Physiological effects of undernutrition on postpartum anoestrus in cows

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The focus of this review is the physiological effects of undernutrition on ovarian follicle growth and the occurrence of ovulation in postpartum cows. Evidence suggests that moderate levels of underfeeding, before or after calving, may interfere with the mechanism(s) of final follicle maturation and ovulation, whereas more pronounced nutritional deficiencies may affect the mechanism(s) regulating dominant follicle size and the dynamics of dominant follicle growth and regression. Severe undernutrition may result in the absence of follicles > 5 mm in diameter. These changes are consistent with likely effects of reduced LH or FSH secretion that have been associated with inhibition of both tonic and surge release of GnRH from the hypothalamus. As yet, there is no direct evidence for local effects of undernutrition on ovarian function in cows, and studies examining this issue often ignore dynamic interdependencies between the hypothalamus, anterior pituitary gland, and the ovaries, and possible nutritional modulation of ovarian feedback mechanisms that affect gonadotrophin secretion. Further research is required to elucidate nutritional effects on the positive feedback regulation of gonadotrophin secretion and on the expression of behavioural oestrus. Further studies are also needed to determine whether nutritional or metabolic state may influence ovarian function directly in a manner that affects the duration of postpartum anoestrus and fertility in postpartum cows.

Introduction

Efforts to improve reproductive efficiency in cattle are often limited by prolonged periods after calving when cows do not ovulate or do not show behavioural oestrus. Factors affecting the resumption of ovulation and ovarian cycles after calving have been extensively reviewed (see articles by Peters and Lamming, 1990; and Short *et al.*, 1990). These include age, breed, nutrition, milk yield, suckling (or the presence of a calf), season, puerperal disorders and the presence of a bull. Undernutrition, or the inadequate intake of nutrients relative to demands, is a major factor contributing to prolonged postpartum anoestrus, particularly among cows dependent upon natural forages for most, if not all, of their feed requirements.

Effects of undernutrition may also interact with effects of these other genetic, environmental or management factors to influence the duration of postpartum anoestrus. Although the precise nature of

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these factors and their interactions is complex and often unclear, many appear to act via common hormonal mechanisms. The study of ovarian follicular growth patterns may yield useful information about such mechanisms, as these reflect the net effect of the prevailing endocrine and metabolic milieu. However, it is important to consider variation in follicular growth patterns in the context of the dynamic feedback relationships between the ovaries and the hypothalamo–pituitary axis, and the need for the coordinated function of these components if ovulation and successful establishment of pregnancy are to occur.

The aims of this review are to examine physiological effects of undernutrition on ovarian follicular growth and ovulation in postpartum cows, and some of the possible mechanisms that may underlie these effects. In contrast to the focus of research that has examined effects of specific dietary components (for example energy, rumen-degradable versus undegradable protein and lipids) in formulated rations (reviewed by Ferguson and Chalupa, 1989; Lucy *et al.*, 1992), the focus here is on effects of under-nutrition among cows grazing natural forages. In this context, intakes of dietary components, such as energy, protein and minerals, such as phosphorus, are interdependent and specific effects of individual components cannot easily be identified or partitioned. Thus it is probably more accurate to describe animals as suffering from undernutrition rather than from specific nutrient deficiencies, although in some cicumstances these may also occur (Entwistle, 1991).

Effects of Undernutrition on Patterns of Growth of Ovarian Follicles

Follicles > 5 mm in diameter are generally absent at the time of parturition in cows (Braden *et al.*, 1986; Ryan *et al.*, 1994), but regular patterns of growth and regression of large follicles similar to those recorded during the oestrous cycle and early pregnancy are evident from as early as one week after calving in most animals (see reviews by Roche *et al.*, 1992; Fortune, 1993). Most lactating dairy cows ovulate the first dominant follicle that develops, 2–3 weeks postpartum, when nutrition is not limiting (Savio *et al.*, 1990). In contrast, recurrent growth and regression of several (range 1–6) dominant follicles may occur before ovulation in adequately fed suckled beef cows in both temperate (Murphy *et al.*, 1990) and tropical (L. A. Fitzpatrick and K. W. Entwistle, unpublished) environments.

These typical patterns of postpartum follicle growth and ovulation can be altered by undernutrition. Wiltbank *et al.* (1964) reported that the size of the largest palpable follicle was consistently smaller before first ovulation in postpartum cows receiving submaintenance diets than in cows receiving 150% of maintenance requirements. Although nutritional effects have not been consistently detected in cross-sectional studies examining follicle populations in postpartum cows at isolated points in time (Henricks *et al.*, 1986; Prado *et al.*, 1990; Rutter and Manns, 1991), longitudinal studies using ultrasonography have consistently revealed effects on the dynamics of follicle growth and regression (Lucy *et al.*, 1991; Perry *et al.*, 1991; Rhodes *et al.*, 1993; Ryan *et al.*, 1994). Moreover, effects on ovarian follicle growth appear to vary with the degree of undernutrition, suggesting that measurement of these may serve as a form of *in vivo* bioassay of the endocrine and possibly the metabolic states prevailing in individual cows during the postpartum period.

At one end of the scale, moderate levels of undernutrition may delay the resumption of cyclicity after calving by apparently affecting the mechanism(s) responsible for final follicle maturation or ovulation, without marked effects on the growth of follicles up to preovulatory size. This is illustrated by results from a recent study on pasture-fed dairy cows that calved in below optimal body condition (McDougall *et al.*, 1995). Large follicles (≥ 10 mm) were evident in the ovaries of all cows by about 10 days after calving, in agreement with previous studies in dairy cows in which nutrition was not limiting (Savio *et al.*, 1990; Roche *et al.*, 1992). However, the first dominant follicle did not ovulate in most of the underfed cows (in contrast to adequately fed dairy cows), and the interval from calving to first ovulation was prolonged (43 ± 5 days). The pattern of postpartum follicle development in these underfed dairy cows was similar to that described for adequately fed suckled beef cows. This was characterized by the successive growth and regression of 1–9 dominant follicles (mean $\pm$ SEM: 4.2 ± 0.6), the maximum diameter of which progressively increased before first ovulation (Fig. 1).
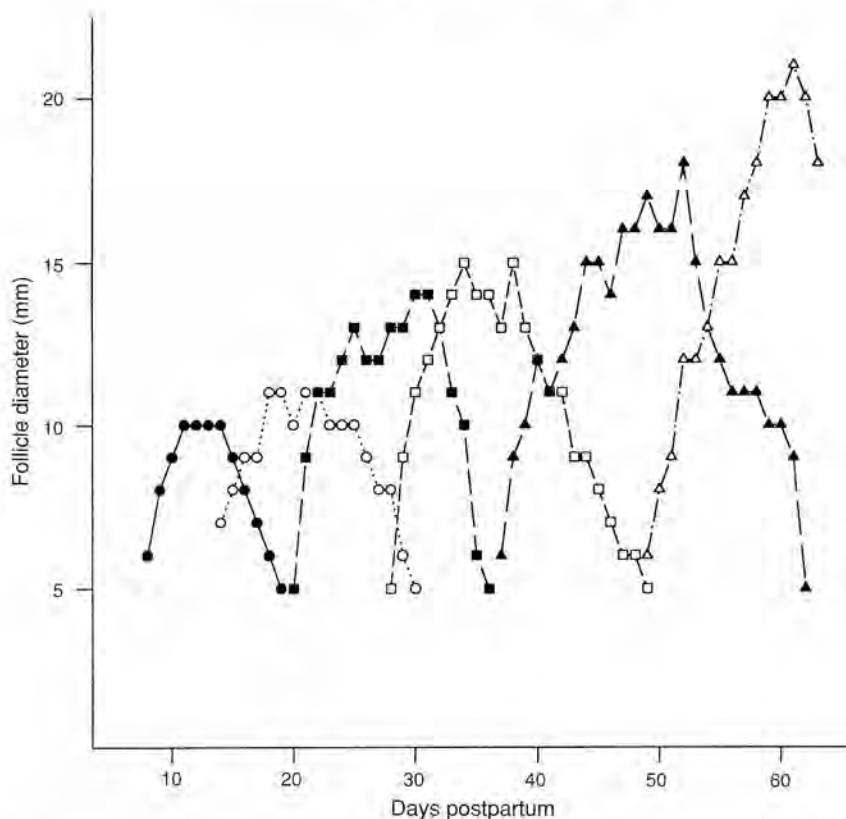
Fig. 1. Pattern of dominant follicle growth and regression from calving to first ovulation in a pasture-fed dairy cow that calved in below optimal body condition. Each symbol represents a separate follicle. In this experiment ($n = 17$ cows), days to first ovulation averaged ($\pm$ SEM) 43 ± 5 (range 13 to 93) following 4.2 ± 0.6 (range 1 to 9) waves of follicle growth. Reproduced with permission from McDougall (1994).

Although spontaneous ovulation did not occur until about 6 weeks after calving in these moderately underfed dairy cows, dominant follicles that developed earlier in similarly managed animals could ovulate in response to an exogenously induced LH surge. Injection of GnRH when a large growing follicle (≥ 10 mm) was present (as identified by daily ultrasonography), 24 ± 3 days postpartum, stimulated an LH surge and ovulation within 24–48 h in 9 of 10 cows, whereas only 1 of 10 saline-treated control cows ovulated within 4 days of treatment (McDougall, 1994). However, spontaneous oestrous cycles resumed after this induced ovulation in only 3 of 9 cows that ovulated in response to GnRH; the remainder experienced a short luteal phase and returned to an acyclic state. Thus, although regular patterns of dominant follicle growth and regression resume soon after calving in moderately underfed dairy cows and these follicles can ovulate, factors inhibiting spontaneous follicle maturation and ovulation may still predominate.

Restriction of prepartum or postpartum feed intake may also affect the maximum size and persistence of anovulatory follicles that develop after calving. These effects may be subtle or pronounced, depending upon the degree of nutritional restriction. In another recent study, the first dominant follicle that developed after calving took longer to attain maximum size (14.0 ± 1.3 versus 10.6 ± 0.7 days); and emergence of the second and third dominant follicles was delayed by 4–6 days; these follicles remained dominant for short periods, and the postpartum interval to first ovulation was prolonged (77 ± 8 days versus 51 ± 9 days) in Friesian heifers that calved in below optimal body condition compared with heifers that calved in higher body condition (condition score 4.5 versus 6.5; scale 0 = emaciated;
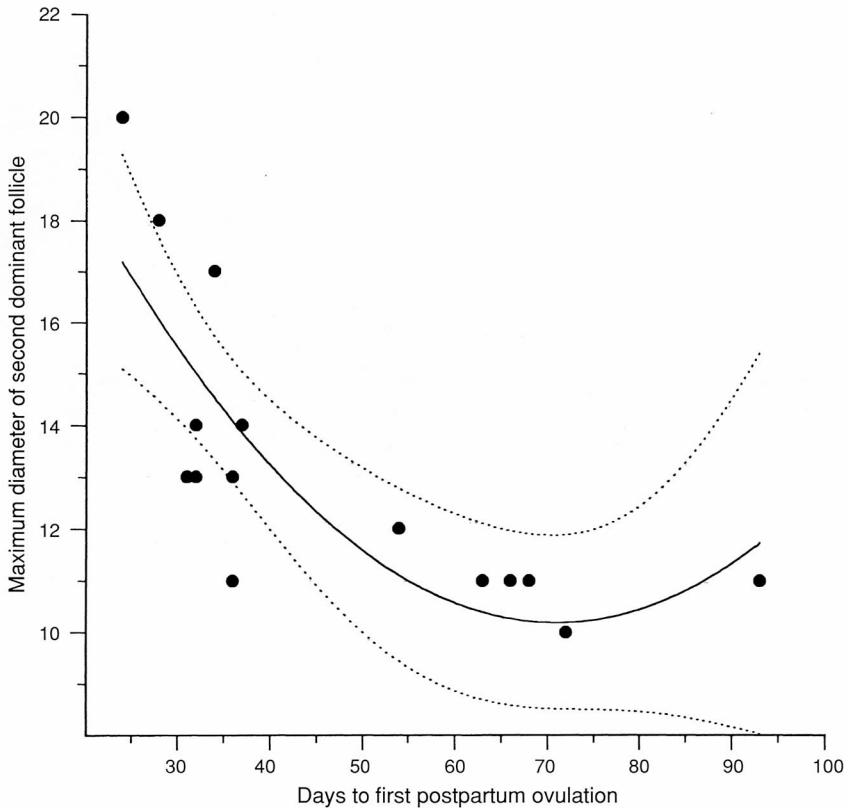
Fig. 2. Relationship between the maximum diameter of the second dominant follicle to develop after calving and days to first postpartum ovulation in moderately underfed dairy cows. The regression line is shown with 95% confidence limits ($y = 27.1 - 0.5x + 0.003x^2$, $R^2 = 0.66$). Reproduced with permission from McDougall (1994).

10 = obese) (C. R. Burke, S. McDougall and K. L. Macmillan, unpublished). This point is further illustrated by two relationships that were evident in the study of moderately underfed cows described in Fig. 1; both the maximum size and the persistence of the second postpartum dominant follicle were inversely related to the interval from calving to first ovulation (Figs 2 and 3; McDougall, 1994; McDougall *et al.*, 1995).

These findings are consistent with effects of chronic undernutrition on patterns of dominant follicle growth and regression in cows undergoing regular oestrous cycles. Long-term energy deprivation was similarly associated with reductions in both dominant follicle diameter and persistence (Murphy *et al.*, 1991). A more striking example comes from a study in which heifers were fed restricted amounts of roughage until spontaneous ovulation ceased (Rhodes *et al.*, 1993). Before the cessation of ovulation, the maximum diameter of successive dominant follicles and corpora lutea that developed decreased in a linear manner that was proportional to the decrease in bodymass of the heifers. Feeding *ad libitum* after a period of anovulation was subsequently associated with linear increases in the maximum diameter and persistence of successive dominant follicles that developed, which were proportional to gain in body mass. Ovulation resumed in these heifers when dominant follicles attained a maximum size equivalent (105 ± 4%) to that of the follicles that ovulated in the period before nutritional restriction began (F. M. Rhodes, personal communication).

At the extreme end of the scale, severe undernutrition in the last trimester of pregnancy and postpartum may result in the absence of ovarian follicles ≥5 mm in diameter or of larger (> 8 mm) follicles that can produce appreciable amounts of oestradiol (Perry *et al.*, 1991; Jolly, 1992). In the first
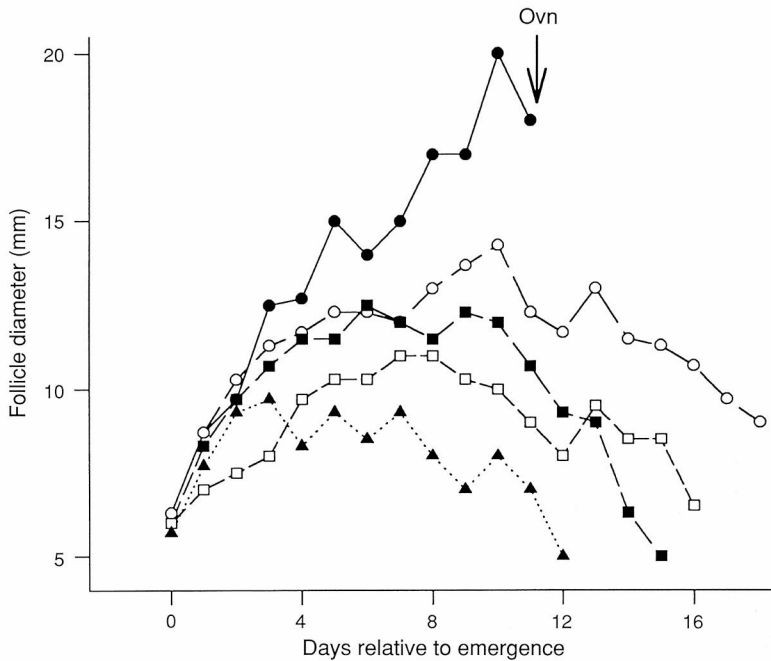
Fig. 3. Patterns of growth and regression of the second dominant follicle (mean diameter) that developed after calving in dairy cows that ovulated their second (●), third (○), fourth (■), fifth to seventh (□), or eighth to ninth (▲) dominant follicles postpartum. Ovn: ovulation. Reproduced with permission from McDougall (1994).

of these studies, growth of follicles ≥ 8 mm was virtually abolished in cows fed a low energy diet before and after calving. Low energy intake after calving also resulted in a reduced rate of appearance of smaller follicles (5.0–7.9 mm), which persisted on the ovaries for prolonged periods, perhaps reflecting the absence of functionally dominant large follicles (Perry *et al.*, 1991). In the second study, no underfed cows ($n = 10$) had oestrogenic follicles > 8 mm and half of these cows had no follicles > 5 mm in diameter present in either ovary 6–10 weeks postpartum. However, in small (2–4 mm) and medium (5–7 mm) sized follicles that were present, oestradiol and progesterone concentrations in follicular fluid, the proportion (within cows) of follicles with follicular fluid oestradiol:progesterone ratios > 1, and oestradiol synthesis by individual follicles in culture were not different between cows fed maintenance or restricted diets (Jolly, 1992). These findings are consistent with others suggesting that chronic undernutrition has little effect on the steroidogenic function of small- or medium-sized follicles in cows (Prado *et al.*, 1990; Rutter and Manns, 1991; Ryan *et al.*, 1994).

In summary, various degrees of nutritional deprivation affect patterns of ovarian follicle development before first ovulation in postpartum cows. Moderate levels of underfeeding, before or after calving, may interfere with the mechanism(s) of final follicle maturation and ovulation, whereas more pronounced deficiencies appear to interfere with the mechanism(s) regulating the size and dynamics of dominant follicle growth and regression. Severe undernutrition may result in the absence of growth of large follicles, and at the extreme, the absence of follicles > 5 mm in diameter. This effect in chronically underfed suckled cows is perhaps analogous to that of hypophysectomy reported in other species (reviewed by Driancourt, 1991).

Mechanisms Underlying Nutritional Effects on Ovarian Follicle Growth

It is clear that undernutrition affects follicle growth and ovulation in postpartum cows, but what are the mechanisms underlying these effects? Questions that frequently arise are whether such effects on follicle

growth simply reflect nutritional effects on gonadotrophin secretion, including the sensitivity of the hypothalamus and anterior pituitary gland to ovarian feedback, or whether nutritional or metabolic state may modulate follicular growth or function directly?

These questions may be addressed by considering what is known about gonadotrophin requirements for follicular development in cows, and about how ovarian follicles, the hypothalamus and the anterior pituitary gland must interact for ovulation to occur.

Gonadotrophin requirements for follicular development

The growth of follicles beyond 4–5 mm in cows and their ability to produce appreciable amounts of oestradiol appears to be absolutely dependent upon adequate FSH and LH support (see Driancourt, 1991; Fortune, 1994). Although the precise gonadotrophin requirements for follicle growth and ovulation remain to be defined in cows, FSH appears to govern the ability of follicles to continue to grow above this apparent threshold size, whereas LH is associated more with the maintenance of dominant follicles and their capacity to produce substantial amounts of oestradiol, necessary for the induction of oestrus, the LH surge and for ovulation (Fortune, 1994). The growth of follicles above 4–5 mm in diameter appears to follow subtle increases (20–40%) in plasma FSH concentrations (Adams et al., 1992, 1993), and can be induced in heifers actively immunized against GnRH by injecting with recombinant bovine FSH (Crowe et al., 1993). In cyclic cows in which high (similar to follicular phase) LH pulse frequencies were maintained without ovulation occurring, by maintaining subluteal concentrations of plasma progesterone, large follicles increased linearly in size and plasma oestradiol concentrations were high for extended periods (15–20 days). In contrast, low-frequency LH pulsatility (as found in the presence of higher concentrations of plasma progesterone) was associated with the turnover of dominant follicles and initiation of new follicle growth at more frequent intervals (Sirois and Fortune, 1990; Stock and Fortune, 1993). The amount of oestradiol a large follicle can produce also depends upon the level of LH stimulation it receives, as the synthesis of oestradiol by granulosa cells is thought to depend upon the supply of aromatizable substrate (predominantly androstenedione in cows) from the adjacent theca interna layer which is governed by LH (McNatty et al., 1984).

Resumption of large follicle growth after calving probably results from increased FSH secretion, which occurs within 3–4 days after calving in adequately fed cows (Lamming et al., 1981). The ability of these follicles to continue to grow and become dominant and the duration of their functional dominance probably depends upon the level of pulsatile LH secretion, which in turn reflects the level of tonic GnRH secretion from the hypothalamus. The recovery of high frequency (hourly) pulsatile LH secretion is necessary to stimulate both the continued development of a large dominant follicle and the secretion of oestradiol by this follicle to levels sufficient to induce a preovulatory gonadotrophin surge. This requires the re-establishment of positive feedback effects of oestradiol on both the hypothalamus and the anterior pituitary gland which are suppressed during the early postpartum period, but which also typically recover within 2–3 weeks of calving when nutrition is not limiting (Schallenberger et al., 1982; Alam and Dobson, 1987; Nolan et al., 1988). Ovulation requires the coordinated interaction between a large dominant follicle, the hypothalamus and the anterior pituitary gland.

It is possible that the effects of undernutrition on ovarian follicle growth and ovulation described above may result from inhibition of gonadotrophin secretion at one or more levels in this hypothetical scenario.

Effect of undernutrition on LH and FSH secretion in postpartum cows

Effects of undernutrition on gonadotrophin secretion in postpartum cows have been reviewed by Short et al. (1990) and Schillo (1992), and considerable evidence suggests that prolonged acyclic intervals caused by undernutrition are associated with suppression of both tonic and surge release of GnRH from the hypothalamus, and hence LH and FSH secretion from the anterior pituitary gland. These effects are thought to be mediated by inhibition of oestradiol positive feedback stimulation of the gonadotrophin-surge mechanism, or by enhanced negative feedback effects of oestradiol on the hypothalamus, as well as by direct (ovary independent) inhibition of GnRH release (see Schillo, 1992).

Recovery of the GnRH surge mechanism after calving is inhibited by suckling (Nolan *et al.*, 1988), and by undernutrition in milked cows (Schallenberger and Prokopp, 1985; Alam and Dobson, 1987). The LH surge response to exogenous oestradiol occurs more rapidly with increasing time postpartum (Alam and Dobson, 1987; Nolan *et al.*, 1988) but may also be delayed by undernutrition (Echternkamp *et al.*, 1982). Failure of the mechanism(s) governing final follicle maturation and ovulation in cases of moderate undernutrition may therefore involve inhibition of the high frequency pulsatile LH release necessary for stimulating high levels of oestradiol secretion, and (or) central inhibition of the GnRH surge response to oestradiol. Progressive increases in the maximum diameter of dominant follicles before first ovulation, such as those evident in Fig. 1, may reflect increasing pulsatile LH secretion over time postpartum. However, the failure of these follicles to ovulate may reflect persistent inhibition of the hypothalamic GnRH surge mechanism. This hypothesis may explain reports of high LH pulse frequencies, similar to those measured in the follicular phase of the oestrous cycle, which have been measured in some postpartum cows that remained anovulatory for considerable periods (Haresign *et al.*, 1983; S. R. D. Sutherland and P. D. Jolly, unpublished). Similarly, prolonged acyclic intervals that were correlated with magnitude of postpartum loss of bodymass in autumn calving dairy cows have been attributed to the failure of follicular phase concentrations of oestradiol to induce GnRH surge responses rather than to inadequate oestradiol production (Gyawu and Pope, 1990).

However, the recovery of high frequency pulsatile LH secretion postpartum is also inhibited by undernutrition (Echternkamp *et al.*, 1982; Nolan *et al.*, 1988; Wright *et al.*, 1990, 1992; Perry *et al.*, 1991). In suckled beef cows, effects of body condition at calving on LH pulse frequency decreased, whereas effects of postpartum nutrition (maintenance versus low) became more predominant, with increasing time postpartum (Wright *et al.*, 1992). In lactating dairy cows, the transition from decreasing to increasing energy balance in early lactation was associated with an increase in LH pulse frequency (Canfield and Butler, 1990). This finding suggests that pulsatile LH secretion may be inhibited until a negative energy balance nadir is reached, after which time LH pulse frequency increases, before first ovulation. Given the relationships described above between LH pulse frequency and dominant follicle growth, reductions in the size and persistence of dominant follicles associated with undernutrition may be reflecting inhibitory effects on the frequency of pulsatile LH secretion.

Inhibition of LH pulsatility may also explain lower concentrations of oestradiol measured in the follicular fluid of large follicles recovered from cows fed maintenance or submaintenance diets, compared with *ad libitum* fed controls (Henricks *et al.*, 1986; Prado *et al.*, 1990). Oestradiol and testosterone concentrations in follicular fluid were lower in dominant follicles recovered at the same stage of growth (as determined by ultrasonography) from postpartum dairy cows that were still acyclic two months after calving, compared with cows that had resumed regular oestrous cycles, and both were linearly related to LH pulse frequency measured just before ovariectomy (McDougall, 1994; Fig. 4). However, ratios of oestradiol:testosterone were similar in follicular fluid obtained from dominant follicles among these two groups of cows, suggesting that they had similar capabilities to aromatize testosterone to oestradiol, and that lower oestradiol concentrations in acyclic cows resulted primarily from reduced levels of LH stimulated testosterone production.

Nutritional effects on plasma FSH concentrations in postpartum cows are less consistently reported than are effects on LH concentrations, most likely reflecting differences in the mechanisms regulating the secretion of these two hormones. Terqui (1985) suggested that low FSH together with low LH concentrations may be associated with a more profound level of suppression of the reproductive axis ('deep' anoestrus) in postpartum cows and ewes than low concentrations of LH in the presence of moderate levels of FSH secretion. This contention is supported by data from postpartum beef cows fed low quality roughage diets (Jolly *et al.*, 1991). Mean plasma LH and FSH were suppressed to very low values, as was pulsatile LH secretion (< 1 pulse in 8 h), in suckled cows for prolonged periods (> 70 days) after calving. Ovarian negative feedback effects predominated in cows fed *ad libitum* or restricted amounts of roughage, and totally suppressed increases in LH pulse frequency and plasma FSH concentrations that occurred in ovariectomized cows in response to weaning. However, in ovariectomized cows, strong inhibitory effects of both suckling and undernutrition remained that were independent and additive in their effects on FSH, but interactive in their effects on LH secretion (Jolly *et al.*, 1991; Jolly, 1992; Fig. 5). These results are consistent with effects of undernutrition and suckling
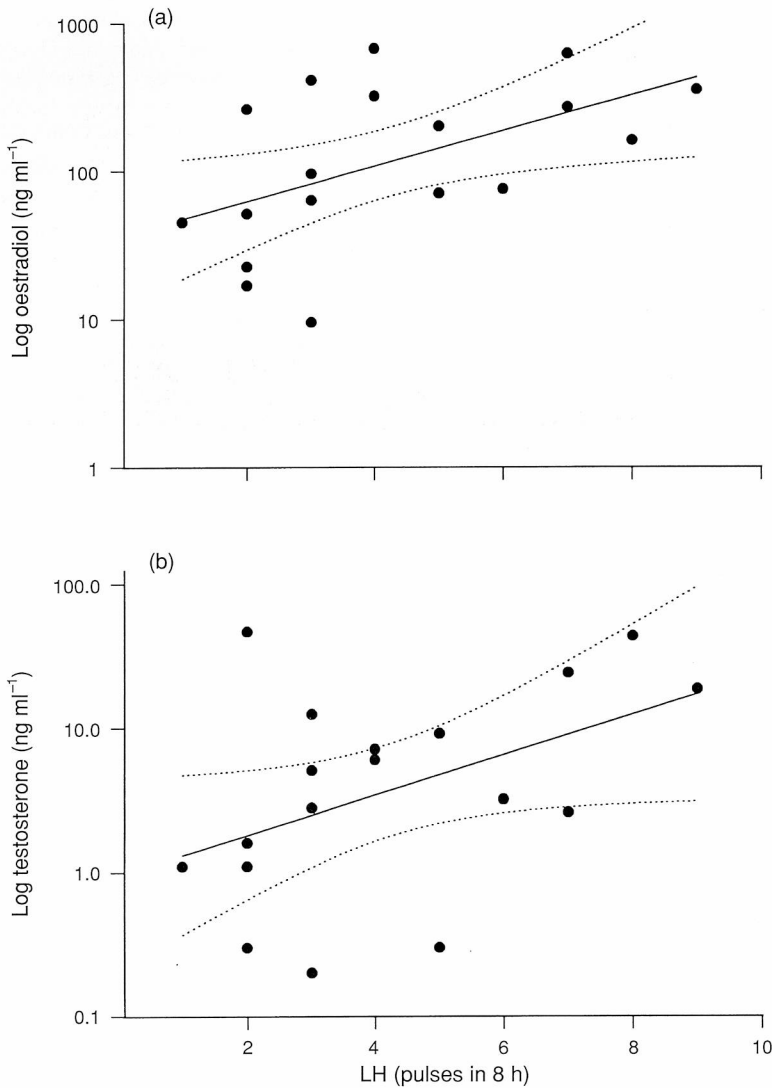
Fig. 4. Relationships between dominant follicle fluid (a) oestradiol (b) testosterone concentrations ($\log_{10}$ scale) and LH pulse frequency in the 8 h preceding ovariectomy in postpartum dairy cows, determined by linear regression analysis ($R^2 = 0.22$, $P < 0.05$, and $R^2 = 0.16$, $P = 0.07$, respectively). Reproduced with permission from McDougall (1994).

previously reported in chronically ovariectomized animals (Imakawa *et al.*, 1987; Ebling *et al.*, 1990). Taken together, these data support the hypothesis that both FSH and LH secretion may be inhibited by ovary-independent mechanisms, in addition to those mediated by enhanced sensitivity to ovarian (predominantly oestradiol) negative feedback.

Suppression of both FSH and LH synthesis and secretion has been attributed to profound suppression of hypothalamic GnRH release in chronically underfed ovariectomized ewe lambs (Foster *et al.*, 1989; Ebling *et al.*, 1990). A similarly marked level of suppression of hypothalamic and pituitary function may have occurred in the postpartum cows described above that had no oestrogenic follicles > 8 mm in diameter and half of which had no follicles > 5 mm in diameter present in their ovaries. Indeed, no LH pulses were detected in sampling periods of 8 h either before or 9 days after ovariectomy
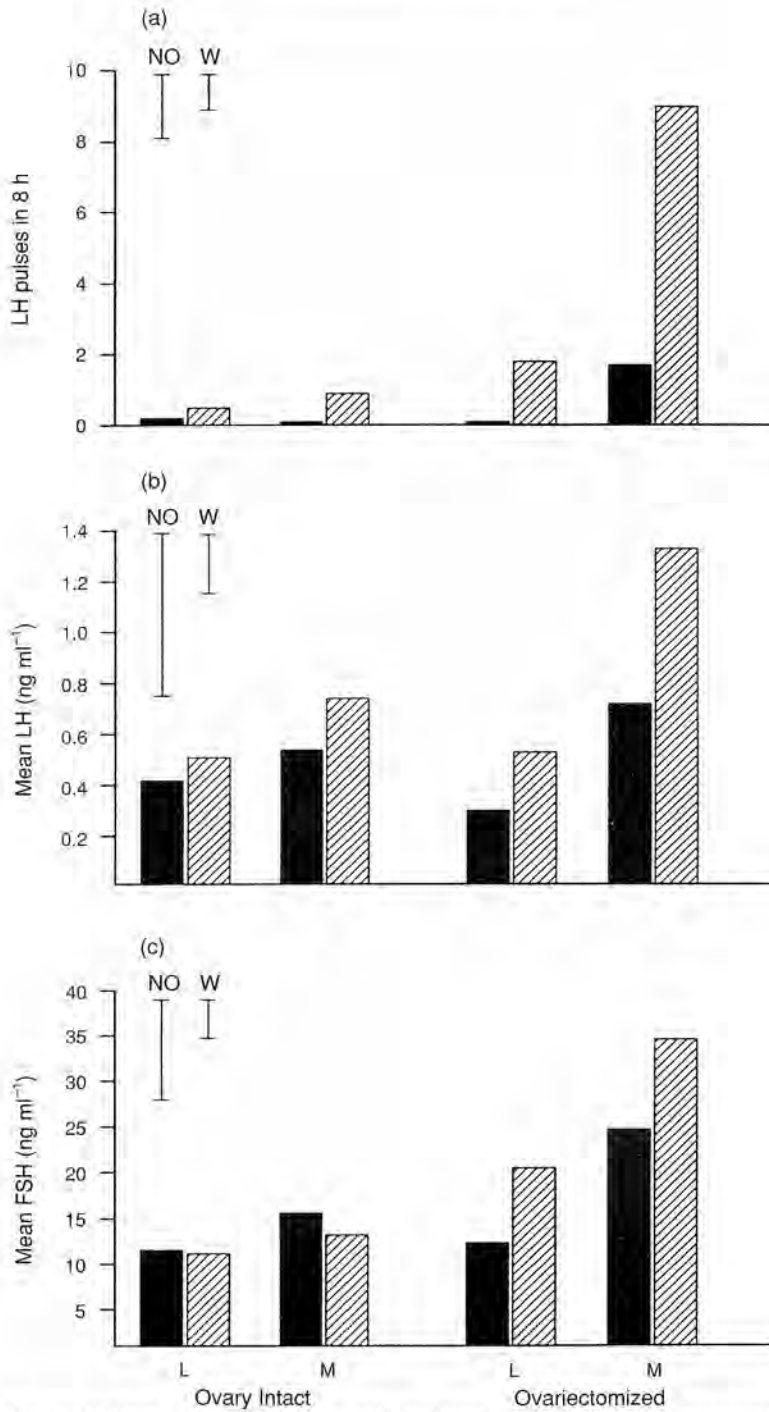
Fig. 5. (a) Mean LH pulse frequency, and (b) plasma LH and (c) FSH concentrations before (■) and 4 days after (▨) calves were weaned at 70 days postpartum, in beef cows that were either ovary intact or had been ovariectomized 10 days before weaning. Cows were fed to maintain (M) or lose (L) bodymass in the preceding 120 days. Vertical bars represent least-significant differences for effects of nutrition and ovariectomy (NO), or weaning (W). Reproduced with permission from Jolly (1992).
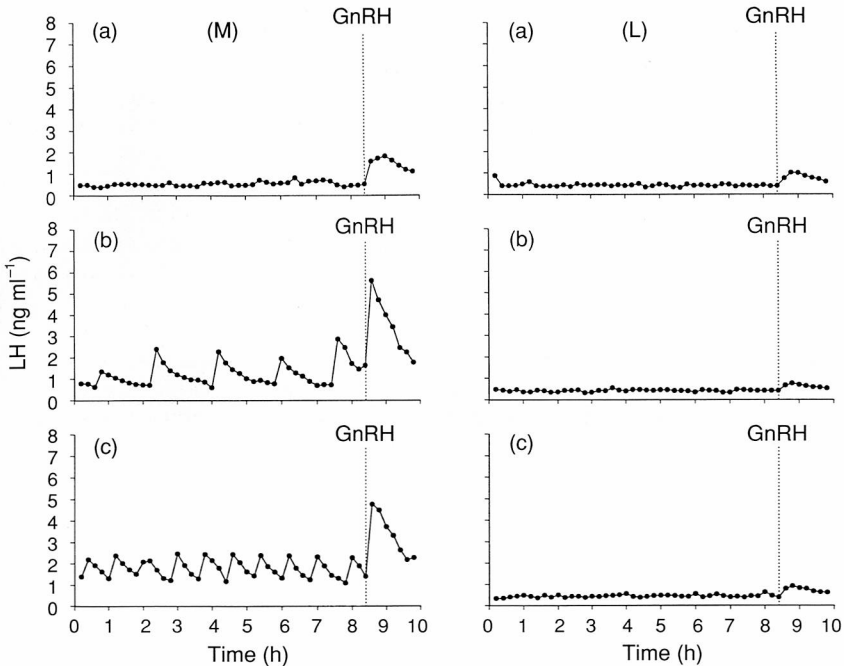
Fig. 6. Representative LH profiles for postpartum beef cows fed to either maintain (M) or lose approximately 20% (L) their bodymass for 6 weeks before and 8 weeks after calving. Samples were taken (a) when cows were ovary intact and suckled, 8 weeks postpartum; (b) 10 days later, after ovariectomy but while still suckled; and (c) 4 days later after weaning. At the end of each 8 h sampling period, 5 μg GnRH was injected i.v. and sampling continued for a further 90 min. Increases in LH pulse frequency after both ovariectomy and weaning in maintenance-fed cows were markedly suppressed in underfed cows. Reproduced with permission from Jolly (1992).

(Fig. 6), and neither mean plasma FSH nor LH concentrations increased after ovariectomy in these severely underfed cows. Moreover, plasma LH concentrations measured after a single exogenous GnRH challenge were consistently lower in underfed than in maintenance-fed cows in that study (Jolly, 1992). These data, together with those from the growth-restricted ewe–lamb model, suggest that severe undernutrition may suppress endogenous GnRH release to values below those required to maintain sufficient pituitary FSH and LH synthesis and secretion for the stimulation of ovarian follicle growth above the size at which they become totally dependent on gonadotrophins (for example ≥ 5 mm in cows).

As FSH appears to be essential for stimulation of growth of large follicles and for the induction or maintenance of aromatase activity (Greenwald and Roy, 1994), the absence of follicles > 5 mm in diameter or of large oestrogenic follicles reported in severely underfed cows (Perry *et al.*, 1991; Jolly, 1992) may reflect inadequate FSH stimulation or inhibition of FSH-dependent processes within the ovary. Similarly marked suppression of hypothalamic and pituitary function may underlie the syndrome of prolonged (3–6 months or longer) postpartum anoestrus in lactating cows during adverse seasonal conditions in the dry tropics, which markedly constrains reproductive rates in this environment (Entwistle, 1991; Jolly, 1992).

Other possibilities that require further consideration include possible nutritional modulation of FSH or LH microheterogeneity or steroid hormone metabolism, which may affect the physiological actions of circulating hormones. For example, lack of difference in FSH immunoactivity reported in some studies does not preclude possible effects of undernutrition on FSH bioactivity that may affect the ability of FSH to stimulate follicle growth and steroidogenesis. The regulation of FSH microheterogeneity has been

best studied in prepubertal ewe lambs. Although nutritionally regulated changes in endogenous GnRH secretion did not alter FSH isoform distribution in ovariectomized lambs (Padmanabhan *et al.*, 1992a), changes in isoform distribution may be regulated by changes in effects of ovarian feedback regulation of pituitary FSH synthesis or secretion (Padmanabhan *et al.*, 1992b; Hassing *et al.*, 1993). However, this speculation has yet to be tested, and current understanding of the functional significance of heterogeneity amongst LH and FSH isoforms is limited.

Potentiation of oestradiol negative feedback effects on gonadotrophin secretion in underfed heifers may also be related to increased concentrations of unbound ('free') steroid in plasma, due to reduced metabolic clearance or plasma protein binding. Low energy intakes have been associated with reduced binding capacity of sex-steroid-binding protein(s) in plasma of intact, but not ovariectomized, heifers, and with suppression of the increase in binding capacity stimulated by growth hormone and oestradiol (Lermite and Terqui, 1991). A positive relationship has also been demonstrated between the level of nutrition and the metabolic clearance rate of plasma progesterone in ewes, which was related to effects of intake on liver blood flow (Parr *et al.*, 1993). Although comparable studies have not been conducted in cattle, plasma concentrations of progesterone in lactating cows treated with an intravaginal progesterone releasing device were less than half of those in non-lactating ovariectomized cows and in non-parous heifers (Macmillan *et al.*, 1991; K. L. Macmillan, unpublished). It is likely that circulating concentrations of steroid hormones that affect feedback regulation of gonadotrophin secretion or the maintenance of early pregnancy are determined not only by ovarian outputs, but also by factors affecting their plasma protein binding or metabolic clearance rates, which may in turn vary with lactational and nutritional status.

Mechanisms Linking Nutritional State to Reproductive Function

A schematic summary of possible mechanisms by which undernutrition may inhibit the resumption of ovarian cycles in postpartum cows is presented in Fig. 7. As discussed, evidence suggests that the predominant inhibition is of GnRH secretion from the hypothalamus. However, little is known about the specific ways in which information about nutritional state is translated into neuroendocrine signals that affect GnRH secretion. Candidate mechanisms have been reviewed for sheep and cattle (Schillo, 1992) and include possible roles for metabolic hormones involved in the regulation of intermediary metabolism (particularly insulin), as well as the availability of specific amino acids (for example tyrosine) and oxidizable metabolic fuels. Recent studies have suggested that central effects of undernutrition on hypothalamic GnRH secretion may be partly mediated by a recently discovered peptide, neuropeptide Y, that also appears to play an important role in the regulation of feed intake and, consequently, energy balance in ruminants (Miner, 1992; McShane *et al.*, 1993).

Whether nutritional effects on follicular growth and function in monovular species such as cows are due simply to changes in gonadotrophin secretion, or involve local effects within the ovary, possibly mediated by metabolic hormones or growth factors, is unknown. Evidence reviewed here for nutritional effects on the growth and functional capacity of large follicles is consistent with likely effects of reduced LH or FSH secretion. Chronic undernutrition in suckled postpartum cows may induce a state analogous to that of hypophysectomy, totally suppressing the growth of follicles that are totally dependent upon gonadotrophin support (Perry *et al.*, 1991; Jolly, 1992). Nutritional inhibition of follicle growth and ovulation may be overcome by treatment with exogenous gonadotrophins in cows (Bishop and Wettemann, 1993). Similarly, chronically undernourished prepubertal lambs treated hourly with injections of LH could secrete oestradiol, elicit an LH surge, and form a corpus luteum in the same manner as well-nourished lambs (McShane and Keisler, 1991). Likewise, marked differences in body condition between groups of ewes chronically treated with a GnRH agonist to suppress endogenous LH and FSH secretion did not affect ovarian responsiveness to exogenous FSH infusion, in the presence or absence of concurrent treatment with exogenous oestradiol (Rhind *et al.*, 1993). In the last model, chronic undernutrition did not affect the numbers of gonadotrophin-dependent follicles (i.e. ≥ 2.5 mm in diameter), the proportion of these follicles that were oestrogenic, or their mean rate of oestradiol secretion *in vitro*, after FSH infusion. However,
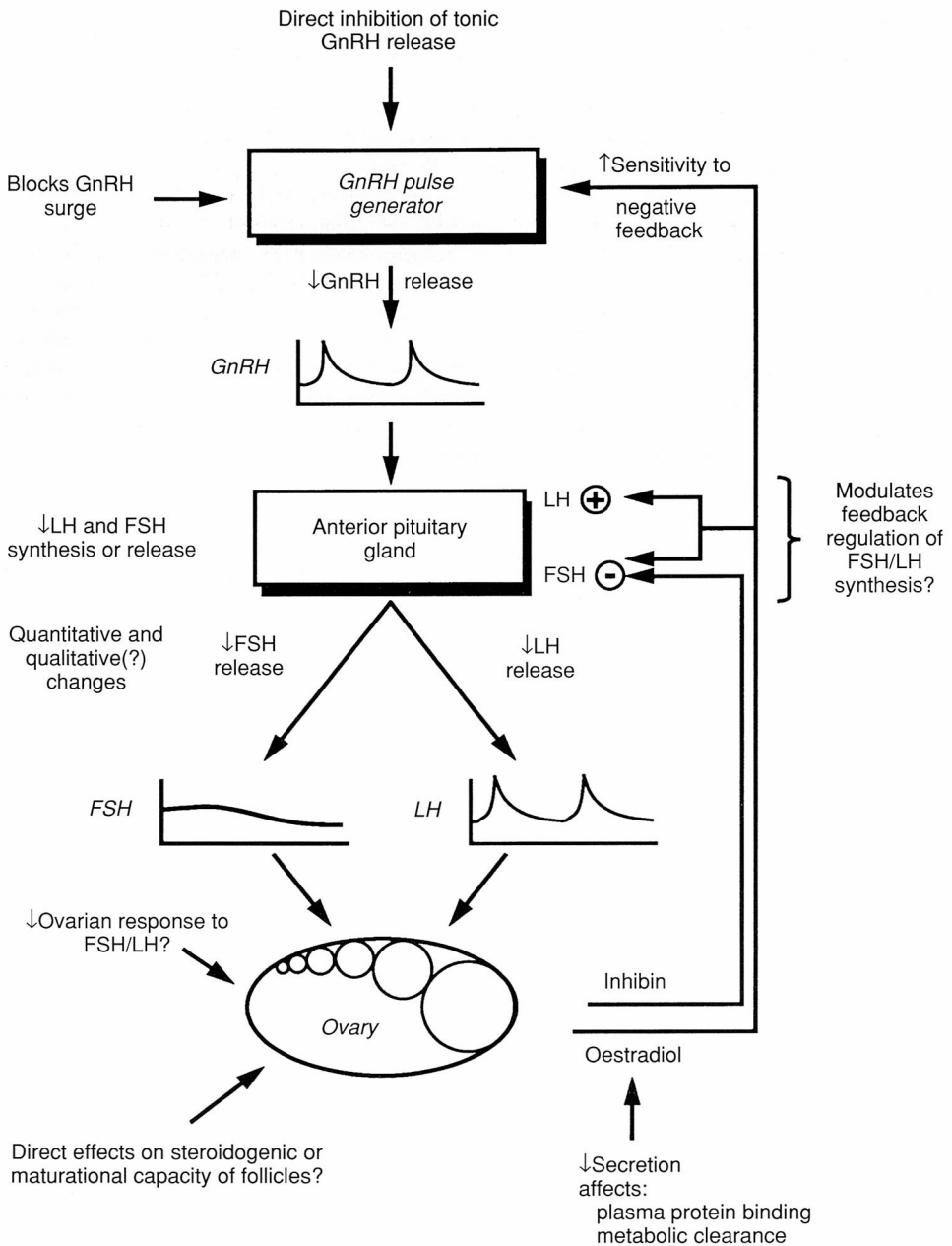
P. D. Jolly et al.

Fig. 7. Schematic summary of putative mechanisms by which undernutrition may inhibit the reproductive axis in postpartum cows. Tonic or surge release of GnRH from the hypothalamus may be inhibited by mechanisms that regulate sensitivity to ovarian feedback as well as through mechanisms that are independent of ovarian control. Reduced GnRH input to the anterior pituitary gland results in reduced LH pulse frequency and reduced LH and FSH synthesis and FSH secretion if pronounced. Inhibition of dominant follicle growth and persistence may reflect effects of reduced FSH or LH secretion, or effects of other endocrine or metabolic signals acting directly within the ovary. Undernutrition may also modulate ovarian feedback regulation of FSH and LH synthesis or secretion by effects on ovarian output of, plasma protein binding or metabolic clearance of, or hypothalamic or pituitary sensitivity to, feedback hormones such as oestradiol and inhibin.

none of these studies has examined whether nutrition may modulate the concentration of exogenous LH or FSH required to induce these effects, and hence ovarian sensitivity to gonadotrophin stimulation.

The gonadotrophic actions of LH and particularly FSH are known to be modulated by other hormones and growth factors acting in endocrine, paracrine and autocrine ways (Greenwald and Roy, 1994; Tsafriri and Adashi, 1994). These include insulin, GH and insulin-like growth factor I (IGF-I), which play key roles in intermediary energy metabolism and which are themselves nutritionally regulated (Bass *et al.*, 1992). It is likely that the fate of individual follicles is determined not only by the level of gonadotrophin stimulation, but also by the balance of numerous other stimulatory and inhibitory factors during critical phases of their development (Driancourt *et al.*, 1991). It is possible that suppression of key processes such as follicle recruitment and selection, or the ability of follicles to produce large amounts of oestradiol, may result from combined effects of reduced gonadotrophin stimulation and reduced potentiation, or inhibition, of gonadotrophin actions within follicle cells. However, most of the data that would support this contention come from studies of polytocous species such as rats and pigs *in vitro*. There is no direct evidence for such local effects in cows, and the role of metabolic hormones or growth factors in the local mediation of nutritional effects on follicle growth or steroidogenic function in this species remains speculative.

There is evidence that in ewes specific nutrients, such as glucose or branched chain amino acids, or metabolic signals such as insulin or IGF-I, can modulate ovulation rate independent of circulating FSH concentrations, by direct effects on ovarian follicle development (Downing and Scaramuzzi, 1991; Smith, 1991). Likewise, twinning rate in cattle (although low) may be increased by treatment with recombinant bovine growth hormone, probably via similar effects on the recruitment of small follicles mediated by direct ovarian effects of IGF-I or insulin (Gong *et al.*, 1993; Webb *et al.*, 1994). However, it is important to note that the principal mechanisms involved in the control of ovulation rate are different from those determining whether ovulation will occur. It may be that mechanisms for direct (intraovarian) nutritional modulation of ovarian function play a more important role in polytocous species such as rats, pigs and (to a lesser extent) ewes, in which nutrition is a major determinant of litter size, than in monotocous species such as cows.

Conclusions

The nature of genetic, environmental and husbandry factors and their interactions that affect the duration of postpartum anoestrus in cows is complex, but many of these factors appear to act via common hormonal mechanisms. The net effect of these is reflected by the dynamics of ovarian follicle growth and regression before first postpartum ovulation. Moderate levels of underfeeding, before or after calving, may interfere with the mechanism(s) of final follicle maturation and ovulation, whereas more pronounced nutritional deficiencies appear to interfere with the mechanism(s) regulating the size and dynamics of dominant follicle growth and regression. Severe undernutrition may result in the absence of follicles > 5 mm diameter. These changes are consistent with probable effects of reduced LH or FSH secretion that have been associated with inhibition of both tonic and surge release of GnRH from the hypothalamus. There is no direct evidence for local effects of undernutrition on ovarian function in cows. Studies that examine this issue often ignore the dynamic interdependencies between the hypothalamus, anterior pituitary gland, and the ovaries, and possible nutritional modulation of ovarian feedback mechanisms that affect gonadotrophin secretion. Further research is required to elucidate nutritional effects on the positive feedback regulation of gonadotrophin secretion and on the expression of behavioural oestrus, and to determine whether nutritional or metabolic state may influence ovarian function directly in a manner that affects the duration of postpartum anoestrus or fertility in postpartum cows.

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