Physiological and health factors affecting fertility in beef and dairy cows

Crowe, M.A., Williams, E.J., Mulligan, F.J., UCD Veterinary Sciences Centre; School of Veterinary Medicine, University College Dublin, Belfield, Ireland

ABSTRACT

It has been well documented that genetic selection for milk yield over the last 50 years has been associated with reduced fertility in dairy cows. There is a variable anoestrous period following parturition in cows. Follicular growth generally resumes within 7-10 days in the majority of cows associated with a transient follicle-stimulating hormone (FSH) rise that occurs within 3 to 5 days of parturition. Dairy cows that are not nutritionally stressed generally ovulate their first post partum dominant follicle (~15 days), whereas beef suckler cows in good body condition normally have a mean of 3.2 ± 0.2 dominant follicles (~30 days) to first ovulation; and beef cows in poor body condition have a mean of 10.6 ± 1.2 dominant follicles (~70-100 days) to first ovulation. The lack of ovulation of dominant follicles during the post partum period is associated with infrequent luteinising hormone (LH) pulses, with both maternal-offspring bonding and low body condition score at calving being implicated as the predominant causes of delayed resumption of cyclicity in nursed beef cows. The normal pattern of early resumption of ovulation may be delayed in high yielding Holstein-type dairy cows generally due to the effects of severe negative energy balance, dystocia, retained placental membranes and uterine infections. First ovulation, in both dairy and beef cows, is generally silent (i.e. no behavioural oestrus) and followed by a short inter-ovulatory interval (>70%). Stress caused by production diseases in high yielding dairy cows also contributes to the problems of poor fertility. Lameness results in reduced intensity of oestrus, and can contribute to ovulation failure, which is largely due to reduced pre-ovulatory oestradiol secretion and failure of the LH surge. Mastitis has been associated with prolonged intervals to dominant follicle selection and, in animals with uterine infection, the dominant follicle grows more slowly and produces less oestradiol. In conclusion, the key to optimising resumption of ovulation in both beef and dairy cows is appropriate pre-calving nutrition and management so that cows calve down in optimal body condition (body condition score (BCS) 3.0 to 3.25) with post partum body condition loss minimised. The adverse effects on fertility associated with genetic selection for increased milk yield in dairy cows is, in part, associated with increased incidences of production disease-induced stress, subclinical metabolic adaptions to negatively altered nutritional status and a true genetic predisposition to reduced fertility in some high milk yield genotypes.

INTRODUCTION

Fertility in dairy cows has decreased from the 1960’s to the present day. This had been associated with a period of intense selection for cows having increased production of milk (Butler 1998). The causes of this decline in fertility are multifactorial and revolve around the issue of supplying sufficient nutrients for milk production at the expense of other physiological processes including reproduction (Walsh and others 2011). Conception rates to a single service for post partum dairy cows have decreased from about 65% to 38% over the last 40 years. For beef cows trends for fertility are not so clear cut. The major issues for beef cows are achieving return to cyclicity, detection of oestrus (if AI is being used) and successful re-establishment of pregnancy.

Reproductive efficiency in dairy and beef cows is dependent upon achieving high submission rates and high conception rates per service. However, to achieve good submission and conception rates cows must resume ovarian cycles, have normal uterine involution, be detected in oestrus, and be inseminated at an optimum time. In seasonally calving herds the aim is to achieve conception by 75-85 days following parturition so that calving-to-calving intervals are maintained at 365 days. This may be extended somewhat for non-seasonal herds in indoor management systems.

POST PARTUM RESUMPTION OF OVULATION

The pattern of resumption of ovarian function in both dairy and beef cows has been previously reviewed (Roche and others 1992, Crowe 2008, Crowe and others 2014). Resumption of ovarian cyclicity is largely dependent on LH pulse frequency. Both dairy and beef cows have early resumption of follicular growth within 7 to 10 days post partum. The fate of all dominant follicles is dependent on the LH pulse secretion pattern.

Follicle growth during pregnancy

During pregnancy follicular growth continues during the first two trimesters (Ginther and others
1989, Ginther and others 1996) at regular 7 to 10 day intervals. In late pregnancy (last 22 days) the strong negative feedback of progestagens (mostly from the CL of pregnancy and partly of placental origin) and oestrogens (mostly of placental origin) suppresses the recurrent transient FSH rises that stimulate follicle growth (Ginther and others 1996, Crowe and others 1998; Figure 1) so that the ovaries are largely quiescent during the last 20–25 days of gestation. At parturition, pituitary stores of LH are low due to the effects of elevated circulating concentrations of oestradiol, of placental origin, in late pregnancy (Nett 1987).

**Physiology of resumption of ovarian cyclicity in post partum cows**
At the time of parturition progesterone and oestradiol concentrations reduce to basal concentrations. Parturition allows the removal of the negative feedback effects of elevated oestradiol and the recommencement of the synthesis of FSH and LH. The synthesised FSH is released into peripheral circulation as evidenced by the almost immediate resumption of recurrent transient increases in blood concentrations of FSH (within 3 to 5 days of parturition) that subsequently occur at 7 to 10 day intervals (Crowe and others 1998). The first of these increases stimulates the first post partum wave of follicle growth that generally produces a dominant follicle by 7-10 days post partum (Murphy and others 1990, Savio and others 1990a, Crowe and others 1993). The re-accumulation of anterior pituitary stores of LH is slower than that of FSH and takes 2-3 weeks to complete. During this period circulating concentrations of LH, and LH pulse frequency, are both low primarily due to lack of releasable pools of LH in the gonadotroph cells of the anterior pituitary. This is the case in all cows irrespective of whether they are milked or suckled (Silveira and others 1993, Griffith and Williams 1996). The synthesis and sequestration of LH requires only a low level of GnRH pulsatility. Between days 10 and 20 post partum the pulsatile release of LH increases in dairy cows (or in beef cows that are weaned). The concurrent LH pulse frequency determines the fate of the first follicular wave dominant follicle, which is dependent on its ability to secrete sufficient oestradiol to induce a gonadotrophin surge. The capacity for oestradiol secretion is in turn dependent on the prevailing LH

![Figure 1. Follicle-stimulating hormone (FSH), progesterone (P4), oestradiol (E2) and follicular diameter profiles in two representative beef cows from ~30 days prepartum until 50 days postpartum (Crowe and others 1998).](image-url)
pulse frequency during the dominance phase of the follicle wave, the size of the dominant follicle and IGF-I bioavailability (Austin and others 2001, Canty and others 2006). Thus the major driver for ovulation of a dominant follicle during the post partum period is the GnRH/LH pulse frequency. In suckled beef cows, the suppressive effect of suckling and maternal offspring bonding on hypothalamic GnRH secretion prevents the establishment of the requisite LH pulse frequency that is required for oestradiol synthesis, induction of a pre-ovulatory LH surge and ultimately ovulation (Murphy and others 1990, Crowe and others 1993, Duffy and others 2000). Eventually, the suckled beef cow will escape the effect of suckling and maternal offspring bonding resulting in an increased frequency of LH pulses and ovulation (Stagg and others 1998). The major physiological difference between suckled beef and dairy cows at 15-20 days post partum is the lower frequency of pulsatile release of LH in beef cows nursing their own calves (Silviera and others 1993, Griffith and Williams 1996) compared with dairy cows.

The LH pulse frequency required to stimulate a dominant follicle towards ovulation is one LH pulse per hour. This has been tested and validated by the LH pulsatile infusion studies of Duffy and others (2000) in early post partum anoestrous beef cows. Figure 2 depicts the likely fate of the early post partum dominant follicles in beef and dairy cows. In beef cows the first dominant follicle, and frequently a successive number of dominant follicles, generally fail(s) to ovulate (Murphy and others 1990, Stagg and others 1995), rather undergoing atresia. With beef cows in good body condition the first post partum dominant follicle to ovulate is generally from wave 3.2 ± 0.2 (~30 days; Murphy and others 1990); whereas for beef cows in poor body condition there are typically 10.6 ± 1.2 waves of follicular growth before ovulation occurs (~70-100 days; Stagg and others 1995; Figure 3). In the case of dairy cows ovulation of the first post partum dominant follicle typically occurs in 30 to 80% of cows, while it undergoes atresia in 15 to 70% of cows or becomes cystic in 1-5% of cows (Savio and others 1990a, Beam and Butler 1997, Sartori and others 2004, Sakaguchi and others 2004). There is no evidence that lack of FSH or a delayed resumption of ovarian follicle waves are causes of prolonged post partum anoestrous intervals in either beef (Crowe and others 1998, Stagg and others 1998) or dairy cows (Beam and Butler 1999).

First ovulation in both dairy and beef cows is generally silent (i.e. no behavioural oestrus; Kyle and others 1992) and is generally (>70%) followed by a short cycle, usually containing just one follicle.
wave. This first luteal phase is of short duration due to the premature release of prostaglandin F2\(\alpha\) (PGF\(2\alpha\)) (Peter and others 1989a), thought to be induced by increased oestradiol produced from the formation of the post-ovulatory dominant follicle on days 5-8 of the cycle inducing premature oestradiol and oxytocin (Zollers and others 1993) receptors. Thus the corpus luteum, which also secretes lower quantities of progesterone, regresses prematurely around days 8-10 of the cycle, with the second ovulation occurring around days 9-11 after the first ovulation. This second ovulation is generally associated with the expression of oestrous behaviour and followed by a luteal phase of normal duration generating normal concentrations of progesterone (Crowe and others 1998).

Cyclic cows may have 2, 3 or very occasionally 4 follicle waves during the oestrous cycles that occur in the post partum period (Savio and others 1990b, Sartori and others 2004). Unlike non-lactating heifers, lactating Holstein post partum dairy cows tend to have two follicle waves per 18-23 day cycle (Sartori and others 2004). Blood concentration of progesterone is the major factor that affects LH pulse frequency in cyclic animals. Generally, lactating Holstein dairy cows tend to have lower blood concentrations of progesterone during the cycle than cyclic heifers (Sartori and others 2004, Wolfenson and others 2004). These lower progesterone concentrations tend to allow a subtle increase in LH pulse frequency and also for prolonged growth of each dominant follicle rather than the faster atresia that occurs in cyclic heifers. Cows with prolonged luteal phases tend to have a fourth follicular wave (Savio and others 1990a). The number of follicular waves or rate of turnover of dominant follicles is directly related to the duration of dominance of each dominant follicle, and cattle with shorter durations of dominance for the ovulatory dominant follicles tend to have higher conception rates (Austin and others 1999, Santos and others 2004). Therefore, nutrition, by altering metabolic clearance of progesterone, can affect the duration of dominance of a dominant follicle, the number of follicular waves per cycle and have an indirect effect on conception rates.

Factors contributing to GnRH/LH pulse frequency in early post partum beef cows

The regulation of LH secretion is the key driver of resumption of ovulation in post partum cows. A decrease in the concentration of LH and a suppression in the frequency of LH pulses had been reported in nutritionally induced anoestrous beef cows (Richards and others 1989) and heifers (Imakawa and others 1986), and occurs as a result of reduced GnRH secretion from the hypothalamus.

The major factors that control the GnRH/LH pulse frequency (and therefore the fate of early post partum dominant follicles) in post partum beef cows include maternal bond/calf presence (presumably due to effects on opioid release), suckling inhibition (Myers and others 1989, Stagg and others 1995), and poor body condition (Canfield and Butler 1990). Calf presence has a very clear negative effect on resumption of ovulation in beef cows nursing calves. Restricted suckling of beef cows (once per day) from day 30, where calves were in an isolated pen away from sight of the cows, significantly shortened the interval from calving to first ovulation (51 days) compared with cows where the calves had ad libitum access (79 days; Stagg and others 1995). The effect of calf presence can be further compartmentalised into suckling stimuli (mammary sensory pathways) and maternal behaviour/bonding effects (Silveira and others 1993, Williams and others 1993) but requires positive calf identification by either sight or olfaction (Griffith and Williams 1996). Beef cows that calve down in poor BCS (<2.5) are more likely to have a prolonged anoestrous period (Stagg and others 1995) due presumably to lower LH pulse frequency (Stagg and others 1998).

As beef cows (with prolonged anovulatory anoestrous) approach their first post partum...
ovulation, LH pulse frequency increases (observed during each sequential follicular wave from 6 waves before ovulation until the ovulatory wave; Stagg and others 1998). Concentrations of IGF-I increased linearly from 75 days before first ovulation until ovulation, which was associated with a linear decrease in growth hormone concentrations during the same period (Stagg and others 1998). These increased circulating concentrations of IGF-I help to stimulate dominant follicle maturation and growth so that there is sufficient secretion of oestradiol to induce an LH surge and ovulation. Management may be used to encourage earlier ovulation by restricting suckling/access of the cows to the calves from approximately day 30 post partum (Stagg and others 1998) or by increased plane of nutrition and body condition. The available evidence would indicate that the majority (85%) of beef cows are capable of ovulating by 35 days post partum (Murphy and others 1990, Crowe and others 1993, Duffy and others 2000, Mackey and others 2000). Removal of the suckling/maternal calf bond results in a doubling of LH pulse frequency within 48 hours of calf separation followed by subsequent ovulation of the concurrent or next DF. Interestingly, in the small proportion of cows that fail to respond to the removal of the suckling/maternal calf bond show no increase in LH pulse frequency, and no evidence of an increase in the circulating concentrations of oestradiol. These non-responders typically had prolonged post partum anoestrous intervals and could be described as being in “deep anovulatory anoestrus” (Sinclair and others 2002).

Factors contributing to LH pulse frequency in early post partum dairy cows
In dairy cows the major factors affecting resumption of ovulation include BCS and energy balance (milk energy output and energy intake), parity, season and disease (Bulman and Lamming 1978, Beam and Butler 1997, Opsomer and others 2000, Wathes and others 2007). Energy intake, BCS and milk yield interact to affect energy balance in dairy cows. There is evidence to link many of these factors to reduced LH pulse frequency. A number of studies have been conducted in dairy cows of various yield potential that have categorised the pattern of resumption of ovarian function by measuring milk progesterone. These range from a study by Fagan and Roche (1986) using traditional moderate yielding Friesian cows (4,000–5,000kg milk per lactation) to that of Opsomer and others (1998) using modern high yielding Holstein type cows (6,900–9,800kg milk per lactation). The data from these two studies are summarised in Table 1. Furthermore, this pattern of resumption of ovarian function has been validated in a series of equivalent papers and the two key problem categories (prolonged interval to first ovulation and prolonged luteal phase) are summarised in Figure 4. Risk factors for these two ovarian abnormalities have been determined in a large epidemiological study by Opsomer and others (2000). The major risk factors for a prolonged interval from calving to first ovulation included (odds ratio in parentheses): acute body condition score loss up to 60 days post calving (18.7 within

### Table 1. Pattern of resumption of ovarian cyclicity in postpartum dairy cows (traditional moderate yielding Friesians vs modern high yielding Holsteins), using milk progesterone profiling (samples collected twice weekly).

<table>
<thead>
<tr>
<th>Item</th>
<th>Traditional moderate yielding Friesian cows¹</th>
<th>Modern high yielding Holstein cows²</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cows/postpartum periods</td>
<td>463</td>
<td>448</td>
</tr>
<tr>
<td>Normal cyclic patterns (%)</td>
<td>78</td>
<td>53.5*</td>
</tr>
<tr>
<td>Prolonged interval to 1st ovulation (%)</td>
<td>7</td>
<td>20.5*</td>
</tr>
<tr>
<td>Prolonged luteal phase (%)</td>
<td>3</td>
<td>20*</td>
</tr>
<tr>
<td>Temporary cessation of ovulation (%)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Short cycles (%)</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>Other irregular patterns (%)</td>
<td>4</td>
<td>2.5</td>
</tr>
</tbody>
</table>

¹Fagan and Roche 1986
²Opsomer and others 1998
*Categories with a major disparity between the two studies

Figure 4. Percentage of cows defined as having either i) delayed resumption of ovulation or ii) prolonged luteal phases based on evaluation of milk progesterone profiles across a number of studies in dairy cows (compiled by Grimard and others 2005; Grimard personal communication).
blood non-esterified fatty acids, and are mobilising tissue at a high rate have increased lactation (Whelan and others 2012). Cows that correctly in their ratio of energy and protein in early BCS (3.0 to 3.25) at calving and balancing diets maximised and by having cows in an appropriate matter intake in the early post partum period is important. This is best achieved by ensuring that dry duration of severe negative energy balance post calving is minimised and by having cows in an appropriate BCS (3.0 to 3.25) at calving and balancing diets correctly in their ratio of energy and protein in early lactation (Whelan and others 2012). Cows that are mobilising tissue at a high rate have increased blood non-esterified fatty acids, and β-hydroxy butyrate, but reduced concentrations of insulin, glucose and IGF-I (Grummer and others 2004). The metabolic status associated with high rates of tissue mobilisation increases the risk of hypocalcaemia, acidosis, fatty liver, ketosis and displaced abomasum (Gröhn and Rajala-Schultz 2000, Maizon and others 2004, Overton and Waldron 2004). Cows affected by these metabolic disorders are more prone to anoestrus, mastitis, lameness and subsequently reduced conception rate to AI (Fourichon and others 1999, Gröhn and Rajala-Schultz 2000, Lucy 2001, Lopez-Gatius and others 2002, Maizon and others 2004). It is hypothesised that serum IGF-I concentration could be useful as a predictor of nutritional status and hence reproductive efficiency in dairy cows (Zulu and others 2002a). Plasma IGF-I concentrations before calving and in the first few weeks of lactation have been linked to subsequent cyclicity and conception rate (Diskin and others 2006, Taylor and others 2006). This emphasises the critical role of correct nutritional management to ensure that the deficit in energy balance post calving is mild rather than severe, and kept to as minimum duration as possible. Current approaches to minimise the energy balance deficit post calving includes: the optimisation of BCS at calving (3.0 to 3.25), shorter dry periods and the maintenance of an appropriate energy to protein ratio in the diet of early lactation cows (Mulligan and others 2006).

In dairy cows one of the main drivers of negative energy balance is BCS at calving because cows calving at BCS >3.5 have both reduced appetite and mobilise in excess of 1 BCS unit (Alibrahim and others 2010), with subsequent detrimental effects on fertility (Mulligan and others 2007). Recently, several laboratories have examined the effects of glucogenic–lipogenic feeding strategies for dairy cows on reproductive function (Garnsworthy and others 2009, Friggens and others 2010). There is evidence that the administration of a glucogenic diet, which promotes increased circulating concentrations of insulin and glucose (Van Knegsel and others 2005), would be expected to improve several reproductive variables. Gong and others (2002) found that the administration of a glucogenic diet increased circulating concentrations of insulin and increased the proportion of cows ovulating by 5 days after calving, which is highly desirable. Further support for this concept has come from New Zealand studies (Burke and others 2010) who also recorded a shorter post partum anoestrous interval in pasture/grass silage fed dairy cows supplemented with 5kg/day of corn and barley based concentrate. Furthermore, Al Ibrahim and others (2013) demonstrated positively altered metabolic status for cows fed a high starch TMR in comparison with grazing cows in the first 3 to 4 weeks of the lactation. However, there is evidence that high circulating concentrations of insulin have negative effects on oocyte quality (Garnsworthy and others 2008a). In contrast, lipogenic diets increased the oestradiol-secreting capacity of pre-ovulatory follicles, thus providing enhanced substrate for progesterone production (Leroy and others 2008) and improved blastocyst development rates. Garnsworthy and others (2008b) proposed a strategy of glucogenic diets during the early post partum period to hasten the onset of regular oestrous cycles followed by more lipogenic diets to lower circulating insulin and improve oocyte quality at breeding. Although this has a strong physiological basis, there is a need for confirmatory studies of the concept. The switch to lipogenic type diets should coincide with lesser adipose mobilisation as the lactation progresses.

Disease state may also regulate follicle fate via LH pulse frequency and other mechanisms. Uterine conditions such as retained foetal membranes (RFM), endometritis and metritis contribute to reproductive efficiency via various mechanisms. Other diseases such as mastitis (Huzenicza and others 1999) and lameness (Pettersson and others 2006) delay resumption of luteal activity by 7 to 17 days, respectively. For these there is considerable...
evidence that this is mediated via acute stressors reducing GnRH and hence LH pulse frequency, leading to decreased oestradiol production by dominant follicles and preventing or reducing the gonadotrophin surge, thus delaying ovulation.

Role of Insulin
Insulin is primarily involved in glucose homeostasis but also serves as a metabolic signal influencing pituitary release of LH (Monget and Martin 1997) and ovarian responsiveness to gonadotrophins (Stewart and others 1995). Plasma insulin concentrations are influenced by both BCS and level of nutrition (Vizcarra and others 1998), and may serve as a more sensitive indicator of nutritional status than BCS. In a multinational study, Sinclair and others (2002) showed that post partum anoestrous beef cows with low (<5 mIU/l) plasma concentrations of insulin were unable to ovulate a dominant follicle in response to restricted suckling, unlike cows with higher (>5 mIU/l) plasma concentrations of insulin, notwithstanding an increase in LH pulse frequency. The results of that study are consistent with those of Gong and others (2001, 2002) who showed that dairy cows fed a diet which increased circulating concentrations of insulin during the first 50 days post partum had shorter post partum anoestrous intervals, independent of any effects on LH or FSH and without affecting milk yield or energy balance. The latter three studies would argue for a direct effect of insulin at the ovarian level. The inability to respond to increased LH pulse frequency may be due to a lack of granulosa cell LH receptors, which are known to be dependent on the combined actions of FSH and oestradiol-17β (Bao and Garverick 1998). Follicular oestradiol-17β is in turn dependent on LH-stimulated production of androgens from thecal cells that are dependent on peripheral concentrations of insulin and IGF-I (Stewart and others 1995). Therefore, low plasma concentrations of insulin could reduce androgen and oestradiol production and thus compromise the ability of follicles to acquire LH receptors.

PROLONGED LUTEAL PHASES
Following resumption of ovarian cyclicity, prolonged luteal phases are the main cause of irregular oestrous cycles in cows. The incidence of prolonged luteal phases in dairy cows has increased from 3% (Fagan and Roche 1986) to 11-22% (Lamming and Darwash 1998, Opsomer and others 1998, Shrestra and others 2004a,b, Figure 4). It is generally considered that prolonged luteal phases are associated with an abnormal uterine environment that disrupts endometrial prostaglandin F2α production. Interestingly, in the study of Opsomer and others (1998), where the incidence of cows with prolonged luteal phases was 20% (89/448 cows), only 43/89 cows had abnormal uterine content, 2/89 had ovarian cysts and 44/89 had no detectable abnormalities. However, in that study abnormalities were identified only by rectal palpation. The major risk factors for a prolonged luteal phase in cows having resumed ovulation included (odds ratio, in descending order of importance, in parentheses)(Opsomer and others 2000): metritis (11.0), abnormal vaginal discharge (4.4), retained placenta (3.5), parity (2.5 for parity 4+ vs primiparous), earlier resumption of ovulation (2.8 for resumption <19 days post partum, 2.4 for resumption 19-24 days post partum). These data support the concept that prolonged luteal phases are related to uterine problems rather than ovarian problems.

FOLLICULAR CYSTS
Follicular cysts occur where dominant follicles in the early post partum period (often the first dominant follicle post partum) fail to ovulate. Cysts typically continue to grow to diameters >20-25mm over a 10 to 40 day period in the absence of a CL (Savio and others 1990a, Gümen and others 2002, Hatler and others 2003). This continued growth appears to be due to lack of positive feedback induced by oestradiol and thus failure of induction of the LH/FSH pre-ovulatory surge, despite increased LH pulse frequency (to an intermediate level). At this time systemic concentrations of progesterone are low, while concentrations of oestradiol are elevated above normal pro-oestrus concentrations (Savio and others 1990a, Hatler and others 2003), and may result in strong exhibition of oestrous behaviour by cows in the early phases of a follicular cyst. The elevated oestradiol in conjunction with elevated inhibin suppresses blood concentrations of FSH, so that no new follicle waves emerge during the early active phase of a follicular cyst. The cyst then becomes oestrogen inactive (despite being morphologically still present) and a new follicular wave emerges. The dominant follicle of this new wave may either ovulate, undergo atresia or also become cystic. Many cows with follicular cysts correct themselves, but some develop sequential follicular cysts. The metabolic risk factors associated with dairy cows developing cysts in the early post partum period are over body-conditioned cows, a reduction in systemic insulin (Vanholder and others 2005) and IGF-I, and increased non-esterified fatty acids (NEFA) (Zulu and others 2002b). The typical incidence for follicular cysts in dairy cows
is between 1 and 5% (Opsomer and others 1998, Beam and Butler 1999).

**POST PARTUM UTERINE HEALTH**

Post partum uterine infection is a major contributor to reduced fertility in dairy cattle. Following parturition, the uterus becomes contaminated with bacteria and, whilst many animals can clear this contamination, infection persists in up to 20% of cows as endometritis (Sheldon and others 2009). There is evidence that uterine infections contribute to reduced fertility via a number of mechanisms. Bacterial products or immune mediators produced in response to infection suppress pituitary LH secretion and are associated with inhibition of folliculogenesis, decreased ovarian steroidogenesis, abnormal luteal phases and a higher incidence of cystic ovarian disease (Peter and others 1989b, Huszenicza and others 1999, Opsomer and others 2000, Mateus and others 2002, 2003, Sheldon and others 2002, Williams and others 2007). Furthermore, Bareille and others (2003) reported reduced feed intake for cows with puerperal metritis and retained foetal membranes (RFM). In a study of 82 clinically normal post partum cattle with no risk factors for uterine disease, 75% of the animals had high numbers of uterine pathogens on day 7 post partum, the predominant isolate being *Escherichia coli*. These animals also had retarded ovarian follicle growth; the first post partum dominant follicle grew slower and produced less oestradiol (Figure 5; Williams and others 2007, 2008a). Furthermore, in the animals that ovulated, the corpus luteum (CL) was smaller and produced less progesterone (Figure 6; Williams and others 2007). Within the uterus *E. coli* may disrupt the mechanisms of prostaglandin (PG) induced luteolysis in cyclic cows and, therefore, contribute to prolonged luteal phases by switching PG synthesis away from PGF2α towards PGE2 (Herath and others 2009, Williams and others 2008b).

These observations show a direct correlation between the presence of uterine pathogens, particularly *E. coli*, on day 7 post partum and suboptimal ovarian function for the following 3 weeks. However, the evidence shows that early post partum uterine disease contributes to infertility in cows by disrupting ovarian function and that these adverse effects may persist after uterine disease has resolved. The specific mechanisms by which uterine infection disrupts ovarian function are many and diverse but there is substantial evidence that the endotoxin lipopolysaccharide (LPS) is a key disruptor of ovarian function. As well as being detected in the uterus and peripheral
circulation, LPS has been detected in follicular fluid of cattle with uterine disease; unsurprisingly, concentrations of LPS are directly correlated with bacterial load (Peter and others 1989b, Mateus and others 2003, Herath and others 2007, Williams and others 2007). Administration of intravenous LPS disrupts neuroendocrine activity and results in interference with the oestrous cycle. In heifers given an intrauterine infusion of LPS, the pre-ovulatory LH surge was blocked, resulting in the formation of cystic follicles (Peter and others 1989b). Intravenous administration of LPS in sheep, suppressed hypothalamic GnRH secretion, inhibited pulsatile LH secretion and reduced pituitary responsiveness to GnRH (Williams and others 2001). Ovarian function can also be disrupted following LPS administration in the absence of gonadotrophic effects. An inhibition of peripheral plasma oestradiol concentrations was observed despite normal plasma LH concentrations (Xiao and others 1998, Battaglia and others 2000). In heifers, LPS delayed ovulation by interrupting the preovulatory oestradiol rise, thus delaying the LH surge (Suzuki and others 2001). Infusion of low concentrations of LPS in utero delayed the time interval over which follicles attained dominance and ovulation; while having no effect on LH or FSH concentrations (Williams and others 2008a). Furthermore, in cows, uterine infection does not affect peripheral plasma FSH concentrations or the consequent emergence of a wave of growing follicles (Sheldon and others 2002, Williams and others 2007). These studies provide evidence for localised effects of LPS in the ovary. Indeed, in vitro LPS has been shown to disrupt granulosa cell oestradiol secretion via reduced expression of aromatase enzyme expression (Herath and others 2007) and acute exposure to LPS increases follicular atresia and reduces the primordial ovarian follicle pool (Bromfield and Sheldon 2013).

In beef cows, the effect of uterine infection on ovarian function has not been studied as extensively as in the dairy cow. However, it could be expected that the mechanisms by which ovarian function are disrupted would be very similar. The incidence of uterine inflammation, as determined by neutrophil infiltration was similar, or even higher in beef cows than in dairy cows in the early weeks after calving (Santos and others 2009), although the incidence of clinical endometritis at this time may be lower in beef cows (Williams, unpublished observations). In contrast, the incidence of cytological endometritis is higher in dairy cattle later in the post partum period and it has been hypothesised that the differences in the timing of the first post partum ovulation between beef and dairy cattle may influence uterine health during this time (Santos and others 2009).

**HEALTH EFFECTS ON FERTILITY**

Cows suffering from health and disease problems are prime candidates for reduced fertility (Dobson and others 2008; Figure 7). Clearly, cows with a range of problems, including caesarean parturition, lameness (Collick and others 1989, Melendez and others 2003, Hernandez and others 2005), endometritis (Borsberry and Dobson 1989), retained fetal membranes (Borsberry and Dobson 1989), dystocia, mastitis (Borsberry and Dobson 1989, Schrick and others 2001), milk fever (Parker 1992), and low body condition scores (Lopez-Gatius and others 2003, Garnsworthy 2006), have extended intervals from calving to subsequent pregnancy establishment or may fail to become pregnant. In many cases, these problems become compounded with one of these conditions increasing the risk of another condition also occurring. In many of these health problems, there are clear risk factors associated with cow management and nutrition (Mulligan and others 2006). It has been well documented that cows subjected to negatively altered lipid or calcium metabolism around parturition have an exacerbation in immunosuppression that predisposes to RFM and uterine infection (Hammon and others 2006, Kimura and others 2006). This is true even where the metabolic alterations do not result in clinical disease (Martinez and others 2014). Specifically, lameness decreases the intensity of oestrous behaviour (Walker and others 2005). This was mediated through interruption of the LH surge, decreased LH pulse frequency, decreased
oestradiol concentrations during the follicular phase, and failure of ovulation (Dobson and others 2008, Morris and others 2011). Mastitis also has a negative effect on reproductive function. Up to 30% of cows with clinical mastitis infection fail to ovulate while infected (Dobson and others 2008, Wolfenson and others 2008). These data provide evidence that the consequences of disease include suppression of reproductive performance.

**INDUCTION OF OESTRUS AND OVULATION IN ANOVULATORY ANOESTROUS COWS**

From the previous sections it is clear that in many cases (especially with dairy cows) anovulatory anoestrus is associated with management risk factors and other diseases (excessive loss of BCS, severe lameness, uterine disease, displaced abomasum, etc). Therefore, before embarking on a specific treatment for anoestrus, the underlying factors and diseases should always be first addressed before commencement of specific treatments for the ovarian problems.

**GnRH**

The major cause of delayed ovulation in post partum cows is an infrequent LH pulse frequency (and by inference GnRH pulse frequency). GnRH treatment was used with variable effectiveness in numerous studies of post partum cows when the follicle status of the animals was unknown. A single injection, two injections 10 days apart, or frequent low dose injections at 1 to 4h intervals of GnRH or GnRH analogues failed consistently to induce ovulation in over 90% of treated anoestrous cows (Mawhinney and others 1979, Riley and others 1981, Walters and others 1982, Edwards and others 1983). However, when a GnRH analogue (20µg buserelin) was used at known stages of follicle growth (determined by daily ultrasound scanning) of the first post partum DF, all cows ovulated when administered during the growing phase of the DF (12/12) and the majority (7/10) ovulated when the first post partum dominant follicle (DF) was in its plateau/early declining phase of growth (Crowe and others 1993). In a further study conducted by Ryan and others (1998), 250 µg GnRH resulted in ovulation in 20 of 20 cows when given at dominance of a follicular wave, this was followed by emergence of a new wave of ovarian follicular growth 1.6 ± 0.3 days later and dominance of the subsequent wave was attained in 5 ± 0.3 days. However, there was no effect of GnRH on follicular dynamics when given at emergence of a follicular wave. The existing cohort of follicles continued to develop unaffected in 17 of 17 cows, and dominance occurred 3.6 ± 0.5 days later. Thus, GnRH may cause ovulation or no effect on follicle development depending on the animal’s stage of follicle development at treatment. As a result, when GnRH is used as part of an Ovsynch protocol (GnRH-PGF2α-GnRH treatment) in post partum anoestrous cows the effectiveness of the treatment is wholly dependent on the presence or absence of a DF at the time the first GnRH injection is administered.

**Progesterone**

Treatment of anoestrous cows with progesterone (and oestradiol) will induce oestrus and shorten the post partum interval to conception (Rhodes and others 2003). Currently, use of oestradiol is banned in food producing animals within the EU and elsewhere (Lane and others 2008). Anoestrous cows require progesterone pre-treatment to ensure that the first ovulation is associated with expression of oestrus and a normally functioning luteal phase. The standard progesterone treatment regime for cows known to be in anoestrus is a 7-9 day intravaginal device with the optional use of equine chorionic gonadotrophin (eCG) on the day of removal of the device (Lane and others 2008). The use of eCG may accompany progesterone treatment in cows that are in “deep anovulatory anoestrus” to ensure ovulation (Mulvehill and Sreenan 1977), but care must be taken not to induce too high an ovulation rate by using doses in excess of 500 iu. In a herd situation where there is a mix of anoestrous and cyclic cows, then it is better to treat all cows as if they were cyclic; i.e. use a 7-9 day progesterone treatment as an intravaginal device that is accompanied with a single GnRH injection at the time of progesterone device insertion, and an injection of prostaglandin F2α on the day before device removal (Lane and others 2008).

**Restricted suckling (beef cows)**

Earlier onset of ovulation in beef cows may be induced by restricting suckling from 30 days post partum (Stagg and others 1998). Restricted suckling involves once or twice daily access of calves to cows for suckling and at other times of the day the calves are isolated and out of direct physical contact and possibly sight of the dams (Stagg and others 1998).

**SUMMARY AND CONCLUSIONS**

Follicular growth generally resumes within 7-10 days post partum in the majority of both dairy and beef cows and is associated with a transient FSH rise that occurs within 3 to 5 days of parturition. A summary of reproductive parameters for beef and dairy cows is presented in Table 2. Delayed
resumption of ovulation is invariably due to a GnRH mediated lack of LH pulse frequency whether it is due primarily to suckling inhibition in beef cows or metabolic related stressors in high yielding dairy cows. First ovulation in both dairy and beef cows is generally silent and followed by a short interovulatory interval (ovarian cycle). The key to optimising resumption of ovulation in both beef and dairy cows is appropriate pre-calving nutrition and management so that cows calve down in optimal body condition (BCS 3.0 to 3.25) with post partum body condition loss restricted to <0.5 BCS units. Genetic selection for increased milk yield in dairy cows increases metabolic and nutritional stressors and, in turn, may affect uterine health and/or uterine immune status that has consequences for clearance of uterine disease.

**ACKNOWLEDGEMENTS**

This current research activities of the authors is supported by Science Foundation Ireland Strategic Research Cluster grant code 07/SRC/B1156 (the opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of Science Foundation Ireland) and European Framework programme 7 (EU FP7 grant code 613689; GplusE).

**REFERENCES**


---

**Table 2. Reproductive parameters in the early postpartum period of dairy and beef suckler cows.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dairy cows</th>
<th>Beef cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergence of the 1st follicle wave (days post partum)</td>
<td>5-10</td>
<td>5-10</td>
</tr>
<tr>
<td>% cows that ovulate the 1st dominant follicle</td>
<td>50-80</td>
<td>20-35</td>
</tr>
<tr>
<td>Postpartum interval to first ovulation (days)</td>
<td>15-25</td>
<td>25-120</td>
</tr>
<tr>
<td>Nature of 1st ovulation</td>
<td>silent</td>
<td>silent</td>
</tr>
<tr>
<td>Postpartum interval to first oestrus (days)</td>
<td>25-45</td>
<td>30-130</td>
</tr>
<tr>
<td>% short cycles after 1st ovulation</td>
<td>&gt;70</td>
<td>&gt;70</td>
</tr>
<tr>
<td>Predominant no. of follicle waves per normal (18-24 day oestrous cycle)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Regulation of LH pulse frequency</td>
<td>• declining energy balance</td>
<td>• suckling</td>
</tr>
<tr>
<td></td>
<td>• BCS at calving</td>
<td>• calf presence/maternal bond</td>
</tr>
<tr>
<td></td>
<td>• dry matter intake</td>
<td>• declining energy balance</td>
</tr>
<tr>
<td></td>
<td>• Disease state</td>
<td>• BCS at calving</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Disease state</td>
</tr>
</tbody>
</table>
Hernandez, J.A., Garbarino, E.J., Shearer, J.K., Risco,


of preovulatory luteinising hormone surges in heifers after intrauterine infusions of E. coli endotoxin. American Journal of Veterinary Research 50: 368-373
and endocrine profiles with fertility in primiparous and
Effect of supplemental concentrate type on milk production
and metabolic status in early-lactation dairy cows grazing
perennial ryegrass-based pasture. Journal of Dairy Science 95:
4541-4549
Williams, C.Y., Harris, T.G., Battaglia, D.F., Viguié, C., Karsch,
F.J. (2001) Endotoxin inhibits pituitary responsiveness to
gonadotropin-releasing hormone. Endocrinology 142: 1915-
22
Williams, E.J., Fischer, D.P., Noakes, D.E., England, G.C.,
between uterine pathogen growth density and ovarian
function in the postpartum dairy cow. Theriogenology 68:
549-559
somatosensory pathways are not required for suckling-
mediated inhibition of luteinizing hormone secretion and
delay of ovulation of cows. Biology of Reproduction 49:
1328-1337
Williams, E.J., Sibley, K., Miller, A.N., Lane, E.A., Fishwick, J.,
Nash, D.M., Herath, S., England, G.C., Dobson, H., Sheldon,
and Tumor Necrosis Factor alpha on ovarian function.
American Journal of Reproductive Immunology 60: 462-473
Williams, E.J., Herath, S., England, G.C., Dobson, H., Bryant,
C.E., Sheldon, I.M. (2008b) Effect of Escherichia coli infection
of the bovine uterus from the whole animal to the cell.
Animal 2: 1153-1157
Wolfenson, D., Inbar, G., Roth, Z., Kaim, M., Bloch, A.,
Braw-Tal, R. (2004) Follicular dynamics and concentrations of
steroids and gonadotropins in lactating cows and nulliparous
heifers. Theriogenology 62: 1042-1055
of delayed ovulation and its relation to follicular functions
and luteinizing hormone concentrations in lactating cows.
In Fertility in Dairy Cows: Bridging the Gaps. Eds. M.D. Royal, R.F.
Smith and N.C. Friggens, BSAS, Penicuik, UK. pp 163-164
Stress and the menstrual cycle: relevance of cycle quality
in the short and long-term response to a 5-day endotoxin
challenge during the follicular phase in the rhesus monkey.
Journal of Clinical Endocrinology and Metabolism 83:
2454–2460
Zollers, W.G. Jr., Garverick, H.A., Smith, M.F., Moffatt,
R.J., Salfen, B.E., Youngquist, R.S. (1993) Concentrations
of progesterone and oxytocin receptors in endometrium
of postpartum cows expected to have a short or normal
oestrous cycle. Journal of Reproduction and Fertility 97:
329-337
Zulu, V.C., Nakao, T., Sawamukai, Y. (2002a) Insulin-like
growth factor-I as a possible hormonal mediator of nutritional
regulation of reproduction in cattle. Journal of Veterinary
Medical Science 64: 657-665
Zulu, V.C., Sawamukai, Y., Nakada, D., Kida, K., Moriyoshi, M.
(2002b) Relationship among insulin-like growth factor-I blood
metabolites and postpartum ovarian function in dairy cows.
Journal of Veterinary Medical Science 64: 879-885