristic though powerful tool to enhance reproductive performance in some herds (Pemberton *et al.*, 2001).

While a lot of recent research is obviously directed towards the investigation of the influence of the energy balance and the stressed metabolism on the expression of heat symptoms, one may not forget to take into consideration all management changes which took place while moving towards high yielding herds. Several studies have clearly shown that primary behavioral signs such as mounting and standing may be seriously depressed by the immediate environmental conditions. For example, it is well known that the expression of heat has seriously decreased since the overall use of concrete floors. Cows furthermore dislike being mounted by herdmates if the floor surface is either slippery or very coarse (Vailes and Britt, 1990). Cows with leg or foot problems, regardless of whether the problem is structural, clinical or subclinical, show much less mounting activity. Many of the foot problems affecting mounting activity can be alleviated by proper foot care and trimming. Other recommendations which should be given to farmers in order to prevent the problem of anestrus include spending more time on estrus detection, looking at both primary and secondary signs of estrus and increasing the amount of light in the stall (Van Vliet and Van Eerdenburgh, 1996).

Moment of insemination, insemination technique and sperm quality

The aforementioned decrease in heat symptoms often forces farmers to inseminate their cows based on secondary heat symptoms. The ‘use’ of these symptoms, however, significantly increases the risk of inseminations not being performed at the optimal moment in relation to the moment of ovulation. This may be seen as a significant contributor to the universally recognized decline in preg-

nancy results in modern dairy herds. As has been known for the past 60 years, insemination should take place from 24 to 12 hours before ovulation in order to attain the highest pregnancy rate. Roelofs (2005) also found that insemination during this time interval provided the best chance of ending up with a good quality embryo at day 7 after insemination. The correct use of this rule, however, depends largely on how many times and for how long heat detection is performed during the day, and which heat symptoms are taken into account. If pedometers are used, the correct timing of insemination according to the increase in activity seems to be the best strategy because this increase in activity is observed in most of the estrus periods, and these activity meters are relatively easy to implement in dairy practice.

In addition, the insemination technique and sperm quality are important factors contributing to acceptable fertility. The majority of the dairy herds use artificial insemination (AI) and currently many owners are performing “do it yourself AI”. Proper training and monitoring of the procedures are paramount in getting good results. Sire selection, on the other hand, is usually focused on production and physical traits but should also take into account reproductive efficiency. Only those sires should be used that have above average non-return rates. If natural breeding is used, pregnancy rates are consistently 10-20% higher than with AI. For this reason, farmers often use a bull for their problem cows that have been inseminated 3 or 4 times without getting pregnant.

Housing

Many diseases are associated with the housing of the cows, as is subfertility. For example, slippery floors are very detrimental to estrus expression. The goal in housing design is to provide an environment for the cows that has a positive effect on their health and fertility. Good housing is furthermore essential during calving (Mee, 2004). Parturient cows should be placed in separate, clean maternity facilities to guarantee sanitary conditions. Strict hygienic measures should be used during parturition in order to prevent infections of the genital tract (de Kruif and Opsomer, 2002). If the cow’s housing environment is insufficient, further measures should be taken to meet the animals’ requirements. The bedding of the cubicles, the flooring and the possibilities for guaranteeing optimal hygiene are among the most important factors in this respect.

CONCLUSION

In view of the complex nature of fertility, it is not surprising to find that ideal fertility criteria are extremely difficult to achieve. When infections are involved in a subfertility problem, this can be due either to specific (e.g. BVDV) or non-specific genital infections. The former often strike a whole herd, causing abortions and repeat breeding. The latter are opportunists of unsanitary conditions during calving, dystocia and abnormal puerperium. They often take an insidious course.

It is generally agreed, however, that the main negative influence on the fertility of a dairy herd stems not as much from specific or non-specific infections, but rather from the effects of a host of other factors. These factors seldom exert their effects individually but rather interact together, making it difficult to analyze infertility in a given herd. For example, the advancement of animal husbandry practices has increased both herd size and production, but man hours per cow have dwindled. The direct result of this decrease is that less time remains for detecting heats, instituting hygienic measures and trimming claws. Thus the final fertility status of a dairy herd is the result of interactions of a whole range of factors from environmental conditions such as season, herd size and age composition, to pure managerial factors such as breeding policy, nutrition and estrus detection. Breeding efficiency depends almost totally on whether or not the farmer is able to skillfully cope with these factors in his herd. By way of conclusion, subfertility has been proven to be a multifactorial disease and the optimization of herd fertility often requires the optimization of several interfering managerial factors. There is almost never a single solution. Although poor fertility is becoming more and more common in our top dairy herds, there is a wide variation between herds and sometimes between years within the same herd. This latter fact illustrates that the dairy herd acts as a dynamic structure and may need specific adaptations, depending on the specific situations the herd actually has to face.

Fertility of a dairy herd is thus a relative phenomenon, expressing what the cows have been able to achieve in the face of a host of interacting factors. To avoid a deterioration of fertility below the accepted standards, the advice given to the farmer should enable him to optimally manage his herd under the given environmental and management conditions. Such advice can best be given by paying regular visits to the farmer (Herd Health and Fertility Control Program) so as to impress upon him the relevant factors of management. Hence, the follow-up of the reproductive performance of a dairy herd should be continuous and not only be restricted to the curative interventions when things are really going wrong.

The cornerstone to improving the reproductive performance of lactating dairy cattle also involves the understanding of the biochemical and physiological principles controlling reproductive and lactational processes. The challenge is to integrate this knowledge into nutritional management, production medicine and reproductive management procedures, taking into account the specific obstacles each individual herd has to face, for the purpose of optimizing the fertility of the herd (Thatcher *et al.*, 2006). In the absence of such a holistic approach, the response to traditional veterinary therapies and herd health programs may increasingly diminish.

LITERATURE

- Beam S.W., Butler W.R. (1999). Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *Journal of Reproduction and Fertility Supplement* 54, 411-424.
- Bousquet D., Bouchard E., DuTremblay D. (2004). Decreasing fertility in dairy cows: myth or reality? *Le Médecin Vétérinaire du Québec* 34, 59-61.
- Cook N., Nordlund K. (2004). Behavioral needs of the transition cow and considerations for special needs facility design. *Veterinary Clinics of North America, Food Animal Practice*, 20, 495-520.
- de Kruif A. (1978). Factors influencing the fertility of a cattle population. *Journal of Reproduction and Fertility* 54, 507-518.
- de Kruif A., Opsomer G. (2002). Integrated dairy herd health management as the basis for prevention. In: *Recent developments and perspectives in bovine medicine*; Keynote lectures of the XXII World Buiatrics Congress, 18-23 August 2002, Hannover, Germany; pp 410-419.
- Dobson H., Smith R.F. (2001). What is stress, and how does it affect reproduction? *Animal Reproduction Science* 60-61, 743-752.
- Gong J.G., Lee W.J., Garnsworthy P.C., Webb R. (2002). Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. *Reproduction* 123, 419-427.
- Huime R., Saatkamp H., Bergevoet R. (2002). Economic analysis of common health problems in dairy cattle. In: 'Recent Developments and Perspectives in Bovine Medicine.' Keynote Lectures of the XXII World Buiatrics Congress, 18-23 August, 2002, Hannover, Germany; pp 420-431.
- Kerbrat S., Disenhaus C. (2004). A proposition for an updated behavioural characterisation of the oestrus period in dairy cows. *Applied Animal Behaviour Science* 87, 223-238.
- Leroy J. (2005). Metabolic changes in high producing dairy cows and the consequences on oocyte and embryo quality. PhD Thesis, Faculty of Veterinary Medicine, Ghent University; pp. 252.
- Lopez H., Satter L., Wiltbank M. (2004). Relationship between the level of milk production and estrous behavior of lactating dairy cows. *Animal Reproduction Science* 81, 209-223.
- Mattos R., Staples C.R., Thatcher W.W. (2000). Effects of dietary fatty acids on reproduction in ruminants. *Reviews of Reproduction* 5, 38-45.

- Mee J. (2004). Managing the dairy cow at calving time. *Veterinary Clinics of North America, Food Animal Practice* 20, 521-546.
- Moreels N. (2002). Subfertiliteit bij het Vlaamse melkvee. Scriptie voorgedragen tot het behalen van het diploma van dierenarts, Faculteit Diergeneeskunde, Universiteit Gent, 38 pp.
- Noordhuizen J. (2001). Changes in the veterinary management of dairy cattle: threats or opportunities? *Veterinary Sciences Tomorrow* 2, <http://www.vetscite.com/cgi-bin/pw.exe/issue2/000020/txt000020.htm>
- Opsomer G, Coryn M., Deluyker H., de Kruif A (1998). An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. *Reproduction in Domestic Animals* 33, 193-204.
- Opsomer G, Gröhn Y., Hertl J., Coryn M., Deluyker H., de Kruif A. (2000). Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology* 53, 841-857.
- Opsomer G, Coryn M., de Kruif A. (2002). Postpartum anoestrus in high yielding dairy cows. In: 'Recent Developments and Perspectives in Bovine Medicine.' Keynote Lectures of the XXII World Buiatrics Congress, 18-23 August, 2002, Hannover, Germany; pp 316-323.
- Opsomer G, De Vlieghe S., de Kruif A. (2004). Droogstand en transitieperiode van hoogproductieve melkkoeien: wat met de voeding? *Vlaams Diergeneeskundig Tijdschrift* 73, 374-383.
- Pemberton R., Hart J., Mottram T. (2001). An electrochemical immunosensor for milk progesterone using a continuous flow system. *Biosensors and Bioelectronics* 16, 715-723.
- Roelofs J. (2005). When to inseminate the cow? Insemination, ovulation and fertilization in dairy cattle. PhD Thesis, Institute of Animal Sciences, Wageningen (WIAS), The Netherlands, 149 pp.
- Royal M.D., Mann G.E., Flint A.P.F. (2000). Strategies for reversing the trend towards subfertility in dairy cattle. *The Veterinary Journal* 160, 53-60.
- Sanderson M., Gnad D. (2002). Biosecurity for reproductive diseases. *Veterinary Clinics of North America: Food Animal Practice* 18, 79-98.
- Sheldon I., Wathes D., Dobson H. (2006). The management of bovine reproduction in elite herds. *The Veterinary Journal* 171, 70-78.
- Thatcher W., Bilby T., Bartolome J., Silvestre F., Staples C., Santos J. (2006). Strategies for improving fertility in the modern dairy cow. *Theriogenology* 65, 30-44.
- Thomas C., Leach K.A., Logue D.N., Ferries C., Phipps R.H. (1999). Management options to reduce load. In: *Metabolic stress in dairy cows*. Occasional publication No. 24 – British Society of Animal Science. Edited by J.D. Oldham, G Simm, A.F. Groen, B.L. Nielsen, J.E. Pryce and T.L.J. Lawrence; pp 129-139.
- Vailes L., Britt J. (1990). Influence of footing surface on mounting and other sexual behaviors of estrual Holstein cows. *Journal of Animal Science* 68, 2333-2339.
- Vanholder T. (2005). Cystic Ovarian Follicles in the high yielding dairy cow post partum: role of hormonal and metabolic adaptations in the pathogenesis. PhD thesis, Faculty of Veterinary Medicine, Ghent University; pp 196.
- Van Vliet J., Van Eerdenburgh F. (1996). Sexual activities and oestrus detection in lactating Holstein cows. *Applied Animal Behaviour Science* 50, 57-69.
- Whitaker D. (2002). What to do about modern dairy cows and fertility? *Irish Veterinary Journal* 55, 635-638.
- Zaaijer D., Noordhuizen J.P.T.M. (2003). A novel scoring system for monitoring the relationship between nutritional efficiency and fertility in dairy cows. *Irish Veterinary Journal* 56, 145-151.