

# Follicle growth, corpus luteum function and their effects on embryo development in postpartum dairy cows

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Absent or irregular ovarian cycles in lactating dairy cows are caused by failure to ovulate the dominant follicle at the appropriate time. The follicle then either regresses or develops into a cyst. This process can be triggered by a variety of metabolic and disease factors that act at the hypothalamus and pituitary gland to inhibit pulsatile LH secretion and the LH surge, and at the ovary to reduce follicular growth and oestradiol production. Cows of poor energy status have low circulating concentrations of insulin-like growth factor I (IGF-I). Predisposing factors include calving difficulties, inappropriate diet, reduced intake of dry matter and a high rate of body condition score loss. Various stressors predispose the follicle to cyst development by inhibiting the LH surge and ovulation; these include common infections, such as mastitis. Even when ovulation does occur, poor follicular development may result in production of an inadequate corpus luteum. The timing of the increase in progesterone in the early luteal phase (days 4–5) appears to be a key determinant of fertility, probably because it alters the secretory activity of the reproductive tract, thus influencing embryonic growth and interferon- $\tau$  production. A period of negative energy balance after calving can reduce fertility even though metabolic parameters have apparently improved at the time of service.

## Introduction

Failure to ovulate occurs under two scenarios in the postpartum dairy cow. After calving, a succession of follicles may grow and regress before one follicle reaches sufficient size and produces sufficient oestradiol to trigger an LH surge and ovulation (Roche and Diskin, 2001). The second situation occurs later in the postpartum period when some cows fail to re-ovulate after an initial resumption of cyclicity (Bulman and Wood, 1980; Huszenicza *et al.*, 1988). This situation may lead to an extended inter-luteal interval and in extreme cases the cow reverts to a prolonged anoestrous period. These two conditions have been termed delayed ovulation

type 1 and 2 (DOV1 and DOV2), respectively. Several recent studies have investigated the sequential chain of events that occur within an individual follicle as it achieves dominance or undergoes atresia (for a review, see Bao and Garverick, 1998). The consensus is that the key regulators after calving are an increasing LH pulse frequency acting in synergy with an adequate concentration of the growth factors insulin and insulin-like growth factor I (IGF-I) (Butler, 2001; Roche and Diskin, 2001).

Until recently, little was known about the relationship between pre-ovulatory follicle development and subsequent luteal function after ovulation in naturally cyclic animals, although previous studies in cattle have shown that follicles destined to form short-cycle corpora lutea show reduced oestradiol secretion and have fewer LH receptors (Inskeep *et al.*, 1988). However, early work using embryo transfer and steroid-treated ovariectomized ewes demonstrated that the successful development of an embryo is dependent on adequate luteal function promoting synchronous development of the reproductive tract (for a review, see Wathes, 1992).

In this paper, the factors that influence the interval to first ovulation and the adequacy of luteal function in the dairy cow are reviewed, and particular emphasis is placed on potential metabolic signals that may influence ovarian activity.

## Endocrine control of follicular growth

### *Gonadotrophins*

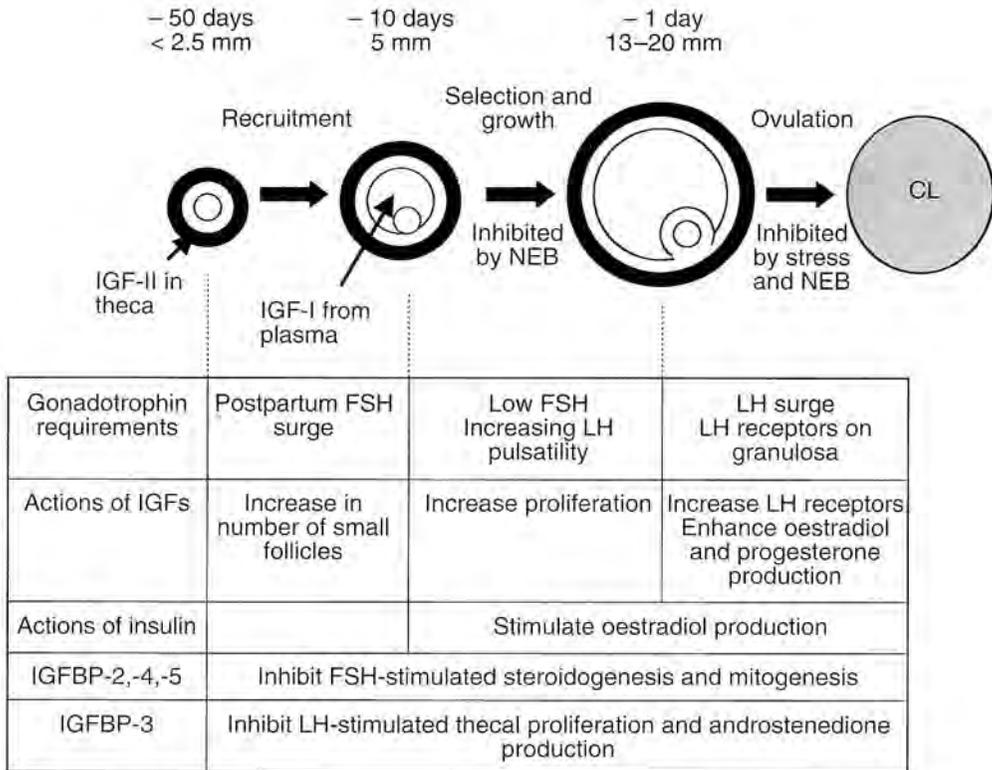
A transient increase in FSH at days 2–3 after parturition is followed by the emergence of a cohort of about three to six follicles of 4–6 mm in diameter; by day 10, one of these follicles will have achieved dominance (Roche and Diskin, 2001). LH pulse frequency increases during the first 2 weeks after parturition and is higher in cows that ovulate their first dominant follicle in comparison with cows that do not (Beam and Butler, 1999). The LH pulse rate is reduced in cows that have not yet reached their nadir in negative energy balance, and in cows with hypoglycaemia or higher milk production (for a review, see Butler, 2001). The ruminant pituitary gland contains high concentrations of the type 1 IGF receptor (IGF-1R) and can respond to exogenous IGF-I by increasing LH release (Adam *et al.*, 2000). The IGF-binding proteins (IGFBP-2, -3 and -5) are also present in the pituitary gland, and their concentration varies according to nutritional status (for data on ewes, see Snyder *et al.*, 1999). These data indicate a direct inhibitory effect of adverse metabolic signals on the LH pulse generator, and that circulating IGF-I, modulated by IGFBPs, may alter the sensitivity of the pituitary gland to both luteinising hormone releasing hormone (LHRH) and to oestradiol feedback.

### *Insulin and IGFs*

The bovine ovary contains specific insulin and IGF receptors but, at supraphysiological concentrations, insulin can also act through the IGF-1R (Spicer and Echtenkamp, 1995). Summarising data from a number of studies the following points emerge (Fig. 1).

(1) Although low expression of IGF-I mRNA may be present in some follicles, several lines of evidence show that the IGF-I measured in bovine follicular fluid is mainly, if not totally, derived from the circulation. The concentration of IGF-I in follicular fluid increases with increasing follicular diameter and is highly correlated with the plasma IGF-I concentration (Spicer and Echtenkamp, 1995; Funston *et al.*, 1996; Perks *et al.*, 1999).

(2) IGF-II mRNA is localized to the theca. Its concentration is higher in small (< 5 mm diameter) follicles and its expression is greater in healthy follicles than in atretic small follicles (Perks *et al.*, 1999).



**Fig. 1.** Actions of insulin-like growth factors I and II (IGF-I and IGF-II) and insulin during different phases of follicular development in cows. NEB: negative energy balance. CL: corpus luteum. IGFBP: insulin-like growth factor binding proteins. Data from Spicer and Echternkamp (1995); Armstrong *et al.* (1996); Funston *et al.* (1996); Bao and Garverick (1998); Perks *et al.* (1999); Austin *et al.* (2001).

(3) IGF-1R are present on both theca and granulosa. Their concentration is also higher in healthy follicles than in small atretic (< 5 mm) follicles and can be increased by epidermal growth factor, oestradiol and FSH (Spicer and Echternkamp, 1995; Perks *et al.*, 1999).

(4) The lower MW IGFBPs (-2, -4 and -5) are synthesized in the follicle wall and their concentration in follicular fluid decreases as the follicles grow and increases as they become atretic, thus regulating the bioavailability of free IGF within the follicle. Exogenous addition of IGFBPs *in vitro* can inhibit FSH stimulated steroidogenesis and mitogenesis in granulosa cells (Spicer and Echternkamp, 1995; Armstrong *et al.*, 1996; Bao and Garverick, 1998; Austin *et al.*, 2001).

(5) IGFBP-3 is also present in follicular fluid, but the main source is probably the circulation. IGFBP-3 can inhibit IGF-I-stimulated thecal proliferation and androstenedione production (Spicer and Echternkamp, 1995; Armstrong *et al.*, 1996).

Although IGF-II is the main locally produced growth factor within the bovine ovary, there is little information available on its role. However, the different IGFBPs present in the follicle have different affinities for IGF-I and -II, which may be physiologically important. IGFBP-2 and -5 both bind IGF-II with a greater affinity than IGF-I, whereas the affinities of IGFBP-3 and -4 for the two peptides are similar (Rajaram *et al.*, 1997). IGFBP-2 and -3 are also nutritionally sensitive, and concentrations of circulating IGFBP-2 increase and IGFBP-3 decrease in response to dietary restriction (Rajaram *et al.*, 1997). After parturition in beef cows

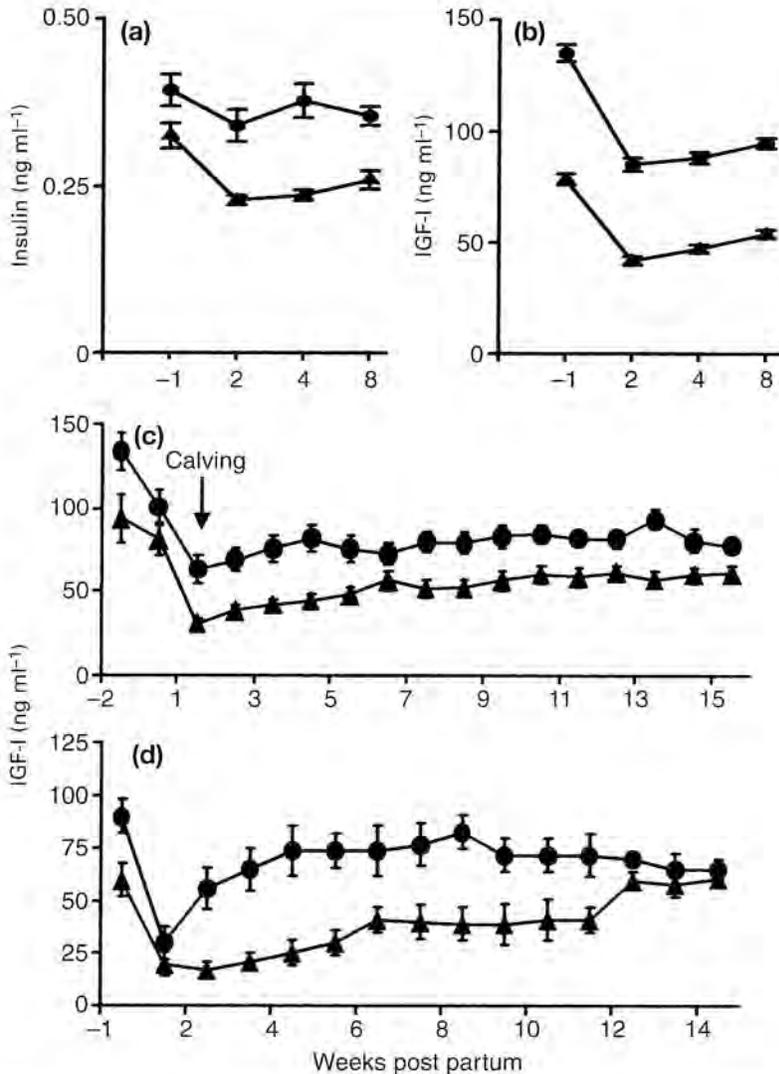
circulating IGFBP-2 at week 2 was higher and IGFBP-3 was lower in cows that remained anoestrus in comparison with cows that resumed cyclicity earlier (Roberts *et al.*, 1997). Thus, it appears that the ability of follicles to grow and achieve dominance is regulated by both intrafollicular and endocrine IGF systems, which can be modified in response to nutritional status.

Although IGF-I and insulin have some functional overlap, there is considerable evidence that they have separate actions. Spicer and Echternkamp (1995) compared their effects on the ovary and they concluded that IGF-I was more mitogenic and that this effect was enhanced by gonadotrophin support in small (< 5 mm), but not in larger follicles. Treatment of cows with bovine somatotrophin, which increases circulating IGF-I, stimulated the growth of small (< 5 mm) follicles and the synergistic action of IGF-I with FSH on granulosa cell proliferation was found only on cells from small follicles (Gong *et al.*, 1993). Both insulin and IGF-I enhanced LH stimulated androgen production by the theca. Insulin at physiological concentrations of  $\leq 10$  ng ml<sup>-1</sup> had the more potent effect on oestradiol production *in vitro*, although there was some stimulation of oestradiol production by IGF-I in large (> 8 mm), but not in small follicles. Although the concentration of oestradiol and IGF-I in follicular fluid both increase as follicles grow, there is little correlation in their concentrations in individual follicles (Spicer and Echternkamp, 1995). Collectively, these data indicate that the growth and oestradiol production of bovine follicles are regulated separately: locally produced IGF-II and systemic IGF-I are mainly responsible for proliferation, particularly of small follicles, whereas insulin is the primary regulator of oestradiol production. Therefore, both factors are required for ovulation (Fig. 1).

It is pertinent to examine the profiles of insulin and IGF-I during this period to understand why some dominant follicles do not ovulate in the postpartum cow. Concentrations of insulin and IGF-I in cows were compared between first lactation and older cows (Fig. 2a,b) and between average and high yielding cows (Fig. 2c). In agreement with other studies (for example, see Butler, 2001), a negative relationship was found between the IGF-I concentration after calving and the interval to the resumption of ovarian cyclicity (Fig. 2d). The trend was similar in multiparous and primiparous cows although IGF-I concentrations were consistently much higher in the younger animals (Fig. 2b). Older cows with either DOV1 or DOV2 had similar insulin values to those in normally cyclic cows but acyclic primiparous cows showed a trend towards lower insulin concentrations ( $P = 0.09$ , V. J. Taylor and D. C. Wathes, unpublished). In some situations, cows may have adequate insulin to promote oestradiol production, but insufficient IGF-I for proliferation; this could lead to the ovulation of a small follicle, resulting in the production of an undersize corpus luteum.

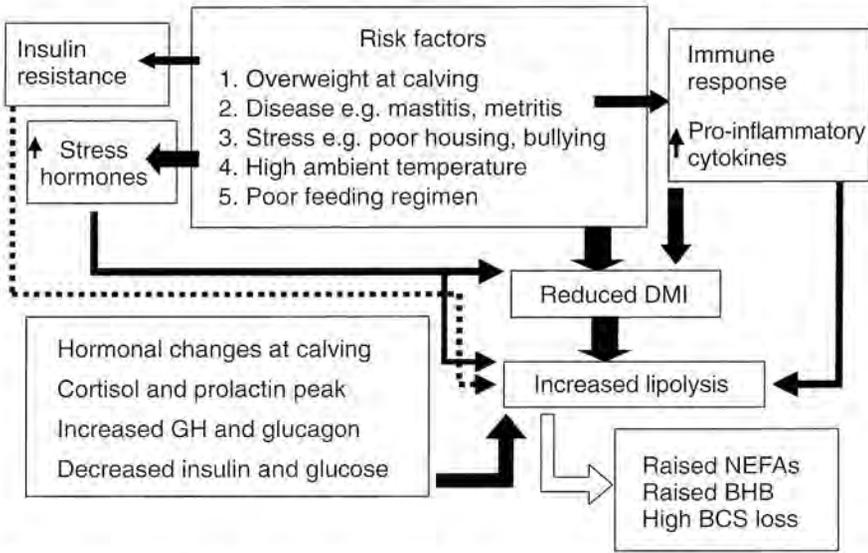
### Leptin

Leptin is a 16 kDa protein produced primarily by adipose cells, although leptin mRNA has been detected in other tissues including the liver and placenta. The circulating leptin concentration shows a positive correlation with the size of the fat depot and also varies with nutritional status, as leptin mRNA expression decreases during fasting (Tsuchiya *et al.*, 1998). Leptin receptors have been identified in both the arcuate nucleus and median eminence of sheep (Keisler *et al.*, 1999) and in bovine ovarian granulosa cells (Spicer, 2001a). In the hypothalamus leptin decreases appetite, thus feed intake is stimulated when leptin concentrations are low. In cattle, leptin concentrations show a marked decrease at about the time of parturition (Kadokawa *et al.*, 2000; Block *et al.*, 2001). In lactating cows, the period of negative energy balance after calving has been linked to reduced leptin concentrations (Kadokawa *et al.*, 2000; Block *et al.*, 2001) and decreased leptin has been linked



**Fig. 2.** Plasma concentrations of (a) insulin and (b–d) insulin-like growth factor I (IGF-I) before and after calving in lactating dairy cows. (a,b) Insulin and IGF-I measured in multiparous (▲,  $n = 160$ ) and primiparous (●,  $n = 158$ ) cows. (c) IGF-I in average genetic merit (●,  $8047 \pm 300$  kg per 305 day lactation,  $n = 20$ ) and high genetic merit (▲,  $10573 \pm 245$  kg per 305 day lactation,  $n = 28$ ) cows. (d) IGF-I in multiparous cows with normal ovarian activity post partum (●,  $n = 27$ ) or in cows with a delayed ovulation type 1 profile (▲,  $n = 6$ ). All values are mean  $\pm$  SEM. Note that both insulin and IGF-I concentrations were significantly higher in the first lactation than in older cows. IGF-I concentrations were significantly lower in high yielding cows and in cows with a delayed start to ovarian activity.

to delayed resumption of cyclicity (Kadokawa *et al.*, 2000). Furthermore, once reproductive cyclicity has been re-established, the occurrence of cycle problems is associated with reduced plasma leptin concentrations (G. E. Mann and D. Blache, unpublished). Therefore, leptin represents another potential metabolic signal linking body condition to reproductive potential.



**Fig. 3.** Hormonal and metabolic changes in periparturient cows associated with high tissue mobilization and loss of body condition which predispose cows to poor fertility. BCS: body condition score; DMI: dry matter intake; GH: growth hormone; NEFA: non-esterified fatty acids; BHB:  $\beta$ -hydroxybutyrate.

## Metabolic signals and ovarian cyclicity

### Background

Dairy cows enter a period of negative energy balance in early lactation, which may last up to 20 weeks in high yielding cows. The shortfall in energy intake is met by extensive mobilization of body tissue. Cows in negative energy balance release non-esterified fatty acids (NEFAs) from fat as an energy source and ketone bodies (acetone, acetoacetate and  $\beta$ -hydroxybutyrate) are produced by the liver as the fatty acids are metabolized. Therefore, circulating concentrations of both NEFA and  $\beta$ -hydroxybutyrate increase after calving to an extent that is related to the degree of fat mobilization (Fig. 3).

The constituents of the lactational diet also influence a number of circulating metabolites which may signal to the reproductive system. These relationships are complex (NRC, 1989) and can be summarized only briefly here. If the balance of concentrate to forage is excessive, cows may develop acidosis, leading to a reduction in dry matter intake (DMI) and a consequent worsening of the negative energy balance. Dairy cow diets typically contain a high crude protein concentration. The rumen degradable component is broken down during rumen fermentation, releasing ammonia which is used for microbial protein synthesis, a process requiring energy. If excess effective rumen degradable protein is fed in relation to the available fermentable metabolizable energy in the diet, then ammonia production will increase. Ammonia must be detoxified to urea by the liver, so urea concentrations increase. The detoxification procedure requires energy, thus exacerbating the negative energy balance (Laven and Drew, 1999).

Fats are included in the lactational diet as an energy source and both the type and amount may be important. Excess fat can depress appetite and, thus, reduce the DMI. The proportion of unsaturated and polyunsaturated fats (PUFAs) that the diet contains also varies. The n-6 PUFAs

are derived from linoleic acid (LA, 18:2, n-6) and predominate in plant oils (for example, soyabean). The n-3 PUFAs are derived from linoleic acid (LNA, 18:3, n-3), which is present in green leaf forages and linseed, and include the longer chain PUFAs eicosapentaenoic acid (20:5, n-3) and docosahexaenoic acid (22:6, n-3), which are constituents of fish oil. As well as providing energy, PUFAs act as precursor molecules for prostaglandin synthesis and the ratio of different types of PUFA in cell membranes can alter the amount and type of prostaglandins produced (for reviews, see Abayasekara and Wathes, 1999; Mattos *et al.*, 2000). Arachidonic acid (AA, 20:2, n-6) has also been implicated in steroidogenesis by increasing the expression of steroidogenic acute regulatory protein (StAR), which mediates the transfer of cholesterol from the cytosol to the inner mitochondrial membrane (Wang and Stocco, 1999).

### *Body condition score and ovulation*

The change in body condition score (BCS) after calving provides a subjective estimate of the energy shortfall in an individual cow. Previous studies have demonstrated a negative relationship between the BCS loss in the early postpartum period and the interval to first ovulation (Beam and Butler, 1999; Wathes *et al.*, 2001a). There is also a clear effect of both the BCS at calving and the BCS loss on the interval to conception (see below). Both first lactation and older cows with a high BCS loss had low IGF-I, increased  $\beta$ -hydroxybutyrate and NEFA concentrations in the early postpartum period (up to 5 weeks) and in the first lactation insulin concentrations were also lower (D. C. Wathes, V. J. Taylor and Z. Cheng, unpublished).

### *NEFAs and $\beta$ -hydroxybutyrate*

Dairy cows with both DOV1 and DOV2 profiles had significantly higher concentrations of NEFA and  $\beta$ -hydroxybutyrate at weeks 1–5 after parturition than normal cyclic cows (Huszenicza *et al.*, 1988). Kruip *et al.* (2001) developed an over-conditioned late pregnant cow model in which animals experienced severe negative energy balance after birth. There were significant relationships between blood NEFA, the liver triacylglycerol concentration and the interval to first ovulation. Primiparous cows with DOV1 also had a higher output of energy into milk (measured in MJ per week) (V. J. Taylor, unpublished). Increased milk fat has previously been noted in ketotic cows, presumably because of the increased availability of  $\beta$ -hydroxybutyrate and fatty acids for milk fat synthesis.

### *Lipids*

Increasing the amount of dietary fat increases both the number of small follicles and the size of larger follicles in the bovine ovary, an effect that is independent of energy supply as it occurs with isocaloric diets (for a review, see Staples *et al.*, 1998). Suggested mediators have included insulin, IGF-I and cholesterol, but metabolic hormone concentrations have not changed consistently in studies in which follicular parameters have altered. First lactation cows were fed diets varying in their PUFA content (Robinson *et al.*, 2002). The high n-6 diet (LA) increased circulating cholesterol and IGF-I concentrations, but did not influence the size or number of follicles, whereas the high n-3 diet (LNA) caused an increase in the number, size, and oestradiol production of follicles, but did not alter either cholesterol or IGF-I. Insulin was unchanged by either diet. The mechanism of action is thus far from clear, but might involve altered follicular prostaglandin production; both PGE<sub>2</sub>

and  $\text{PGF}_{2\alpha}$  have been implicated in the mechanisms controlling follicular blood flow and rupture (see below). A significantly more variable interval in the time from the LH surge to ovulation was found in cows on a high n-6 diet (control, 21–24 h; n-6, 18–44 h), indicating a possible influence on ovulation which warrants further investigation (Robinson *et al.*, 2002).

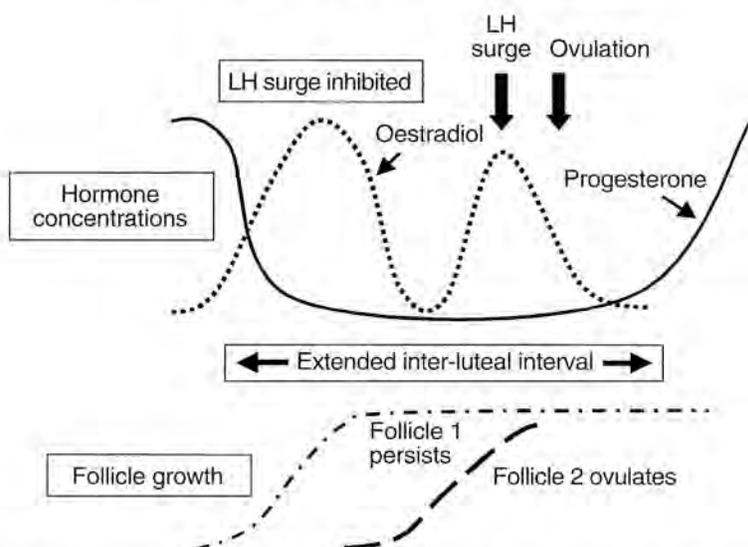
### *Other factors influencing the interval to first ovulation*

Yield, age of cow and season of calving have all been shown to influence the interval to first ovulation in some, but not all, studies. This variability is not surprising as the effects are often interdependent and are influenced by management. First lactation cows produce less milk, but they also eat less and are still growing and, therefore, their energy balance is different from that of older cows (Lucy, 2001). In a study of 400 cows monitored with milk progesterone profiles, half of which were in their first lactation, a significant positive relationship between the interval to first ovulation and both peak and total milk yield was found when first lactation and older cows were assessed separately (D. C. Wathes, Z. Cheng and V. J. Taylor, unpublished). In a previous study in the UK, a seasonal effect and a longer interval to first ovulation was found in cows calving from March to May (Bulman and Lamming, 1978). It was not clear whether this increase was due to altered nutrition at different times of the year (for example, due to spring turn out to grass) or to a direct effect of photoperiod. In hotter climates, summer infertility due to heat stress is a well-recognized phenomenon (al-Katanani *et al.*, 1999). It is likely that in all cases the prime factor determining when an individual cow first ovulates is related to energy balance as discussed above, and this is influenced by diet, yield, age and season in different studies.

## **Ovulation and cyst development**

In dairy cows, follicles generally ovulate after they reach a maximum diameter of 13–20 mm at an interval of 24–48 h after an LH surge. The LH surge is responsible for differentiation of the follicular cells, which includes the switch from oestradiol to progesterone production (for a review, see Juengel and Niswender, 1999). The LH surge also activates an inflammatory reaction involving both hyperaemia and collagen degradation, which are mediated by increasing production of  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$ , respectively, and which are responsible for the thinning and eventual rupture of the follicle wall (Espey, 1980).

During the period after parturition, a number of dominant follicles fail to ovulate at the appropriate time but persist for  $\geq 10$  days. The reported incidence of dominant follicles failing to ovulate is between 6 and 19% (Hooijer *et al.*, 2001; Silvia *et al.*, 2002). There is increasing evidence that the reason for the failure to ovulate is stress-induced inhibition of oestradiol positive feedback to the hypothalamus and pituitary gland, preventing the LH surge (Fig. 4). Stress induced by transportation delayed the onset of the LH surge and reduced its maximum concentration in ewes (Dobson and Smith, 1998). Cows treated with either ACTH or low dose progesterone, which both mimic stress-induced endocrine changes, developed persistent dominant follicles (Noble *et al.*, 2000; Silvia *et al.*, 2002). Ovulation can be inhibited and cyst development promoted in cows that develop mastitis or endometritis. These infections are accompanied by increased cortisol concentrations (Hockett *et al.*, 2000) and extended follicular phases (Huszenicza *et al.*, 1998). The relationship with yield is equivocal: some studies report a link to higher yield (for example, see Hooijer *et al.*, 2001), whereas others do not. In contrast, Nanda *et al.* (1989) reported that cysts developed shortly after an unexpected fall in yield, which could



**Fig. 4.** Proposed events leading to extended inter-luteal intervals and persistent follicles (cysts) in postpartum cows. The LH surge may be inhibited at the hypothalamus if the cow is subjected to stress or develops an infection during the follicular phase, despite an adequate production of oestradiol by the dominant follicle. This follicle then fails to ovulate but persists structurally. When oestradiol production by the first follicle decreases another follicle is selected and grows. After the period of stress has finished, this follicle can initiate an LH surge and ovulate.

have been indicative of some environmental factor (for example, change in diet) causing both events.

## Development of the corpus luteum

### *Links between the pre-ovulatory follicle and subsequent corpora lutea*

When judging the adequacy of the corpus luteum, the interval from ovulation to the progesterone increase, the maximum progesterone concentration reached and the duration of the luteal phase must all be considered. In a population of naturally cyclic cows selected as 'at risk' of exhibiting a delayed progesterone increase after ovulation, such delays were associated with poor oestradiol secretion during the follicular phase leading up to ovulation (Starbuck *et al.*, 2000), but this did not occur in a population of cows exhibiting normal post-ovulatory progesterone increases after ovulation (G. E. Mann, E. C. L. Bleach, G. R. Starbuck and M. D. Fray, unpublished). However, in these cows, the mean plasma concentration of progesterone from day 4 to day 6 after ovulation increased significantly with increasing diameter of the ovulatory follicle and decreased with increasing time from luteolysis to ovulation. These data indicate a link between steroidogenic function in the preovulatory follicle and the post-ovulatory corpus luteum and demonstrate that increased progesterone secretion by the developing corpus luteum is related to the ovulation of a larger follicle that took less time to mature. Progesterone production during the early luteal phase is also dependent on the amount of gonadotrophin support. Inadequate gonadotrophin production after ovulation in cows is accompanied by a marked reduction in LH pulse frequency on day 5 (Starbuck *et al.*, 2000).

### *Nutritional influences on luteal function*

The effects of nutrition on progesterone concentrations in cows are equivocal. In sheep, there is a strong negative correlation between dietary intake and progesterone concentrations (O'Callaghan *et al.*, 2001). However, data from heifers are inconsistent as *ad libitum* feeding has been reported to increase, decrease or to have no effect on progesterone concentrations (for a review, see O'Callaghan *et al.*, 2001). In cows, a negative correlation between intake of dietary crude protein and circulating progesterone has been reported in lactating, but not in non-lactating, cows (for a review, see Butler, 1998). During lactation, luteal phase progesterone concentrations were also lower in higher yielding cows (Lucy and Crooker, 2001) and we have found a delayed increase in progesterone in the early luteal phase in cows with peak milk production of  $> 42 \text{ kg day}^{-1}$  (A. Swali, V. J. Taylor and D. C. Wathes, unpublished), although in lower yielding cows no such relationships are apparent (G. E. Mann, unpublished). Several authors have suggested that the lower progesterone is related to a greater liver mass and associated higher catabolic activity when cattle and sheep receive a greater dietary input (for example, see O'Callaghan *et al.*, 2001).

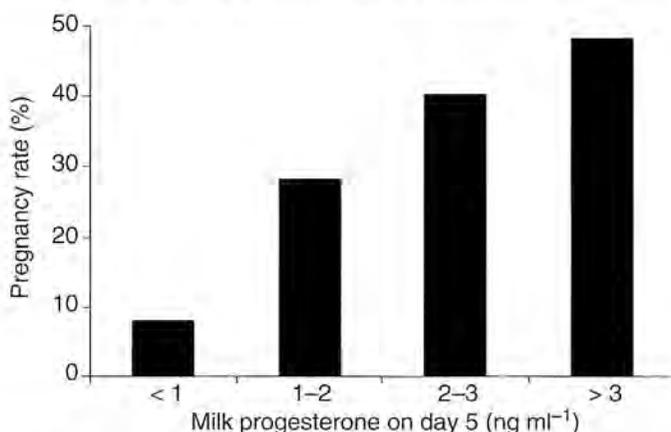
The bovine corpus luteum can synthesize both IGF-I and -II, contains insulin and type 1 IGF receptors and responds to insulin and IGF-I *in vitro* by increasing progesterone output (McArdle and Holtorf, 1989; Perks *et al.*, 1999). Growth hormone receptors are also present in large luteal cells (Lucy *et al.*, 1993). Luteinized granulosa cells from large ( $> 10 \text{ mm}$ ) but not smaller follicles respond to growth hormone by increasing progesterone output, an effect that was not mediated by stimulation of local IGF-I production (Gong *et al.*, 1994; Wathes *et al.*, 1995). Growth hormone treatment of cows (as bovine somatotrophin) can also promote luteal progesterone production *in vivo* (for example, see Schemm *et al.*, 1990), although this effect is inconsistent. Therefore, all of these metabolic signals are clearly able to influence luteal function, although their precise mode of action may differ. During the early postpartum period when cows are in negative energy balance, growth hormone receptors in the liver are downregulated and the endocrine profile is of high growth hormone coupled with low insulin and IGF-I. As cows recover from negative energy balance, IGF-I increases and growth hormone decreases. Therefore, metabolic influences on the corpus luteum will vary according to the time after calving.

Robinson *et al.* (2002) studied the effect of dietary PUFAs on progesterone concentrations in first lactation cows. Both high n-6 and high n-3 diets delayed the timing of the progesterone increase in the luteal phase, and in cows fed the n-3 diet there was also a significant reduction in the peak progesterone concentration. This effect could be mediated by altered luteal prostaglandin production as both  $\text{PGE}_2$  and 6-keto- $\text{PGF}_{1\alpha}$  are luteotrophic in the early luteal phase (Milvae, 1986). Changes in cholesterol availability might also be mediated via regulation of StAR protein (see above).

## **Factors influencing early embryo development**

### *Importance of luteal function during early pregnancy*

In dairy cattle, inadequate luteal function to support normal embryo development is now established as a major reproductive problem. Repeated studies have demonstrated reduced progesterone concentrations during early pregnancy in cows in which pregnancy fails (for reviews, see Mann and Lamming, 1999; Mann *et al.*, 1999). There is now considerable evidence that it is the time at which progesterone concentrations start to increase, rather than the final concentrations achieved, that is the critical determinant of the outcome of



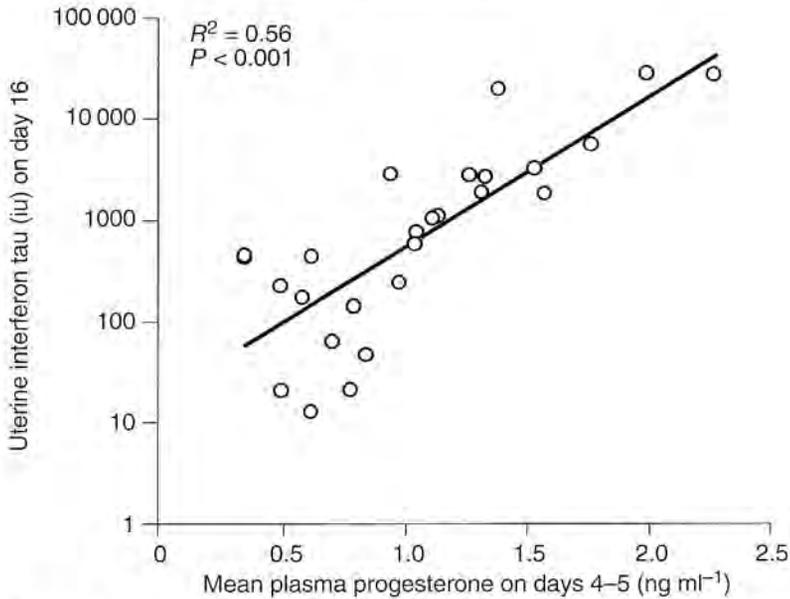
**Fig. 5.** Relationship between milk progesterone concentration at day 5 after mating and pregnancy rate in a survey of 1228 Holstein-Friesian dairy cows. (Adapted from Starbuck *et al.*, 2001).

mating. For example, by monitoring milk progesterone concentrations on day 5 after mating in over 1400 cows, Starbuck *et al.* (2001) showed that cows with 'adequate' milk progesterone ( $> 3 \text{ ng ml}^{-1}$ ) had pregnancy rates of approximately 50–55%, whereas cows with concentrations of  $< 1 \text{ ng ml}^{-1}$  had pregnancy rates  $< 10\%$  (Fig. 5). Larson *et al.* (1997) also demonstrated a delayed onset of the progesterone increase after ovulation in cows failing to conceive.

The importance of early progesterone on the outcome of insemination is further supported by progesterone supplementation studies. In an overall analysis of 17 such studies, Mann and Lamming (1999) demonstrated a highly significant 10.3% improvement in pregnancy rate when progesterone was administered before day 6 compared with only a 1.4% increase when progesterone was administered after day 6. Treatment of cattle with progesterone from day 2 to day 5 resulted in a tenfold increase in conceptus elongation on day 14 (Garrett *et al.*, 1988), whereas later increases in progesterone failed to cause any marked increase in embryo development (Kerbler *et al.*, 1997). The timing of the progesterone increase is important because it acts on progesterone receptors in the uterus to alter the pattern of uterine secretions (for a review, see Geisert *et al.*, 1992). These secretory products in turn provide histiotrophic support for the developing embryo and thus affect its growth rate. The bovine conceptus must produce sufficient interferon tau (IFN $\tau$ ) to prevent oxytocin receptor upregulation on about day 16, which in turn initiates luteolysis (Wathes and Lamming, 1995). IFN $\tau$  is synthesized by the trophoblast, so there is a direct relationship between the size of the embryo and IFN $\tau$  production (Mann *et al.*, 1999; Robinson *et al.*, 2001). We have found a strong correlation between progesterone concentrations on day 4 and day 5 after mating (but not later in the luteal phase) and the uterine concentrations of IFN $\tau$  on day 16, during the period of maternal recognition of pregnancy (Fig. 6). This finding again indicates that it is the timing of the progesterone increase which is critically important for embryo development.

#### *Relationship between metabolic parameters and conception rates*

Studies into the relationships between metabolic parameters and fertility have often been inconsistent (for a review, see O'Callaghan *et al.*, 2001). One reason for this

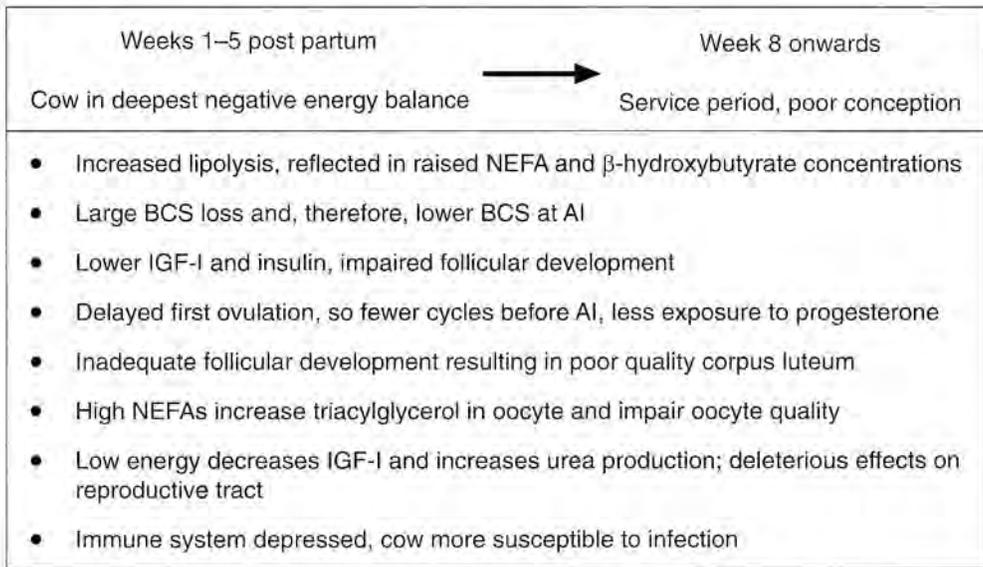


**Fig. 6.** Relationship between plasma progesterone concentration on days 4–5 after mating and total uterine interferon- $\tau$  on day 16 in non-lactating Holstein-Friesian cows. (Based on data from Mann *et al.*, 2002).

is that there appears to be a carry over effect of the period of negative energy balance on to conception rates, which are not reflected by metabolic parameters measured at the time of artificial insemination (Fig. 7). Our own recent work, summarized here, has also revealed major differences between first lactation and multiparous cows, which have often not been considered in previous studies.

A variety of metabolic parameters were measured before and after calving in > 300 multiparous cows and these were related to the interval to conception. Cows failing to conceive at all had consistently lower IGF-I concentrations both before calving and in the early post-partum period (weeks 1–7) than cows that conceived, but differences were generally no longer significant during the service period (Wathes *et al.*, 2001a). Urea concentrations increased after calving in cows with calving to conception intervals of > 150 days, whereas urea values remained stable over this period in cows that conceived more rapidly (Wathes *et al.*, 2001b). Therefore, the differences in urea concentrations between fertile and infertile cows were apparent later than for IGF-I. These metabolic changes in the less fertile cows were generally associated with either a large BCS loss, a low BCS at calving or a lower DMI than in more fertile cows (D. C. Wathes, Z. Cheng and V. J. Taylor, unpublished). In another study, we found that cows failing to conceive to first service had failed to regain significant amounts of condition score during the service period (G. E. Mann, unpublished).

In primiparous cows ( $n = 198$ ), the relationships between calving to conception intervals and metabolic profiles were clearly different. Animals that failed to conceive had the highest IGF-I and urea concentrations before calving, and heifers calving with a pre-calving BCS of > 3 had the longest calving to conception intervals. These cows also experienced a greater BCS loss post partum. These results indicate that fertility problems in the first lactation are triggered principally by the metabolic status at calving. There were no consistent relationships between NEFA,  $\beta$ -hydroxybutyrate or insulin concentrations and calving to conception intervals in either age group.



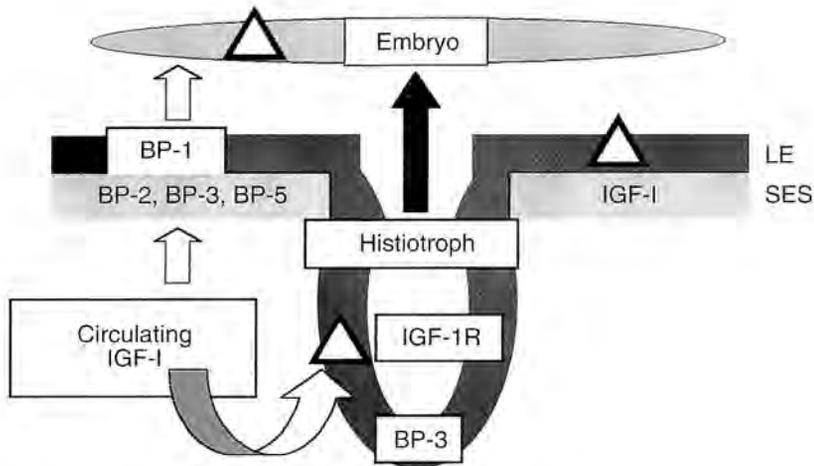
**Fig. 7.** Suggested mechanisms whereby a period of severe negative energy balance in the immediate postpartum period can reduce subsequent fertility in cows. AI: artificial insemination; BCS: body condition score; IGF-I: insulin-like growth factor I; NEFA: non-esterified fatty acids.

The carry-over effects of a negative energy balance on fertility are consistent with the results of other workers who have found that oocyte quality is impaired in cows with a large BCS loss (Snijders *et al.*, 2000) or a high liver triacylglycerol content (Kruip *et al.*, 2001). The increasing urea concentration after birth is probably indicative of an underlying energy imbalance in individual animals, as high urea values in cows in positive energy status do not seem to have a detrimental effect on embryo survival (Laven and Drew, 1999; O'Callaghan *et al.*, 2001).

#### *IGF system in the reproductive tract*

The association of low circulating IGF-I concentrations with poor conception rates supports proposals that the IGF system has an important role in the reproductive tract (Wathes *et al.*, 1998). The embryo is in the oviduct for 4–5 days before travelling to the uterus. The embryo starts to elongate from about day 13, but does not attach to the uterine wall until day 19, so it is entirely dependent on the secretions of the uterine tract to provide both an appropriate environment (for example, in terms of pH) and nutritive support during this critical early period. The oviduct mucosa synthesizes an increasing amount of IGF-I at the time of ovulation, which presumably acts on IGF-1R which are present in both the mucosa and muscle layers (Schmidt *et al.*, 1994; Pushpakumara *et al.*, 2002). IGF-I may also be released into the oviductal lumen to affect embryo growth and metabolism directly (Heyner, 1997). Preliminary evidence indicates that expression of IGFBP-3 within the mucosa is higher in lactating than non-lactating cows. This difference may represent a nutritionally sensitive mechanism for reducing IGF action in the oviduct (Pushpakumara *et al.*, 2002).

There is also an active IGF system within the bovine uterus (Geisert *et al.*, 1991; Keller *et al.*, 1998; Robinson *et al.*, 2000). Local production of IGF-I by the endometrial stroma appears quite low, but there is intense expression of IGF-1R mRNA within the endometrial glands and of IGF-II mRNA in the caruncular stroma. IGFBP-1 is expressed in the luminal epithelium in the late luteal phase and is upregulated in early pregnancy. As IGFBP-1



**Fig. 8.** The insulin-like growth factor (IGF) system in the bovine reproductive tract during conceptus elongation. Circulating IGF-I acts on type 1 IGF receptor (IGF-1R) in the glandular epithelium to promote the secretion of histiotroph. There is also low production of IGF-I in the sub-epithelial stroma (SES). Some IGF-I may transit into the uterine lumen to act directly on IGF-1R in the developing embryo. The actions of IGF-I are modulated by the production of IGF-binding proteins (BP), which are localized to the luminal epithelium (LE), SES or glandular epithelium. Pregnant cows tend to have higher circulating IGF-I concentrations and expression of IGFBP-1 mRNA and lower expression of IGFBP-2 and -3. (Based on data from Robinson *et al.*, 2000).

possesses an RGD domain that can recognize the  $\alpha_5\beta_1$  integrin receptor on trophoblast, its presence in the luminal epithelium at this time may be important for embryo attachment (Han and Carter, 2000). IGFBP-2 mRNA is localized to the sub-epithelial stroma with higher concentrations in cows that did not conceive to insemination. IGFBP-3 is widely expressed in the endometrium in stroma, epithelial cells and blood vessels, but the precise localization varies among cows and there are suggestions that the distribution may be nutritionally regulated and may be lower in cows that conceive. As both IGFBP-2 and IGFBP-3 can reduce IGF bioavailability (Rechler and Clemmons, 1998), changes in binding protein expression may act in concert with alterations in circulating IGF-I to regulate IGF actions in the reproductive tract (Fig. 8).

### Infectious disease and fertility

The immune system is depressed in early lactation due, in part, to increases in cortisol at parturition or in response to other stressors. Therefore, dairy cows are particularly susceptible to bacterial infection causing mastitis or endometritis, which are more likely to occur after a difficult calving (retained fetal membranes, dystocia, twins, calf mortality; Kruip *et al.*, 2001). The endotoxins released induce the liberation of a variety of inflammatory mediators including  $\text{PGF}_{2\alpha}$ , tumour necrosis factor  $\alpha$  ( $\text{TNF}\alpha$ ) and other pro-inflammatory cytokines. These factors mediate a 'reprogramming of metabolism' which preferentially targets nutrients for lymphocyte and macrophage proliferation, and production of antibodies and acute phase proteins, but away from other body functions (Spurlock, 1997). Cows with mastitis have lower circulating concentrations of IGF-I and insulin (Huszenicza *et al.*, 1998), whereas cows with

higher circulating concentrations of IGF-I can mount a better immune response (Mallard *et al.*, 1997). Cows with metabolic disorders, such as hepatic lipidosis, also have reduced immune competence.

Thus, it is not surprising that common infections can impact on fertility. Huszenicza *et al.* (1998) found that the development of mastitis in the follicular phase of the cycle was associated with an extended inter-luteal interval from  $8 \pm 2$  to  $14 \pm 8$  days. A longer anovulatory period after birth and follicular cysts are more common in cows with endometritis (Hussain and Daniel, 1991) indicating that ovarian and uterine function is impaired. This finding is supported by Spicer (2001b) who demonstrated that  $\text{TNF}\alpha$  can decrease IGF-I stimulated follicular steroid production. Inflammatory cytokines may also act at the hypothalamus to inhibit LHRH.

The incidence of persistent luteal phases (increased progesterone for  $> 19$  days) has increased over the past 30 years (Royal *et al.*, 2000). Risk factors for persistent luteal phases are an abnormal calving, retained placenta and metritis, although many cows with persistent luteal phase show no obvious signs of uterine infection. The incidence of persistent luteal phases also increases with age and in cows that have their first ovulation  $< 19$  days post partum, before uterine involution has occurred (Opsomer *et al.*, 1998) and is higher in US Holstein than in New Zealand Friesian cows (Lucy, 2001). Cows with uterine infection have prolonged secretion of  $\text{PGF}_{2\alpha}$  post partum (Bekana *et al.*, 1996), which may be in response to lipopolysaccharide stimulation (Leung *et al.*, 2001). Despite this, the duration of the luteal phase is more often lengthened than shortened, possibly because the pattern of  $\text{PGF}_{2\alpha}$  release is inappropriate to trigger luteolysis. Leung *et al.* (2001) have shown that the pro-inflammatory interleukins -1, -2 and -6 can inhibit development of oxytocin receptors in the bovine endometrium.

The incidence of persistent luteal phases also increases during heat stress, possibly in association with reduced follicular oestradiol production (Lucy, 2001). However, we and others were unable to demonstrate any major changes in metabolic hormone profiles indicative of a prolonged negative energy balance post partum in cows that developed persistent luteal phase profiles (Huszenicza *et al.*, 1988; V. J. Taylor and D. C. Wathes, unpublished). There is a well-established relationship between endometritis and fertility: infected cows have a prolonged interval to first service, more services per conception and an increased calving to conception interval (Dobson and Smith, 1998).

A number of other infectious diseases have been associated with reduced fertility in dairy cattle. For example, bovine viral diarrhoea virus (BVDV) can reduce conception rates by up to 50%. The causal mechanisms have again not been fully elucidated, but experimental infection with BVDV, such that peak viraemia occurred during pre-ovulatory follicle development, caused significantly lower progesterone concentrations during the ensuing luteal phase (Fray *et al.*, 2002).

## Conclusions

Many studies have now shown that ovarian cyclicity in postpartum dairy cows is often extremely irregular. Ovarian cycles may not resume until some considerable time after calving. When they do, long inter-luteal intervals and both short and long luteal phases are all commonly observed. The steady increase in yield over the past 30 years has placed cows under increasing metabolic demands which challenge their ability to supply adequate nutrients to body systems other than the mammary gland. All mammals have evolved strategies to prevent reproduction at times of nutrient shortage or stress. In dairy cows this manifests itself as inhibition of pulsatile LH secretion, reduced follicular and luteal growth, impaired

oocyte quality and a poor environment in the reproductive tract related to the time of the progesterone increase. It is hardly surprising that fertility rates are often so poor. Under these conditions, many aspects of dairy cow management become crucial in determining whether an individual cow will conceive at the desired time after calving. The diet needs to contain the correct protein:energy ratio and sufficient dry matter intake must be achieved to counteract the drain of lactation. Many individual dietary constituents also seem to influence reproductive performance, although the mechanisms of action in many cases remain elusive. It is also increasingly apparent that nutritional management before calving plays a critical role in determining subsequent fertility, via alterations in milk yield, BCS loss and calving difficulty. The process of calving followed by the metabolic drain of lactation also impact on the immune system, making cows susceptible to diseases such as mastitis and metritis. These diseases also reduce fertility through inhibition of hypothalamic and pituitary function as well as having direct effects on the ovary and uterus.

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