



Approaches to increase reproductive efficiency in artificially inseminated dairy cows

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Abstract

Infertility has been linked to numerous factors in high producing dairy cattle in the last decades. The detection of estrus continues to present difficulties and, although progress has been made in regards to estrus synchronization and artificial insemination, the reproductive performance of dairy cows has not improved substantially. Moreover, in warm countries, summer heat stress is a major factor impairing fertility. This presentation expresses our views on factors of a non-infectious nature that affect the fertility of lactating dairy cows following AI. Special attention is paid to factors related to the cow and its environment and to some approaches to increase reproductive efficiency such as confirmation of estrus at insemination and the insemination procedure.

Keywords: bovine, cornual insemination, estrus, fertility, management, season.

Introduction

In the past decades, infertility has been linked to numerous factors in high producing dairy cattle (Beam and Butler, 1999; Royal *et al.*, 2000). Reasons for the lower fertility have not been entirely linked to increased milk production (Lucy, 2001; López-Gatius, 2003; López-Gatius *et al.*, 2006; Garcia-Ispuerto *et al.*, 2007b). Therefore, a main objective in recent years has been to preserve fertility of dairy herds (Gosden and Nagano, 2002). New management practices (leading to the improved well-being of cows) can improve the health and fertility of dairy cows (Windig *et al.*, 2005), and there is a tendency towards a higher level of management in high producing compared to lower producing herds (Calus *et al.*, 2005). However, in spite of the progress of the knowledge in the reproductive physiology of the cow over the last several years, fertility has not substantially improved. Moreover, in warm countries, summer heat stress is a major factor impairing fertility. This presentation expresses our views on factors affecting fertility in high producing dairy herds. Special attention is paid to factors related to the cow and its environment and to some approaches to increase reproductive efficiency such as confirmation of estrus at insemination and the insemination procedure.

Management factors

Infertility has been often associated with high milk production, but this problem is multi-factorial and cannot be solely attribute to milk yield (Lucy, 2001; López-Gatius, 2003). In three extensive studies including 24,366 AI we could not detect a negative effect of milk production on fertility (López-Gatius *et al.*, 2005a, b; Garcia-Ispuerto *et al.*, 2007b). However, extensive studies have made possible to link high milk production in individual cows to high fertility (Lucy, 2001; López-Gatius *et al.*, 2006). For example, early fertile cows (cows that become pregnant before 90 days postpartum) were those who produce more milk at day 50 postpartum (López-Gatius *et al.*, 2006).

The question is why higher producing cows are more likely to conceive at the beginning of lactation? Maybe because good management practices allow the expression of the genetic potential of these animals, whereas lower producer cows receive inadequate care. If genetic progress is linked to fertility declining, low producer cows should have a major chance for becoming pregnant earlier, and this is not the case. Probably, highly fertile and producer cows had the highest genetic merit within the herd and suffered a low negative energy balance during postpartum period (López-Gatius *et al.*, 2003). Conception rate and calving interval do not appear to be affected by the genetic merit of a herd (Mayne *et al.*, 2002), and the highest producing dairy cows in the herd are not necessarily those with the greatest negative energy balance or the lowest body condition score (Lucy, 2001; Grohn and Rajala-Schultz, 2000). Anyway, the findings of studies that have identified factors promoting fertility such as cows becoming pregnant during the first trimester postpartum should be incorporated in routine checks conducted on herds. Data derived from these types of study are often more interesting than those of studies examining factors related to fertility failure.

The effects of milking frequency on fertility have been extensively explored in dairy cattle (Barnes *et al.*, 1990; Erdman and Varner, 1995; Stelwagen and Knight, 1997). Some studies conclude that reproductive performance is unaltered by milking three times per day (Amos *et al.*, 1985; Barnes *et al.*, 1990; Kruij *et al.*, 2002), whereas others have related this milking frequency to reduced fertility (DePeteres *et al.*, 1985; Gisi *et al.*, 1986; Smith *et al.*, 2002). In a more recent

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study (García-Ispuerto *et al.*, 2007b) analyzing 10,965 inseminations, we demonstrated that a three times daily milking regimen reduced fertility by an odds ratio of 0.58, compared to milking two times per day. The strong negative effect of the three times daily milking routine observed in our study adds more fuel to the controversy over the effects of milk production on reproductive performance. Milking frequency was a risk factor for infertility, whereas milk production at AI not. This indicates that the decrease in fertility associated with milking frequency seems not to be directly related to the increased milk yield. It is likely that cows milked three times per day will be more influenced by the luteolytic effects of oxytocin release in response to udder massage at each milking (Silvia *et al.*, 1991). An equally plausible explanation is the additional stress induced by the extra milking event each day.

The bull and AI technician effects on fertility have been extensively reported. In several studies performed in our area we could determine that some bulls decreased fertility at an odds ratio range of 0.31 to 0.44 (López-Gatius *et al.*, 2005a; García-Ispuerto *et al.*, 2007b), whereas one single bull increased fertility by a factor of 4.7 (López-Gatius *et al.*, 2005b). According to DeJarnette *et al.* (2004), we should consider whether the decline on fertility of high producing dairy herds can be attributed sometimes to the male, as a logical question. The problem is that many environmental and herd management factors will affect fertility estimates of an inseminating bull (Foote, 2003). Therefore, a continuous control of seminal doses entering in a herd should be useful to promptly locate a negative bull. Concerning the AI technician, we could only assess this factor in one single study including 10,965 inseminations (García-Ispuerto *et al.*, 2007b). The different AI technicians caused great variation in pregnancy rates. The likelihood of pregnancy decreased by a factor of 0.25 when a cow was inseminated by the worst compared to the best inseminator. Two of 13 AI technicians performed bicornual insemination of all cows (half of the seminal dose, 4-5 cm deep into each uterine horn), whereas the remaining 11 AI technicians performed uterine body insemination. Better results were obtained from technicians performing deep AI, in agreement with previous reports (López-Gatius and Camón-Urgel, 1988; Senger *et al.*, 1988). This variation in pregnancy rates could be an important practical limitation for the success of AI and also for herd fertility. It is clear that some AI technicians inseminate cows less efficiently than others. Deep cornual AI requires more training of inseminators and, therefore, favors results (Senger, 1993; López-Gatius, 2000). However, irrespective of the use of deep AI, retraining and continuous control of inseminators should be considered.

Environmental factors

Environmental factors such as the use of a bull in the herd; poor nutrition or the loss of the body

reserves (negative energy balance); and housing elements (concrete slatted or dirty floors) can affect fertility. However, most studies report the seasonal effect as a major environmental factor affecting fertility. Although heavy rain, strong wind or high humidity can reduce fertility, high temperatures have been strongly linked to low fertility (see review by López-Gatius, 2012).

The fertility indicators conception and pregnancy rate to first service have suffered a fall of 0.5-1% per year (Royal *et al.*, 2000; López-Gatius, 2003). However, when the results were stratified according to season, warm period resulted in an increased infertility, while cool period preserved fertility. Thus, average conception rates to first AI in 2000 were 43 and 22% for the cool and warm periods, respectively (López-Gatius, 2003). The continued increase in milk production has been the result of improvements of genetics, nutrition and management practices, which probably cope with the negative effect of milk production. During warm period, any stressor such as high temperatures, could compromise the benefits of milk production. Heat stress seems to induce the premature aging of oocytes (Edwards *et al.*, 2005; Schrock *et al.*, 2007; Andreu-Vázquez *et al.*, 2010). Metabolic demands due to high milk production added to stressful factors such as high temperatures can compromise the reproductive functions of cows (Labèrnia *et al.*, 1998; De Rensis and Scaramuzzi, 2003; López-Gatius, 2003).

In a study including the temperature humidity index (THI; García-Ispuerto *et al.*, 2007a), a negative effect of high maximum THI was found, especially 3 days before AI and at the day of AI. This index incorporates the effects of both ambient temperature and relative humidity (RH) in an index (Thom, 1958) and is widely used in hot areas worldwide to assess the impact of heat stress on dairy cows (Hahn, 1969; Fuquay, 1981). However, when temperature was analyzed alone, it was demonstrated that high temperatures on day 3 before insemination and 1 day after were correlated with low fertility. Thus, climate factors seem to be highly relevant for conception rate, especially during the period encompassing 3 days before to 1 day after AI. The use of the THI or temperature to control a farm environment would depend on the individual farm and on each environmental situation, but it is important to check temperature and humidity to know when to adopt cooling measures.

Confirmation of estrus

Inseminating the cow is the final, but by no means the least important, step in the process of a good estrus detection practice in the herd. Despite the significant progresses in the development of estrus detection aids during the last decades, detection of estrus remains a major problem in the XXI century (Roelofs *et al.*, 2010). Incorrect estrus detection is the



most common and expensive cause of failure of AI programs. Cows are often falsely identified as being in estrus and inseminated when conception cannot occur (López-Gatius and Camón-Urgel, 1991; López-Gatius, 2000, 2011; Sturman *et al.*, 2000). Although professional inseminators palpate the reproductive tract of numerous cows every day, most are not trained to examine the uterus and ovaries and, therefore, to confirm estrus. This situation poses a serious practical limitation to the success of estrus detection procedures and AI. Estrous signs in pregnant cows make the situation even more difficult. Pregnant cows stood willingly to be mounted by another cow or bull at all stages of pregnancy (Thomas and Dobson, 1989) and the insemination of pregnant cows can cause embryonic mortality or abortion (Vandemark *et al.*, 1952). In fact, 19 (Sturman *et al.*, 2000) to 40% (Nebel *et al.*, 1987) of AI have been incorrectly performed in pregnant cows. Finally, the most accurate external sign of estrus, standing to be mounted, was only registered in 58% of estrous periods in a more recent study (Roelofs *et al.*, 2005). Thus, the first goal of any estrus confirmation program should be to positively identify estrus and to reject cows for insemination that are not ready for service or are pregnant. Through rectal examination of the bovine reproductive tract either by hand or by ultrasonography, an animal can be correctly diagnosed as being ready for service (Roelofs *et al.*, 2010).

Feeling the ovaries

As ovulation approaches, the follicle feels very soft separating itself from the remainder of the ovary (Studer and Morrow, 1981; Keenan, 1984). Hereafter, the ovulatory follicle is rapidly evacuated during the process of ovulation, and this ovulatory depletion may be difficult to recognize, especially 12 h after ovulation (Hanzen *et al.*, 1999). Thus, the sequence of changes that the dominant ovulatory follicle goes through at palpation during the periovulatory period is: firm/soft follicle (young preovulatory follicle), followed by very soft follicle (mature preovulatory follicle), followed by evacuated follicle (follicle associated with ovulation). Since one of these three follicle types are usually present at the time of insemination, a recent study on 2,365 AI was designed to determine possible differences between the types of follicle firm/soft, very soft or evacuated in terms of their effects on fertility (López-Gatius, 2011). The likelihood of pregnancy decreased significantly by factors of 0.86 or 0.82 in cows with a firm/soft follicle inseminated during the cool or warm period, respectively, and by a factor of 0.09 in cows with evacuated follicles inseminated during the warm period, using as reference cows with a very soft follicle inseminated during the cool period (yielding the highest pregnancy rate). The state of the periovulatory follicle at insemination was clearly related to fertility and masked the effects of factors commonly affecting fertility such

as parity, days in milk at AI and inseminating bull. More importantly, these results suggest that by including ovarian follicle checks in artificial insemination routines, the success of this procedure could be improved.

Site of semen deposition

During mating, the bull deposits several billion spermatozoa into the anterior vagina. However, since the cervix is a major obstacle for sperm transport, the number of spermatozoa that finally reach the uterine body usually does not exceed 1% of the ejaculated spermatozoa. In artificial insemination, semen is generally deposited directly into the uterine body, thus bypassing the cervix and permitting the use of a considerably reduced number of spermatozoa (López-Gatius, 2000). One of the most significant contributions to the successful commercial application of AI in dairy cattle breeding has been attributed to the highly trained inseminator (Foote, 1996). However, there has been a tendency to adopt routine insemination techniques and to ignore inseminator-related factors that, as it has been discussed above, can dramatically affect fertility (García-Ispuerto *et al.*, 2007b). Presently, the pregnancy rate after a single AI service is rarely higher than 40%, which is far from the 60% or higher rate commonly recorded in the 1960s (Olds, 1978). This drop in AI efficiency has prompted the suggestion of changing the site of semen deposition in cattle, based on the idea that deep uterine insemination should ensure the deposition of spermatozoa nearer to the uterotubal junction, which is thought to be the main sperm reservoir prior to ovulation (Hunter and Greve, 1998; López-Gatius, 2000). Therefore, several studies were designed to evaluate deep bicornual and unicornual uterine insemination in an attempt to approach inseminate to the uterotubal junction could favor the results (López-Gatius, 2012).

For the bicornual method, the inseminating catheter tip is guided into one uterine horn until resistance is met, and half of the semen dose deposited. In a similar manner, the remaining half dose is deposited in the opposite horn (Senger *et al.*, 1988). For deep unicornual insemination, ovaries are palpated per rectum to determine the side of ovulation, then the major curve of the uterine horn ipsilateral to the side of the preovulatory follicle is straightened out by gentle manipulation per rectum and the semen introduced into the cranial half of the horn (López-Gatius and Camón-Urgel, 1988). Significantly better results have been obtained after deep bicornual or unicornual insemination, although some authors question the efficiency of this technique (López-Gatius, 2012). Perhaps, the advantage of deep uterine insemination, either bicornual or unicornual, is that it allows the deposition of semen nearer to the uterotubal junction and reduces the chances of cervical deposition.



Concluding remarks

From a scientific stand point, one should pay attention to cows becoming pregnant within 90 days as well as the state of the follicle at AI; whereas from a herd management stand point, one should pay attention to the training and re-training of the AI technicians, be careful not to inseminate pregnant animals and improve estrus detection. Semen providing bull should be frequently monitored; and THI and/or temperature measurements should allow us to decide to establish better cooling systems in the herds.

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