



OPTIMIZING DAIRY COW REPRODUCTIVE PERFORMANCES BESIDES THE USE OF HORMONES

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1. INTRODUCTION

Despite the considerable expansion of our knowledge about the bovine reproductive cycle and the increasing possibilities to intervene herein, fertility in high yielding dairy herds has unremittingly been reported to decline since last decades. Since studies reporting about this fertility decrease originate from regions all over the globe, this 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 (Beam & Butler, 1999). In the UK this decrease has been in the order of 1% per year (Royal *et al.* 2000). As comparable results from several other countries continue to appear, these reports have provoked an alarm response that goes on unabated (Bousquet *et al.* 2004).

Currently, most routine contact by veterinarians with a dairy herd is via herd health visits including fertility, the principal aim of which is to assist the herd to achieve its preset targets. Hence, managing reproduction and treating infertility have become dominant foci of dairy veterinary practitioners and this seems not to change in the near future (Noordhuizen, 2001). Obviously, the next question arises as what are veterinarians doing wrong or why are they running behind facts, as they are apparently not able to stop the so costly fertility decline. It is clear that veterinarians currently fail to transfer the increasing theoretical knowledge about the pathophysiology of fertility disorders in high yielding dairy cows towards the validation of this knowledge in the field by offering ready to use solutions.

Basically, nothing has changed about the way in which we can get the cows in calf as 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, basic factors that have since long time been recognized to be important for the fertility of a cattle population, are still valid (De Kruif, 1978). The cows as well as the way in which they are kept have however changed considerably over time. The latter is mainly illustrated by the marked increase in milk production as noticed during last decades. Hence, the aforementioned fertility decline seems to be associated with the marked increase in milk production.

Whether this association can only be seen as a direct drawback of the current production level, or whether it is confounded by factors that go along with the managerial adaptations on the modern dairy herds, or factors that can be seen as the consequence of the increase in production, is not clear (Leroy, 2005). Probably both are important and may even interfere at several levels.

The present paper aims to highlight some practical points to keep in mind when advising farmers in their endeavour 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 world widely carried out research however continues to highlight ever returning basic points valid for all high producing herds. We should focus on these basic points and try to incorporate them in the herd’s management package of each individual herd, in order to improve fertility.

2. DECREASED FERTILITY?

Currently, the debate about the (economically) optimal length of the calving interval still goes on and it probably will do so for a long time. While the overall advice still needs to be to strive for the shortest possible calving interval (Huirne *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, objectives and targets should be set dealing with specific features both at herd and cow level. Thresholds should be set however to be able to score the reproductive performances reached, based on the preset objectives of the individual herd (Sheldon *et al.* 2006). The latter implies the availability of recent and accurate data, based on a clear animal identification, at all times.

In Flanders, the mean calving interval of Holstein cows has increased considerably during last 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 herein, also the high yielding dairy cow herself contributes to this problem as abnormalities in the resumption of ovarian cyclicity post partum were 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 recognised 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, not only the interval from parturition to insemination 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 oestrus 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, currently reached production levels go along with a deeper and more prolonged NEB as can be measured by lowered levels of glucose, IGF1 and insulin, and elevated levels of metabolites like 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. The latter reflects a significant herd variation in the occurrence of risk factors for reduced fertility, and proves that acceptable fertility performances are still feasible even on herds with very high productions.

3. 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 programme in reaching an acceptable level of fertility. As modern herd health control programmes should focus on taking preventive measures rather than on increasing the number of curative treatments (de Kruif & Opsomer, 2002), not only modern cows but also their ‘coaches’ have to adapt to the current high level of milk production. This adaptation has to do with an optimization of management! For practising veterinarians, it should be clear that implementing a dairy herd fertility control programme should definitely be more than putting their arms in cows’ rectums to examine problem cows. Giving advice upon the management of the dairy “topathletes” to prevent health and fertility problems for sure needs even more energy, knowledge and experience.

4. FACTORS INFLUENCING FERTILITY AND MEASURES TO BE TAKEN TO OPTIMIZE THEM

4.1 Infectious diseases affecting reproduction

The currently encountered herd expansion and intensive housing conditions increase the risk for transmission and maintenance of infectious diseases which are known to compromise reproductive efficiency (Bovine Herpes Viruses (IBR), Bovine Viral Diarrhoea 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 on the herd. Hence, expanding and large herds need to specifically target and eradicate infectious diseases as a priority in the maintenance of both herd health and fertility. Details of systematic preventive herd health programmes against BVDV, IBR, Leptospirosis, Johne’s disease, Salmonellosis and Neosporosis should clearly be outlined and thoroughly be discussed with the herd manager. Appropriate biosecurity plans should furthermore be implemented to prevent the introduction of new agents into the herd (Sanderson & Gnad, 2002).

4.2 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 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) however varies with the magnitude and rate of increase of milk yield compared to energy intake, 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. 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, the endeavour towards an optimal production level can be the next goal. In practice, in their enthusiasm to reach top productions, farmers often forget this basic principle.

To optimize energy intake 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 and 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 hereof. For example the ratio of n6:n3 fatty acids provided in the diet can influence the synthesis of 2-series of prostaglandins, desirable after calving to speed up uterine involution, but undesirable after insemination as they can attribute to the break down of the corpus luteum of pregnancy. Hence, the practical implementation of our current knowledge needs to be a better timing of the implementation of rumen protected fats into the diet, according to the reproductive stage of the cow. Although primary results seem to be promising, these studies need further confirmation before definite conclusions can be made and results can be transferred into practical advices.

Nutrient or dry matter intake is highly dependent on a lot of both cow and environmental factors. Among the cow factors general health status and body condition score are of major importance. Hence, transition cow programmes 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 to detect ill cows as soon as possible and to treat them properly.

Efforts must furthermore be made to remove any environmental restrictions to feed intake, as the environment must be conducive to high intakes. 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 intakes cows will achieve (Cook & Nordlund, 2004). Intakes 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. Grouping of cows and social behaviour also have their implications. A lot of attention should be paid hereon because during the transition period cows are several times transferred 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 to establish 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 hence also be avoided (Cook & 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 on the herd (Zaaijer & Noordhuizen, 2003). Besides the use of production data that usually are readily available on the herd, the use of clinical scoring systems has 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 on a regular time interval.

Today's dairy cows may furthermore 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 hence be avoided (Dobson & 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 regularly carried out 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 some eye-openers to be confronted with.

4.3 Heat detection

As mentioned since long time, under European conditions where it is not feasible to generally use synchronization protocols, one of the most important limitations of the reproductive performance of a cattle herd is poor detection of oestrus (de Kruif, 1978; Opsomer *et al.* 2002). Besides the higher incidence of ovarian disturbances in high yielding dairy cows, also the expression of heat symptoms is reported to be diminished. For example, less than 50% of the oestrous cows "stand to be mounted" (Lopez *et al.* 2004). Therefore some authors propose to use other or more than one behavioural sign besides this ever known gold standard to declare a cow in heat (Van Vliet & Van Eerdenburgh, 1996; Kerbrat & Disenhaus, 2004). The fact that the "stand to be mounted" reflex is significantly less displayed, implies that the aids to detect oestrus which are based hereon currently loose their applicability to a large extent. The use of other aids such as activity meters however seems to give promising results. Also systems to automatically collect diagnostic data may 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 performances on some herds (Pemberton *et al.* 2001).

Whilst 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 clearly showed that primary behavioural signs such as mounting and standing, may be seriously depressed by the immediate environmental conditions. It is for example well known that the expression of heat 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 & Britt, 1990). Cows having 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 advices which should be given to farmers in order to prevent the problem of anoestrus, are to spend more time to oestrus detection, to look at both primary and secondary signs of oestrus and to increase the amount of light in the stall (Van Vliet & Van Eerdenburg, 1996).

4.4 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 latter however significantly increases the risk that inseminations are not performed at the optimal moment in relation to the moment of ovulation. This may be seen as a significant contributor towards the universally mentioned decline in pregnancy results on modern dairy herds. As already has been stated since 60 years, insemination should take place 24 to 12 hours before ovulation in order to attain the highest pregnancy rate. Also Roelofs (2005) found that insemination during this time interval gave the best chance to end 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, 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 oestrous periods and these activity meters are relatively easy to implement in dairy practice.

Also 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 perform “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 focussed on production and physical traits but should also take into account reproductive efficiency. Only those sires should be used with 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 which have been inseminated 3 or 4 times without getting pregnant.

4.5 Housing

Many diseases are associated with the housing of the cows as is subfertility. For example slippery floors are very detrimental to oestrus 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 separated into 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 & Opsomer, 2002). If housing of the cow’s environment is insufficient, measures should be taken to meet the requirements of the cows. The bedding of the cubicles, the flooring and the possibilities to guarantee optimal hygiene are among the most important factors herein.

5. CONCLUSION

Realizing the complex nature of fertility, it is not surprising to find that ideal fertility criteria are extremely difficult to reach. When infections are involved in a subfertility problem, it can be due to either 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 fertility of a dairy herd stems not as much from specific or non-specific infections, as from the effects of a host of other factors. These factors seldom exert their effects individually but interact together, making it difficult to analyse 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, age composition, to pure managerial factors like breeding policy, nutrition, oestrus detection etc. Breeding efficiency depends almost totally on whether or not the skill of the farmer can cope with these factors in his herd. Conclusively, subfertility is proven to be a multifactorial disease and optimization of herd fertility often needs an optimization of several interfering managerial points. There hardly ever is a single solution. Although poor fertility is more and more common in our dairy top herds, there is a wide variation between herds and sometimes between years within the same herd. The latter illustrates that the dairy herd act as a dynamic structure and may need specific adaptations depending on 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 hinges on how he can best manage his herd under given environmental and management conditions. Such advices can best be given by paying regular visits to the farmer (Herd Health and Fertility Control Programme) 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 improve reproductive performance of lactating dairy cattle furthermore 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, to finally optimize 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 programmes may become increasingly diminished.

6. SUMMARY

The present article aims to provide veterinarians with some important topics on which they should focus when giving their dairy herds advice to strive for optimal fertility. Before using drugs, veterinarians should focus on providing their clients with advice about adapting the management towards their level of production. 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 factors herein. To do so, rations should not only be calculated on paper but it should also be checked in the stable whether the calculated amount is really consumed by the cows. Veterinarians should furthermore use their “clinical eyes” as well as other diagnostic tools to assess the general health status of the cows and to assess at which level things are going wrong and need to be adjusted. Besides the control of the negative energy balance and health status, other management factors which need to be maximized are heat detection, cow comfort, insemination technique, time of insemination during oestrus and sperm quality. Only if management is on a very high level, high milk production and good fertility are a feasible combination!

7. KEY WORDS

Dairy cow, milk production, reproductive disorders, management.

8. RESUME

Cet article a pour objectif d'attirer plus particulièrement l'attention des vétérinaires sur certains points importants qu'ils devraient considérer lorsqu'ils sont amenés à donner leur avis pour optimiser la fertilité des troupeaux laitiers. Avant tout usage de médicaments, les vétérinaires devraient conseiller leurs clients quant à l'adaptation des méthodes de management à des fins de production laitière. Des recherches précises ont été conduites dans notre Département de Recherche afin de comprendre l'association entre un haut niveau de production laitière et les performances de reproduction de la vache laitière actuelle, le prochain objectif étant de traduire cette connaissance en termes pratiques afin d'offrir des possibilités et des stratégies à même de minimiser la décroissance de la fertilité. Un équilibre énergétique défavorable et un mauvais état de santé général de l'animal après vêlage sont connus pour affecter d'une manière extrêmement importante la fertilité, et il est évident qu'éviter les deux constitue la mesure préventive la plus importante à mettre en oeuvre.

L'amélioration du statut énergétique par ingestion de matière sèche et la fourniture d'une alimentation optimale et bien équilibrée pendant la période de transition et en début de lactation sont des éléments fondamentaux. Pour cela les rations ne doivent pas être seulement calculées sur le papier mais évaluées en étable afin de vérifier que les quantités ingérées sont réellement consommées par les vaches. En outre, les vétérinaires devraient utiliser leurs yeux de clinicien aussi bien que les outils de diagnostic pour évaluer l'état général de la vache et à quel niveau il y a des erreurs qui ont besoin d'être corrigées. En addition du contrôle d'une balance énergétique défavorable et de l'état de santé, d'autres facteurs de conduite de troupeau ont besoin d'être optimisés tels que la détection des chaleurs, le confort de la vache, la technique d'insémination, le moment d'insémination durant l'oestrus et la qualité du sperme. C'est seulement si les conditions de conduite de management sont performantes que l'association d'un haut niveau de production laitière et une bonne fertilité est réalisable.

9. MOTS CLES

Vache laitière, production de lait, troubles de reproduction, gestion.

10. REFERENCES

Beam SW, Butler WR. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *J Repro Fert*, 1999; 54:411-424.

Bousquet D, Bouchard E, DuTremblay D. Decreasing fertility in dairy cows: myth or reality? *Med Vet Q*, 2004; 34:59-61.

Cook N, Nordlund K. Behavioral needs of the transition cow and considerations for special needs facility design. *Vet Clin North Am, Food Anim Practi*, 2004; 20:495-520.

De Kruif A. Factors influencing the fertility of a cattle population. *J Repro Ferti*, 1978; 54:507-518.

De Kruif A, Opsomer G. Integrated dairy herd health management as the basis for prevention. *Proc XXIIth WBC*, 2002:410-419.

Dobson H, Smith RF. What is stress, and how does it affect reproduction? *Anim Repro Sci*, 2001; 60-61:743-752.

Gong JG, Lee WJ, Garnsworthy PC, Webb R. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. *Repro*, 2002; 123:419-427.

Huirne R, Saatkamp H, Bergevoet R. (2002). Economic analysis of common health problems in dairy cattle. *Proc XXIIth WBC*, 2002:420-431.

Leroy J. Metabolic changes in high producing dairy cows and the consequences on oocyte and embryo quality. PhD Thesis, Faculty of Veterinary Medicine, Ghent University, 2005.

Kerbrat S, Disenhaus C. A proposition for an updated behavioural characterisation of the oestrus period in dairy cows. *Appl Anim Behav Sci*, 2004; 87:223-238.

Lopez H, Satter L, Wiltbank M. Relationship between the level of milk production and estrous behavior of lactating dairy cows. *Anim Repro Sci*, 2004; 81:209-223.

Mattos R, Staples CR, Thatcher WW. Effects of dietary fatty acids on reproduction in ruminants. *Rev Repro*, 2000; 5:38-45.

Mee J. Managing the dairy cow at calving time. *Vet Clin North Am, Food Anim Pract*, 2004; 20:521-546.

Moreels N. Subfertiliteit bij het Vlaamse melkvee. Scriptie voorgedragen tot het behalen van het diploma van dierenarts, Faculteit Diergeneeskunde, Universiteit Gent, 2002.

- Noordhuizen J. 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>. 2001.
- Opsomer G, Coryn M, Deluyker H, de Kruif A. An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. *Reprod Dom Anim*, 1998; 33:193-204.
- Opsomer G, Gröhn Y, Hertl J, Coryn M, Deluyker H, de Kruif A. Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenol*, 2000; 53:841-857.
- Opsomer G, Coryn M, de Kruif A. Postpartum anoestrus in high yielding dairy cows. *Proc XXIIth WBC*, 2002:316-323.
- Opsomer G, De Vliegher S, de Kruif A. Droogstand en transitieperiode van hoogproductieve melkkoeien: wat met de voeding? *Vlaams Dierg Tijdschrift*, 2004; 73:374-383.
- Pemberton R, Hart J, Mottram T. An electrochemical immunosensor for milk progesterone using a continuous flow system. *Biosensors Bioelectronics*, 2001; 16:715-723.
- Roelofs J. When to inseminate the cow? Insemination, ovulation and fertilization in dairy cattle. PhD Thesis, Institute of Animal Sciences Wageningen (WIAS), The Netherlands, 2005.
- Royal MD, Mann GE, Flint APF. Strategies for reversing the trend towards subfertility in dairy cattle. *Vet J*, 2000; 160:53-60.
- Sanderson M, Gnad D. Biosecurity for reproductive diseases. *Vet Clin North Am Food Anim Pract*, 2002; 18:79-98.
- Sheldon I, Wathes D, Dobson H. The management of bovine reproduction on elite herds. *Vet J*, 2006; 171:70-78.
- Thatcher W, Bilby T, Bartolome J, Silvestre F, Staples C, Santos J. Strategies for improving fertility in the modern dairy cow. *Theriogenol*, 2006; 65:30-44.
- Thomas C, Leach KA, Logue DN, Ferries C, Phipps RH. Management options to reduce load. In: *Metabolic stress in dairy cows*. Occasional publication No. 24 - British Society of Animal Science. JD Oldham, G. Simm, AF Groen, BL Nielsen, JE Pryce and TLJ Lawrence Eds, 1999:129-139.
- Vailes L, Britt J. Influence of footing surface on mounting and other sexual behaviors of estrual Holstein cows. *J Anim Sci*, 1990; 68:2333-2339.
- Vanholder T. 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, 2005.
- Van Viet J, Van Eerdenburg F. Sexual activities and oestrus detection in lactating Holstein cows. *Appl Anim Behav Sci*, 1996; 50:57-69.
- Whitaker D. What to do about modern dairy cows and fertility? *Irish Vet J*, 2002; 55:635-638.
- Zaaijer D, Noordhuizen JPTM. A novel scoring system for monitoring the relationship between nutritional efficiency and fertility in dairy cows. *Irish Vet J*, 2003; 56:145-151.