

## Challenges to fertility in dairy cattle: from ovulation to the fetal stage of pregnancy

*Desafios na fertilidade de gado leiteiro: da ovulação ao estágio fetal da gestação*

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### Abstract

One critical determinant of fertility is the establishment and maintenance of pregnancy after insemination or embryo transfer. Pregnancy success is low in lactating dairy cows. Reduced fertility of the dairy female is only expressed in the lactational state but the magnitude of depression of fertility is not related to milk yield. Pregnancy failure occurs throughout gestation with the greatest frequency of pregnancy losses being in the period from fertilization to day 5-7 after insemination. Poor fertility in lactating cows represents, in part, failure of genetic selection for reproductive traits. In addition, the uterus of the lactating cow is less able to support embryonic and fetal development than the uterus of the non-lactating female. There is little evidence that the oocyte is compromised in lactating cows. Among the determinants of pregnancy success in lactating cows is progesterone secretion in the post-ovulatory period, environment of the ovulatory follicle during its terminal growth, energy balance, and amounts of specific nutrients in the diet. Progress in improving reproductive function of the lactating cow is being made and the historical decline in reproductive function is beginning to be reversed.

**Keywords:** Dairy cattle, fertility, lactation, genetic selection, uterus, oocyte.

### Resumo

*Um determinante crítico da fertilidade é o estabelecimento e manutenção da gestação após inseminação ou transferência de embrião. O sucesso na gestação é baixo em animais lactantes. A fertilidade reduzida da vaca leiteira é expressa apenas no estágio de lactação, mas a magnitude da diminuição da fertilidade não está relacionada à produção de leite. A falha na gestação ocorre ao longo da gestação com a maior frequência de perda de gestação no período entre a fertilização e o dia 5-7 após inseminação. Fertilidade pobre em vacas lactantes representa, em parte, a falha da seleção genética para traços de reprodução. Além disso, o útero da vaca lactante é menos capaz de suportar desenvolvimento do embrião e do feto que o útero de uma fêmea não lactante. Há pouca evidência de que o oócito seja comprometido em vacas lactantes. Entre os determinantes de sucesso na gestação de vacas lactantes está o crescimento, equilíbrio energético e quantidade de ingredientes específicos na dieta. O progresso na melhoria da função reprodutiva da vaca lactante está sendo feito, e o declínio histórico na função reprodutiva está começando a ser revertido.*

**Palavras-chave:** Gado leiteiro, fertilidade, lactação, seleção genética, útero, oócito.

### Introduction

Fertility of lactating dairy cattle has declined throughout much of the world in the last fifty years or so (Fig. 1). Although accompanied by large increases in milk yield, the reduction in fertility has not been due simply to higher milk production. Indeed, estimates of the relationship between milk yield and fertility vary from negative to positive (Eicker et al., 1996; Loeffler et al., 1999; García-Ispierto et al., 2007; Caraviello et al., 2006; López-Gatius et al., 2006; Demetrio et al., 2007). Similarly, there was no effect of milk yield on oocyte quality as assessed by in vitro fertilization (Snijders et al., 2000; Roth et al., 2008). Nonetheless, poor fertility in dairy cattle is dependent upon the lactating state. While non-biased comparisons are difficult to make, fertility appears not to be greatly different between nonlactating dairy and beef females. Thus, for example, pregnancy rates to fixed-time artificial insemination (AI) after the 5-day controlled internal drug releasing device (CIDR) synchronization program ranged from 46-59% in dairy heifers (Rabaglino et al., 2010a, b) and 54-84% in beef heifers (Peterson et al., 2011).

As shown in Fig. 1, the decrease in fertility occurring since the 1960s has been stemmed since 2000 and, in some breeds including the Holstein, the trend has been reversed slightly. Recent improvements in fertility can be ascribed to intensive efforts in development of new technologies to improve fertility, particularly timed artificial insemination, and increased emphasis on genetic selection for reproduction and health traits (note the increase in sire breeding value for daughter pregnancy rate in Fig. 2).

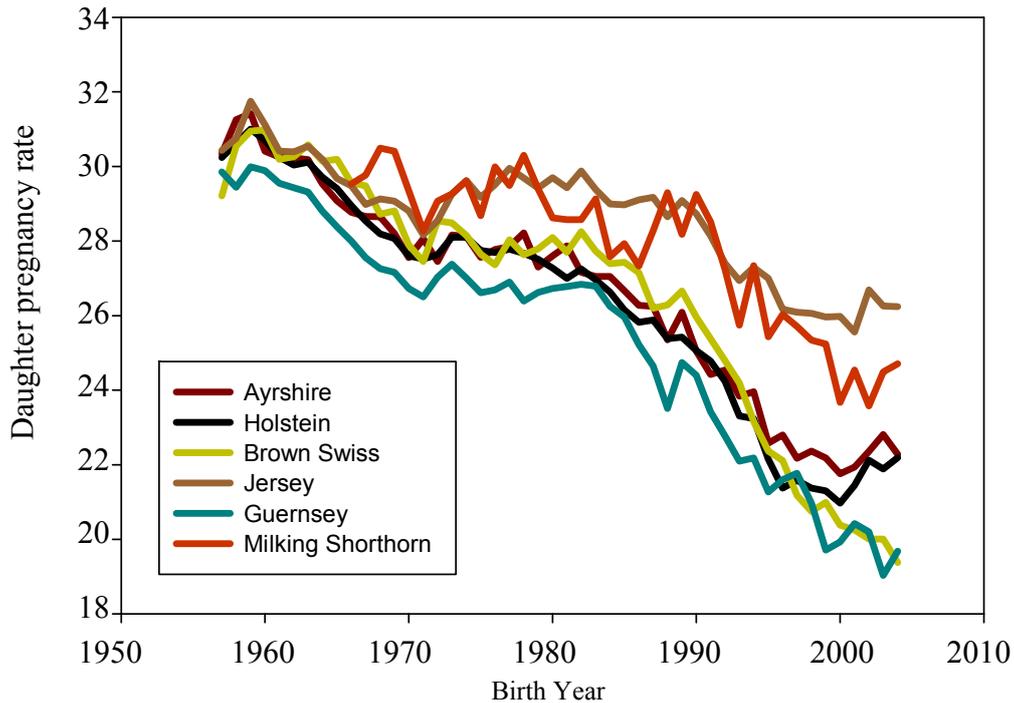


Figure 1. Historical changes in phenotypic measurements of daughter pregnancy rate in United States dairy breeds from 1957-2005. Data were obtained from United States Dept. of Agriculture Animal Improvement Program Laboratory (<http://aipl.arsusda.gov/eval/summary/trend.cfm>).

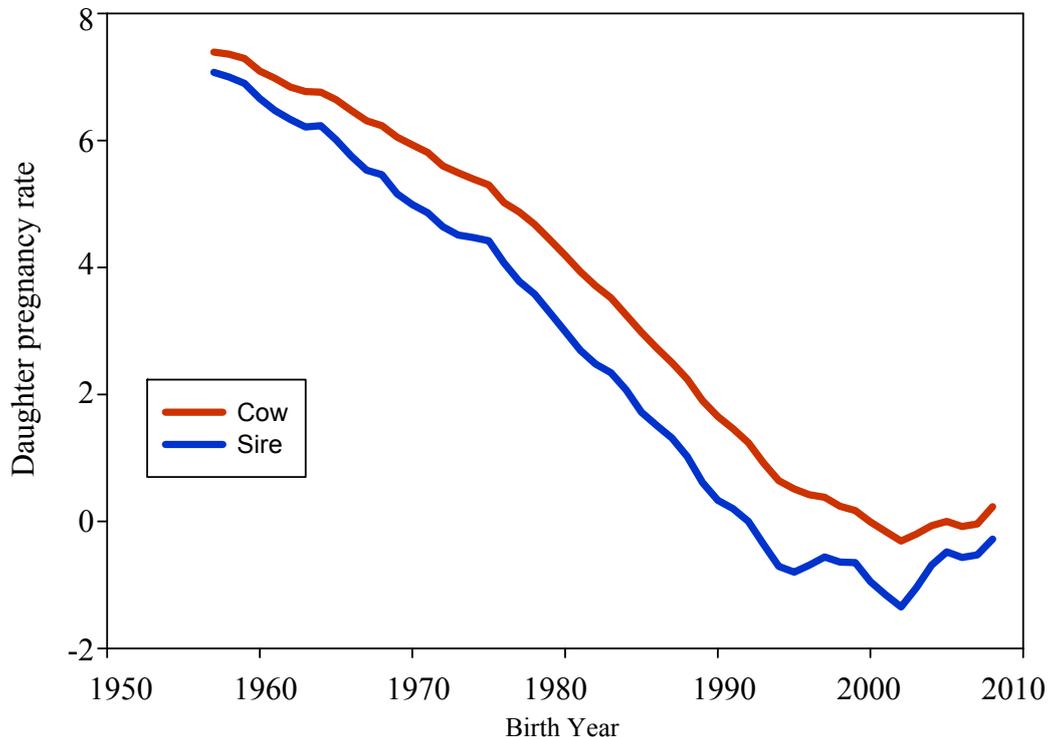


Figure 2. Historical changes in estimated breeding value for daughter pregnancy rate in United States Holsteins from 1957-2008. Data were obtained from United States Dept. of Agriculture Animal Improvement Program Laboratory (<http://aipl.arsusda.gov/eval/summary/trend.cfm>).

Further improvements will be aided by a better understanding of the physiology of the lactating dairy cow. Many excellent reviews have been written about reproductive biology and management of the dairy cow (see for example, Lucy, 2007; Leroy et al., 2008a, b; Robinson et al., 2008;; Santos et al., 2008; Binelli et al., 2009; Sartori et al., 2010; Walsh et al., 2011) and no attempt will be made to recapitulate all the conclusions made in those papers. Poor fertility in dairy cattle involves anovulation, inadequate expression of estrus,

irregular estrous cyclicity and a low probability of pregnancy success after insemination (Lucy, 2007). The approach in this paper will be to examine one crucial aspect of fertility – the establishment and maintenance of pregnancy - and address the question of when and how the events leading to a successful pregnancy after insemination are disrupted. Particular emphasis will be placed on events occurring from the time of ovulation to the beginning of the fetal period (day 42).

### When does pregnancy fail?

Pregnancy failure occurs throughout gestation with the greatest losses occurring early in pregnancy. A graph illustrating the timing of pregnancy failure in the lactating dairy cow is depicted in Fig. 3. The values at different stages of pregnancy, which are approximations only and vary depending on environmental and genetic factors, are based on reports in the literature.

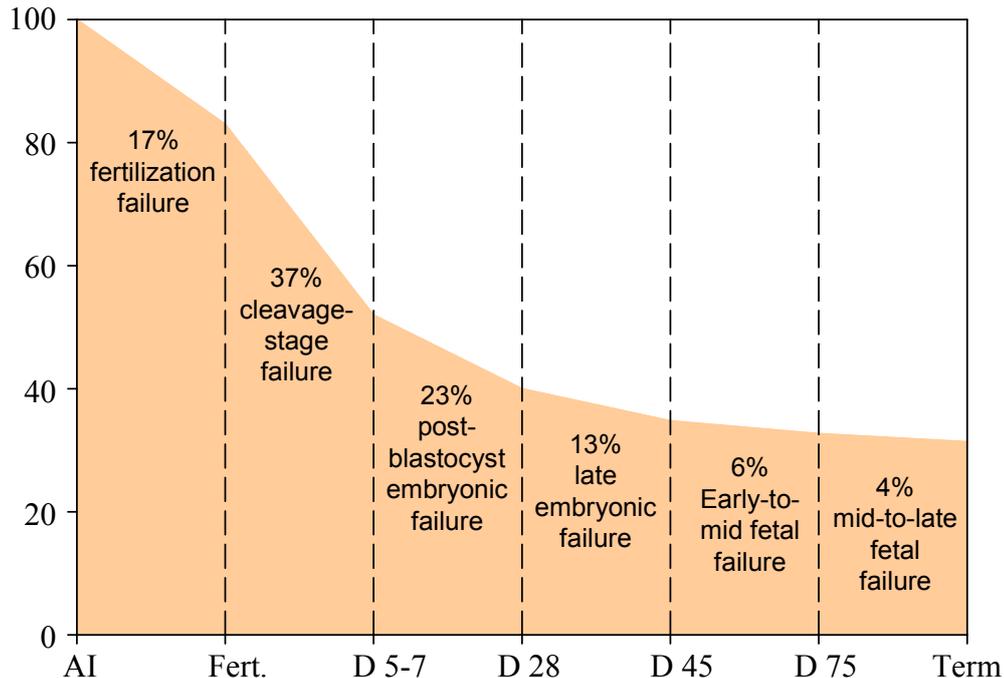


Figure 3. Conceptual representation of pregnancy failure as a function of stage of pregnancy in lactating cattle not subject to heat stress. Note D = day.

The first loss of potential pregnancies results from an insemination that is not accompanied by fertilization of the newly ovulated oocyte. Sartori et al. (2010) used data from papers based on 732 cows to estimate that 83% of oocytes are fertilized in lactating dairy cows after insemination. Fertilization rate appears similar for lactating and non-lactating females (Sartori et al., 2002; Santos et al., 2004).

Using data from papers representing 413 single-ovulating lactating cows, Sartori et al. (2010) estimated that 37% of embryos become non-viable by day 5-7 after insemination. Pregnancy failure during this time is greater for lactating cows than for non-lactating females (Sartori et al., 2002; Rizos et al., 2010).

If we assume, as did Santos et al. (2004), that pregnancy rates at day 28-32 range from 35-45% (average 40%), then an average of about 23% of viable embryos at day 5-7 are lost by day 28-32. One key event during this period is the rescue of the corpus luteum by embryonic secretion of interferon- $\tau$  (IFNT2). There has been much interest in improving pregnancy rate in cattle by facilitating embryonic rescue of the corpus luteum (Robinson et al., 2008; Santos et al., 2008). There are not, however, good estimates of how many pregnancies fail because of inadequate IFNT2 signaling by the embryo. In a study with 78 lactating cows, 25% of cows diagnosed pregnant at Day 18 of gestation based on measurements of expression of interferon-stimulated gene 15 (*ISG15*) in blood cells were not pregnant when diagnosed by ultrasound on Day 32 (Han et al., 2006). Assuming *ISG15* is 100% accurate for pregnancy (a fact not established), it would appear that most of the pregnancy loss from Day 5-7 to Day 28-32 could be accounted by losses after the conceptus had produced sufficient IFNT2 to cause upregulation of peripheral blood cell expression of *ISG15*. Additional studies with *ISG15* or other gene products induced by IFNT2 are needed to confirm this conclusion. However, a very high percentage (71-73%) of inseminated lactating cows have elevated progesterone concentrations in the peripheral blood at Day 21 (Chebel et al., 2003; Han et al., 2006) and this observation would also suggest that many embryos that subsequently die are nonetheless capable of extending lifespan of the corpus luteum.

Based on literature representing data from 4870 cows (Santos et al., 2004), pregnancy losses from day 27-31 to day 38-50 of gestation are about 13%. Pregnancy losses after day 40-50 have been estimated using data from 950 cows as 6.3% to Day 70-80 of gestation and 3.7% thereafter until term (Jousan et al., 2005). Pregnancy failure after day 40-50 of pregnancy is greater for lactating cows than for non-lactating females (Santos et al., 2004; Jousan et al., 2005).

Some late embryonic and fetal loss is likely the result of events occurring much earlier in gestation. For example, treatment of embryos with colony stimulating factor-2 from Day 5-7 reduced the percent of pregnancies diagnosed at Day 30-35 that were lost before term (Loureiro et al., 2009). Similarly, diet affected pregnancy loss between Day 32 and 60 of pregnancy (Silvestre et al., 2011).

### **Why does pregnancy fail – Inherent defects in the embryo or inadequate maternal environment?**

To some degree, the embryo's fate is already determined at the moment of conception by virtue of its inheritance of genes that control development as well as non-genetic contributions from the oocyte and sperm. Thereafter, the extent to which the conceptus can execute its developmental program is determined by the environment of the oviduct and uterus in which the embryo resides, and the systemic maternal physiology which regulates reproductive tract function. There is evidence that embryonic and fetal mortality in the dairy cow is due to inherent defects in the embryo as well as alterations in maternal tract environment.

#### *Genetic inheritance*

Despite the low heritabilities of most reproductive traits, reproductive function is under genetic control. The historical decline in fertility in dairy cattle has been accompanied by a reduction in sire and dam breeding values for daughter pregnancy rate (Fig. 2). In addition, improvements in fertility are possible by selection for quantitative trait loci associated with fertility (Coyral-Castel et al., 2010).

Specific genes that affect embryonic survival are now being identified. Among the genes in which allelic variants affecting development of embryos in vitro are *STAT5A*, *FGF2*, *GHR*, *PGR*, *PRLR*, and *SERPINA14* (Khatib et al., 2008a, b, 2009; Driver et al., 2009). There is a report that alleles for *FGF2* and *STAT5A* are also associated with bull fertility (Khatib et al., 2010) but Oikonomou et al. (2011) failed to find an effect of allelic variation in *FGF2* or *STAT5A* on female fertility in vivo.

It is possible that selection for milk yield has resulted in inadvertent selection for alleles that also compromise reproductive function although the genetic relationships between production and reproductive traits have not been characterized well. Pimentel et al. (2010) found 16 single nucleotide polymorphisms (SNPs) that were related to both production and fertility traits. In 10 cases, relationships were antagonistic. Nonetheless, SNPs were found in which there were positive effects on production and fertility and so selection for fertility without compromising milk yield is theoretically possible.

#### *Oocyte quality*

Evidence from experiments where oocytes were collected from lactating cows or nulliparous heifers and then either chemically activated or fertilized does not support the idea that lactation is associated with reduced competence of the oocyte to be fertilized or to become an embryo capable of developing to the blastocyst stage. Thus, Rizos et al. (2005) found no difference between oocytes from nulliparous heifers vs early postpartum cows in fertilization or blastocyst production rates when used in an in vitro fertilization system and Roth et al. (2008) found no difference between nulliparous heifers vs lactating cows in oocyte morphology or development in vitro after chemical activation. Caution must be taken when interpreting results from these papers, which involved examination of oocytes from a limited numbers of animals and follicles at various stages of growth. It is possible that the oocyte from the preovulatory follicle is compromised by lactation even though most oocytes are not. Indeed, effects of protein content of the diet on oocyte competence in heifers depended on the type of follicle from which the oocyte was derived (Sinclair et al., 2000) and other environmental effects on oocyte quality may depend on the physiological status of the follicle.

#### *Damage to sperm as a cause of infertility*

The fact that fertilization rates in lactating cows appear similar to that of heifers (Sartori et al., 2010) would argue that sperm are not being preferentially damaged in the reproductive tract of the lactating cow. However, sperm damage can affect not only fertilization rate but competence of the resulting embryo to develop to the blastocyst stage (Brocas et al., 1997; Silva et al., 2007; Hendricks and Hansen, 2010). Sperm survival in vivo depends upon interactions with the oviductal epithelium (Pollard et al., 1991) and, as shown in the sheep (Hawk, 1983), is under endocrine control. Accordingly, the effect of lactation on changes in the sperm before fertilization warrant additional investigation.

### *Alterations in maternal function*

There are two lines of evidence that the oviductal or uterine environment is degraded during lactation. The first are the results of experiments in which embryos were transferred at ~ day 7 after estrus to lactating cows, heifers or dry cows. Except for one study (Putney et al., 1988), pregnancy rates were lower when recipients were lactating (Hasler, 2001; Chagas e Silva et al., 2002; Wilson et al., 2006). The reproductive tract appears to be suboptimal as early as the period from Day 2-7 after estrus. Rizos et al. (2010) transferred groups of embryos into the oviduct at day 2 and found that lactating cows experienced lower embryo recovery at Day 7 and a lower proportion of recovered embryos that developed to the blastocyst stage than for heifers.

### **Some physiological determinants of fertility**

#### *Progesterone concentrations*

Circulating concentrations of progesterone are higher in nulliparous heifers as compared to lactating cows (Sartori et al., 2004; Wolfenson et al., 2004; Rizos et al., 2010). In part, this physiological difference reflects increased post-prandial metabolism of progesterone by the liver (Sangsritavong et al., 2002; Vasconcelos et al., 2003). The corpus luteum is also different at the molecular level between lactating cows and nulliparous heifers (Pretheeban et al., 2010).

The reduction in circulating progesterone concentrations is likely to be one cause of reduced fertility in lactating dairy cows. Cows with higher progesterone concentrations after insemination have been reported to be more fertile (Butler et al., 1996; Stronge et al., 2005; Demetrio et al., 2007) and cows with a double ovulation had higher progesterone concentrations and tended to be more fertile even when used as embryo transfer recipients (Sartori et al., 2006).

Effects of progesterone are probably mediated directly on the reproductive tract even though the embryo expresses progesterone receptors throughout early development to the blastocyst stage (Clemente et al., 2009). Treatment of in vitro produced embryos with progesterone in vitro did not affect development or gene expression in vitro or development after transfer to recipients (Clemente et al., 2009; Larson et al., 2011). However, treatment of recipients with progesterone beginning at Day 2 altered gene expression in embryos recovered at Day 7 of pregnancy (Carter et al., 2010). Progesterone treatment can also cause extensive changes in the transcriptome of the endometrium (Forde et al., 2009, 2011).

Hastening the post-ovulatory rise of progesterone can enhance conceptus elongation at day 13-16 of pregnancy (Garrett et al., 1988; Carter et al., 2008). This action of progesterone is probably exerted at the level of the uterus because embryos transferred into uteri of recipients treated with a progesterone intravaginal releasing device beginning on day 3 of pregnancy had elongated to a greater extent at day 14 as compared to embryos for control cows (Clemente et al., 2009).

Despite the relationship between progesterone concentrations and pregnancy success, efforts to improve fertility in cattle by modifying progesterone concentrations after ovulation have met with mixed success. Treatment with CIDRs have generally not caused an improvement in fertility (Mann et al., 2001; Rhodes et al., 2001; Sterry et al., 2006; Arndt et al., 2009), perhaps because treatment was initiated too late after ovulation (Day 5-10) or because the increase in circulating progesterone concentrations caused by CIDRs is too low to improve fertility. An alternative way to increase progesterone secretion is through administration of human chorionic gonadotropin (hCG) at days 5-7 after insemination. Injection of 3000-3300 IU hCG at Day 5 increases circulating progesterone concentrations by increasing the size of the preexisting corpus luteum and causing formation of an accessory CL (Santos et al., 2001). As summarized in Table 1, administration of hCG at day 5-7 after to lactating cows either caused a significant increase in pregnancy rate per insemination (Santos et al., 2001; Shabenkareh et al., 2010), a numerically-higher pregnancy rate that was not statistically significant [Schmitt et al., 1996 (autumn); Vasconcelos et al., 2011] or no beneficial effect [Schmitt et al., 1996 (summer); Hanlon et al., 2005; Shams-Esfandabadi et al., 2007].

#### *Follicular origin*

Prolonged dominance of the follicle can compromise fertility. Thus, pregnancy rate per AI was higher in cows with three wave follicles (Townson et al., 2002). In addition, reducing the period of dominance in a timed AI program from 10 to 8 days by reducing the interval between first GnRH injection to prostaglandin injection from 7 to 5 days improved pregnancy rate per AI.

Ovulation of the second-wave dominant follicle resulted in a higher pregnancy rate in a timed AI program than ovulation of the first-wave dominant follicle (Bisinotto et al., 2010). Similarly, embryo quality following superovulation initiated during the second follicular wave was higher than for females subjected to superovulation during the first follicular wave (Rivera et al., 2011). For both AI and superovulation, differences in pregnancy success are due to differences in progesterone concentrations during follicular growth since

supplementation of cows with progesterone during the first follicular wave tended to improve pregnancy rate per insemination (Denicol et al., 2009) and improved embryo quality (Rivera et al., 2011).

Table 1. Effectiveness of administration of human chorionic gonadotropin at Day 5 after breeding on pregnancy rate in lactating dairy cows.

Day of hCG treatment after AI	Time of pregnancy diagnosis (days after insemination)	Pregnancy rate per insemination <sup>a</sup>			Notes	Reference
		Control	hCG	P		
5-6	45-60	13% (5/39)	9% (4/43)	N.S. <sup>b</sup>	Florida, summer	Schmitt et al., 1996
5-6	45-60	30% (19/63)	36% (20/56)	N.S.	Florida, autumn	Schmitt et al., 1996
5	90	32% (65/203)	38% (78/203)	0.008	California	Santos et al., 2001
5	Six weeks after end of AI breeding season	46% (82/177)	44% (78/179)	N.S.	New Zealand, grazing dairy	Hanlon et al., 2005
5	45-50	35% (31/88)	27% (19/70)		Pakistan	Shams-Esfandabadi et al., 2007
5	40-45	30% (142/329)	43% (102/228)	0.001	Iran	Shabenkareh et al., 2010
7	60	26% (40/126)	30% (45/151)	N.S.	Brazil	Vasconcelos et al., 2011

<sup>a</sup>Data are percent pregnant and, in parentheses, the number of cows pregnant/total number of cows.

<sup>b</sup>N.S. = non-significant.

The mechanism by which the presence of progesterone during follicular growth improves fertility is not known. One possibility is that progesterone alters folliculogenesis. Ovulatory follicles were smaller for follicles ovulated during the second follicular wave as compared to those ovulated during the first follicular wave (Bisinotto et al., 2010). There is also evidence that luteolysis in the postovulatory period is affected by progesterone concentrations during growth of the ovulatory follicle. Ovulation of a follicle that grew in the presence of high concentrations of progesterone was associated with reduced prostaglandin  $F_{2\alpha}$  release from endometrium (Shaham-Albalancy et al., 2001) and increased incidence of premature luteolysis (Bisinotto et al., 2010). Differences in fertility are unlikely to represent differences in post-ovulatory progesterone concentrations (Wolfenson et al., 1999).

#### Diet

Pregnancy rate per AI can be compromised by negative energy balance: the probability for first service conception increased as the average energy balance in the first 28 days after calving increased (Patton et al., 2007). Energy status may affect fertility by altering oocyte function because fertilization rate of oocytes *in vitro* was reduced in cows with low body condition (Snijders et al., 2000).

Recently, it has been proposed that manipulation of the fatty acid composition of the diet can affect pregnancy success after insemination. The idea is to feed diets high in n-6 fatty acids during late gestation and early lactation, to increase uterine prostaglandin production and inflammatory responses and facilitate removal of microorganisms in the reproductive tract, and then change the diet to one enriched in n-3 fatty acids, to suppress uterine prostaglandin synthesis at a time when the embryo must prevent luteolysis (Santos et al., 2008). Such an approach has sometimes yielded promising outcomes. In the best test of this hypothesis, cows were fed one of two diets from ~14 days before calving until Day 30 after calving (Silvestre et al., 2011). Diets included calcium salts of either palm oil (low in n-6 and n-3 fatty acids) or safflower oil (high in n-6 fatty acids). Beginning at Day 30, diets were switched to contain calcium salts of either palm oil or fish oil (high in n-3 fatty acids). There was no effect of diet on first service pregnancy rate at 84 days postpartum but diet did affect pregnancy rate for second service. The highest pregnancy rate (41.3%) was for cows receiving an n-6 enriched diet early postpartum (safflower oil) and n-6 enriched diet (fish oil). Values were intermediate for cows receiving palm oil/fish oil (27.3%) and lower for cows receiving either safflower oil/palm oil (22.5%) or palm oil/palm oil (21.3%). The reason why treatment affected second service fertility while not affecting first service fertility is not known but could be related to duration of feeding.

#### Synopsis

Inadequate fertility remains a limitation to achieving optimal efficiency of milk production in many dairy herds throughout the world. Nonetheless, progress in improving reproductive function of the lactating cow is being made and the historical decline in reproductive function is beginning to be reversed (Fig. 1 and 2). Indeed, first service pregnancy rates per AI in lactating cows can be 40% or more when optimized ovulation synchronization protocols are implemented (Bisinotto et al., 2010; Thompson et al., 2010). Additional improvements in dairy cattle reproduction will be achieved as new knowledge about the reproductive biology of



the cow is used to develop improved reproductive management schemes that manipulate genetics, physiology and nutrition of the cow to improve fertility.

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