Effects of Nutrition on Fertility in Dairy Cows

Maurice P. Boland and P. Lonergan

Faculty of Agriculture, University College Dublin, Belfield, Dublin 4, Ireland
E-mail: Maurice.Boland@ucd.ie

Take Home Messages

- Fertility has declined significantly in lactating dairy cows
- There are some effects of nutrition at the endocrine level; these are variable
- Nutrition can influence follicular dynamics, which in turn can alter fertility
- Nutrition influences early embryo development and hence the potential to establish fertility
- Treatments such as bST have both positive and negative effects on several aspects of fertility.
- Methods to manipulate follicular growth and oocyte quality may provide some guidance to improve in fertility in the long-term.

Introduction

Recent genetic improvement in dairy cows has led to a dramatic increase in milk yield, which has been associated with a decrease in reproductive performance (47,50,60). There are probably many reasons for this decline in fertility such as nutrition, management and expression and detection of estrus. High dry matter intake, increased ratio of plasma glucose to 3-hydroxybutyrate are positively associated with fertility, while higher NEFA concentrations are negatively associated with fertility (62). Reduced fertility is particularly obvious in cows where milk yields are above 6000 L per lactation (27, 39), and in cows fed in excess during the previous dry period (18). Early embryo mortality is a significant cause of reproductive failure in ruminants and part of this may be related to nutritional influences around the time of mating (11). Although the final manifestation of a detrimental effect of nutrition on fertility may be the death of the embryo, it is not totally clear if nutrition affects embryo quality through changing the developmental capacity of the oocyte or through changes

occurring during embryo development. Thus, the relationship between nutrition and reproduction is complex and responses are often quite variable and inconsistent. Energy requirements to support follicle growth, ovulation and early pregnancy are extremely low (less than 3 MJ of metabolisable energy (ME) per day) compared with requirements for maintenance and production (60-250 MJ ME per day in a lactating dairy cow). Nevertheless, in the case of lactating dairy cows, inadequate nutrition in the short term, or as a consequence of a prolonged depletion of body reserves during early lactation, can have significant deleterious effects on resumption of ovarian activity postpartum, conception rate and infertility. Deleterious effects of excessive nutrition around the time of mating on embryo development are becoming evident both in unsuperovulated cattle and in superovulated cattle. The objective of this paper is to review the effects of nutrition on some endocrine parameters, follicular development, oocyte quality and embryonic development with particular emphasis on dairy cows.

■ Nutrition and Gonadotrophin Secretion

Energy status is generally considered to be the major nutritional factor that influences reproductive processes, with prolonged low energy intake impairing fertility. In sheep, poor nutrition which results in lower ovulation rate is associated with decreased LH pulse frequency, which is likely due to inadequate hypothalamic GnRH secretion. In cattle, a strong correlation between negative energy balance in early lactation and resumption of ovulation postpartum is evident (5). While ovulation may not occur in animals on low dietary intakes, follicle growth and atresia will occur. Follicle wave turnover without ovulation is often evident in postpartum beef cows in poor body condition (57). The practical significance of this occurrence is a lengthening of the calving-to-first-ovulation interval, and often an extension of the calving-to-conception interval. Long-term restriction in feed intake has been shown to induce anestrus in cattle (48), due to insufficient circulating LH (49), which is likely to suppress follicle growth and oocyte maturation. These effects, however, are not immediately evident and dietary restriction for several months may be required to prevent follicle growth and ovulation. On the other hand, including supplementary fats in inadequate diets, results in increased concentrations of LH (16).

In contrast to the situations in monogastrics such as pigs and monkeys, effects of short-term dietary restriction on LH pulse patterns are more difficult to observe in ruminants. FSH is essential for follicular growth and ovulation. Yet there is little evidence of an effect of nutrition on plasma FSH concentrations. However, Mackey et al. (25) have shown that short-term restriction of dietary intake to approximately 40% of maintenance energy requirements increased FSH in heifers compared with those on 200% of maintenance energy requirements.

■ Effect of Nutrition on Progesterone Concentrations

Feed intake in sheep can influence the concentration of progesterone, with a strong negative correlation between dietary intake and progesterone concentrations (33). This effect is likely due to an increase in the rate of catabolism of progesterone and in hepatic circulation at higher feeding levels (44,45,52). Progesterone is believed to play an important role in oocyte maturation and in early embryo development.

Ad libitum feeding in heifers increased (31) decreased (59) or had no effect (56) on progesterone concentration when compared with restricted feeding. Liver blood flow and metabolic clearance of progesterone have been shown to be positively correlated and affected by increasing energy intake in dairy cows (52). Low progesterone post-breeding can reduce fertility (19). However, as steroids are selectively stored in fat, any dietary regime that results in fat mobilisation will result in the release of stored progesterone. This may account for some of the increased progesterone evident in animals on low dietary intakes. Concentrations of progesterone and embryonic interferon-*tau* have been reported to be positively correlated (28). Thus, minor changes in maternal progesterone concentrations during the initial period of embryo development may alter the secretion of this antiluteolytic agent and may be critical to embryo survival.

In sheep, overfeeding which reduced circulating progesterone concentrations also reduced pregnancy rates and decreased both the rate of development and viability of embryos (44). In cattle, Mann et al. (28) reported that the timing of the progesterone rise after ovulation is of critical importance to the development of the embryo. These authors showed that a delayed rise in progesterone was associated with smaller and potentially less viable embryos at Day 16. A more recent experiment in beef heifers showing the detrimental effect of an acute reduction of energy intake immediately after insemination on embryo survival, failed to find an association between progesterone concentration early in the oestrous cycle and embryo survival (10). Peripheral concentrations of progesterone on Days 0 and 1 relative to the LH peak are important for embryo survival in sheep. This presumably modifies follicular maturation and oocyte quality. However, others have suggested that the effect of progesterone on embryo development is acting primarily through the effect of progesterone in the uterus (1).

■ Nutrition and Ovarian Function

The ability of nutrition to alter ovulation rate and lambing rate of ewes is well known. A rapid improvement in body condition is usually associated with an increased ovulation rate and litter size. Alterations in ovulation rate may be

related to cell-entry-rate of glucose in animals on a high plane of nutrition. Dietary supplements containing high energy and protein have been shown to increase ovulation rate in ewes (7). Similarly, increases in ovulation rate were reported when glucose was infused directly (8). Thus, it is likely that short term energy supply is directly involved in follicle recruitment (15) and perhaps also in follicle growth; however this effect may be of short duration when diet level is altered.

Dietary restriction has been shown to alter follicle growth characteristics in cattle (38) and in superovulated sheep (66). Murphy et al. (38) reported that heifers on a low dietary intake had reduced size and persistence of the dominant follicle compared with animals offered higher energy intakes. Acute nutritional restriction (0.4 times maintenance) for about 12 days will not only decrease the growth rate and maximum diameter of the dominant follicle, but also cause ovulation-failure of the dominant follicle following induced luteolysis with prostaglandin (26). Several reports indicate that feeding fat altered the growth pattern of follicles and this effect is somewhat independent from energy (30). Supplemental fat increased the number of follicles (3,23) and increased the size of the preovulatory follicle (22). This increased size of follicle may have beneficial effects on both oocyte quality (20) and on corpus luteum function (30) resulting ultimately in higher pregnancy rates, as increased concentrations of progesterone during the luteal phase before and after breeding have been associated with higher pregnancy rates (4).

Nolan et al. (40) reported an increase in follicle numbers in heifers on a low dietary intake compared with heifers on a high dietary intake after stimulation with exogenous FSH. This difference in response was predominantly due to an increase in the number of follicles in the 7-10 mm size range when measured around the time of the LH surge. Thus, it is clear that dietary intake can under certain conditions alter the growth characteristics of follicles, but the effect of dietary intake on the number of follicles growing in response to stimulation with a fixed dose of FSH during superovulation is less consistent and thus, in that case it is more difficult to draw firm conclusions.

■ Nutrition and Oocyte Quality

While ultrastructural differences have been reported in oocytes from superovulated compared with unstimulated heifers (2), few studies report effects of nutrition on oocyte quality in detail. McEvoy et al. (33) reported that a higher proportion of ova from ewes on a low energy diet were considered viable when compared with those produced in ewes on a high energy diet. In cattle it has been shown that restricting energy intake before slaughter enhanced the subsequent in vitro development of the oocytes from small follicles (32). Yaakub et al. (67) stimulated heifers with FSH and subjected them to a low (silage alone) or high (silage plus 6 kg concentrates) diet prior to slaughter.

Cleavage rate was improved although no significant difference was evident in the in vitro blastocyst formation rate of oocytes collected from cattle on the low diet. A major problem with this type of experiment is the limitation on the amount of material available from one animal at slaughter. Further research from our laboratory suggests that the in vitro blastocyst yield of oocytes collected over several weeks using transvaginal ovum aspiration could be enhanced by restricting the dietary intake of heifers (41). This indicates that the effect of nutrition on reproduction may occur at the level of the oocyte prior to ovulation.

■ Nutrition and Embryo Quality

Short-term restriction in dietary intake has been shown to increase the subsequent pregnancy rates in cattle (9). Mantovani et al. (29) reported that the yield of transferable embryos following superovulation in beef cattle was significantly reduced when heifers had ad libitum access to concentrates compared with restricted levels. Concentrate type and quantity will affect the subsequent yield of transferable embryos following superovulation in beef cattle (65). Heifers on a restricted diet, where the predominant concentrate supplement was in the form of citrus and beet pulp, produced more transferable embryos compared with those where barley was the predominant concentrate or with those on ad libitum quantities of concentrates. Severe restrictions in dietary intake have been shown to have beneficial effects on embryo development when heifers were superovulated and embryos were collected 7 days after breeding and then cultured for 24 hours (41). This comparison of severe restriction versus ad libitum feeding resulted in an increase in the number of blastocysts after culturing embryos for 24 hours. An increase in the total cell number per blastocyst was also noted.

The effect of extremes of dietary intake on embryo development is evident but the point at which this change occurs is still unknown. When ewes were infused with high levels of glucose and superovulated, yield of good quality embryos was reduced (66). Glucose infusion has also been reported to reduce pregnancy rate. While the reason for such an effect of glucose on embryo development is not clear, it may be due to unusually high plasma glucose concentrations interfering with cell signaling mechanisms during pre-ovulatory follicle growth, oocyte development or early embryo development. It is known that high glucose concentrations are deleterious to embryo development in vitro (13, 34). There are also suggestions that hyperglycaemia in ewes is associated with poor embryo quality, an observation consistent with the relatively high incidence of developmental malformations occurring in diabetic women. Similar effects of excess glucose on pre-implantation embryo development are the basis for the practice in assisted reproduction programs of administering insulin to diabetic mothers to reduce blood glucose before embryo recovery.

Blastocyst formation is well recognized as a key developmental process in the growth of an embryo. The blastocoel cavity forms as a consequence of fluid transport across the trophectoderm. This process is partially facilitated by Na/K-ATPase; messenger RNA for this enzyme has been identified in Day 7 bovine embryos. Dietary intake and diet type can alter the expression of transcripts of genes involved in early embryo development, such as Na/K-ATPase and Cu/Zn SOD (64). Abecia et al. (1) reported a decrease in the in vitro secretion of interferon-*tau* in Day-15 embryos from undernourished ewes. An increase in the in vitro secretion by endometrial tissue of $PGF_{2\alpha}$ was evident in the same animals. Thus, nutrient requirements for optimum follicle growth and embryo development may be quite different. This highlights the importance of diet around the time of mating and in particular the significance of extreme overfeeding or underfeeding post mating, in regulating pregnancy rate.

Table 1: The effect of low (40 MJ ME per day) or high (120 MJ ME per day) dietary intake on mean (±SEM) embryo yield on Day 7 in superovulated heifers, and development capacity in vitro.

Diet	Low	High
No. of corpora lutea	16.3 ± 3.0	14.4 ± 1.9
No. of ova / embryos recovered	11.4 ± 2.4	10.4 ± 1.3
No. of grade 1+2 embryos	4.5 ± 1.3	3.5 ± 1.1
Embryo culture for 24 hours		
Blastocyst % (number)	73 (78/107)[a]	41 (44/106)[b]
Blastocyst cell number	98.3 ± 3.0[c]	75.4 ± 2.3[d]

From: (41). Within rows a, b $P < 0.01$; c, d $P < 0.001$.

■ Effect of Milk Production on Embryo Development

The effect of milk yield on the number of oocytes recovered and blastocysts formed following in vitro maturation, fertilization and culture of bovine oocytes was studied by Snijders et al. (54). Oocytes were collected from high and medium genetic merit dairy cows after slaughter between 125 and 229 days postpartum (Table 2). Ovaries were recovered and follicles of 2 to 10 mm were aspirated. Cleavage rate and number of blastocysts were determined 44 hours and 7 days after insemination, respectively. Oocytes from high merit cows formed fewer blastocysts and had lower cleavage and blastocyst formation rates than those from medium merit cows. In a retrospective analysis, there was no difference in the number of oocytes recovered and subsequent development into blastocysts between the cows in a high milk production group and cows in a low milk production group. Cleavage rate and blastocyst formation rate was greater for oocytes from cows in high body condition score (3.3 - 4.0, n = 20) than for oocytes from cows in low body condition score (1.5 - 2.5, n = 20) (75.7% vs. 61.9% and 9.9% vs. 3.0%, respectively). Therefore,

high genetic merit cows with low body condition score yielded oocytes of lower quality. This observation is consistent with reports of low cell number in embryos collected from dairy cows when embryos were collected between 30 and 90 days postpartum (S. Snijders, unpublished). Moreover, preliminary findings from our laboratory suggest that oocyte developmental competence in vitro is increased in oocytes collected by transvaginal ovum aspiration from cows in the early postpartum period that are maintained on restricted dietary intake (J.M. Lozano, unpublished). Thus, reductions in oocyte quality are likely to contribute to the reduced fertility often evident in high genetic merit dairy cows.

Table 2. The effect of genetic merit on the number of oocytes recovered and their subsequent cleavage and development to blastocysts (mean ± SEM) in dairy cows

	High genetic merit	Medium merit
No. of cows	48	46
No. of oocytes collected per cow	6.7 ± 0.75	7.6 ± 0.91
Cleavage rate % (n)	70.4 (238/338)[a]	77.4 (278/359)[b]
No. of blastocysts per cow	0.36 ± 0.19[a]	0.85 ± 0.22[b]
Blastocyst formation rate % (n)		
from oocytes cultured	6.8 (23/338)[a]	11.4 (41/359)[b]
from cleaved oocytes	9.7 (23/238)	14.7 (41/278)

Values with different superscripts within rows are significantly different [a, b] $P < 0.05$.
From : (54).

■ Effect of Feed Intake on Fertility

Total dietary intake can affect fertility, both at the level of the oocyte and embryo. In dairy cows, oocytes aspirated transvaginally from early postpartum dairy cows fed ad libitum grass silage and 1 kg of concentrate had better morphological grading and cleavage rate and a trend towards a higher blastocyst formation rate than those aspirated from cows on ad libitum grass silage and 5 or 10 kg of concentrate (21; Table 3). It is possible that similar effects of high dietary intake and metabolic load are affecting the quality of embryos produced in high yielding dairy cows. Preliminary evidence in dairy cows suggests that embryo quality as recorded by total cell number after recovery on Day 7, is reduced in dairy cows offered large quantities of food in early lactation (54).

Thus, high intake diets exert a negative effect on the developmental capacity of embryos. The effect of nutrition is exerted very early in development, possibly before fertilization during the acquisition of developmental competence by the oocyte. This negative effect of high dietary intake or metabolic load on fertility is a fundamental challenge that needs to be addressed in high production cows, where fertility is compromised.

Table 3. Percentage of grade-1 oocytes (good oocytes) obtained by transvaginal aspiration and cleavage rate and blastocyst formation rate when cultured in vitro in groups. Oocytes were aspirated from early postpartum dairy cows fed grass silage ad libitum and 10 kg (High), 5 kg (Control) or 1 kg (Low) of concentrate daily. Oocytes obtained from slaughterhouse ovaries were cultured as controls.

	Slaughter-house	High	Control	Low
Good oocytes (%)		94/232 (40.7)[a]	66/192 (34.4)[a]	27/56 (48.2)[b]
Cleavage rate (%)	65	11/28 (39)[a]	22/47 (47)[a]	12/18 (67)[b]
Blastocyst rate (%)	30.5%	1/28 (3.5)	0/47 (0)	4/18 (22)

[a,b]P<0.05 From (21)

■ Effects of Dietary Fat on Fertility

The modern high genetic merit dairy cow prioritizes nutrient supply towards milk production in early lactation. This demand appears to take precedence over providing optimal conditions for reproduction. Early in lactation, milk output rises faster than nutrients can be supplied from feed intake. Thus, in order to meet the nutritional demands of milk production, the cow mobilizes energy reserves from her body and enters a phase of negative energy balance. The energy deficit experienced by cows can be reduced by increasing the energy density of lactating rations. Fats are incorporated into the diet of dairy cows in early lactation to minimize the difference between energy input and energy output.

Many studies have reported positive effects of dietary fat supplementation on reproductive performance in cattle. A review examining the influence of supplemental fats on reproductive tissues and performance of lactating cows (58) showed that 11 of 20 articles reported improvement in either conception rate to 1st AI, overall pregnancy rate, overall conception rate, days open or AI per conception. Studies that report a negative influence of dietary fat supplementation on reproductive performance are often accompanied by large increases in milk production (12,53).

Several putative mechanisms have been suggested to explain the positive effects of dietary fat on reproduction. Dietary fat supplementation has been reported to increase the size of preovulatory follicles (3,24), increase the number of follicles, (61) and increase the growth rate of the dominant follicle (42). Supplemental fat may partially reduce energy deficits, however, early postpartum improvement in fertility occurred in some studies independent of the energy status of cattle (24). Fat supplementation has been reported to increase the lifespan of corpora lutea (63). The concentration of plasma cholesterol, which is a precursor of progesterone, is increased consistently

under regimes of supplemental fat (55). Total progesterone production during an estrous cycle has been increased in cows offered dietary fat (16,55). The increased progesterone was associated with increased lipid accumulation in luteal cells of the corpus luteum and a slower rate of disappearance of progesterone from serum. Increases in cholesterol and progesterone have also been reported in the follicular fluid of cattle on supplementary fat diets (51).

■ Interaction between Follicle Quality and Oocyte and Embryo Quality

It is important to differentiate between optimum conditions for follicle growth, (both in terms of number of follicles and paracrine environment), and optimum conditions for embryo survival. Nutritional conditions may not be similar for both. While the significance of increased glucose on increasing ovulation rate has been identified above, it is possible that increased glucose is also having a deleterious effect of embryo development. Preimplantation mouse embryos exposed to hyperglycaemia have delayed development. High dietary intakes that would be likely to alter circulating glucose have resulted in reduced embryo quality in cattle (29,40,65). While direct infusion of glucose to ewes can increase ovulation rate (8), embryo quality can be dramatically reduced (13,66). It is not clear why high concentrations of glucose have such deleterious effects, but it is possibly due to interactions between insulin, glucose and glucose transport proteins during early embryo development. Before blastocyst formation, metabolic reliance of embryos is on lactate and pyruvate rather than glucose. Indeed it is likely that pre-morula embryos cannot metabolise glucose. Glucose transport was increased in mouse embryos in response to glucose deprivation, and both IGF-1 and insulin can stimulate glucose uptake (43). These authors also suggested that GLUT1 is the regulatable transporter in the mouse blastocyst, and that GLUT1 may be an insulin-stimulated recruitment. Moley et al. (36) suggest that elevated concentrations of glucose may alter activity of the polyol pathway and Krebs cycle, resulting in retarded embryo development. These types of observation indicate the significance that changes in the glucose-insulin-IGF-1 axis may have on early embryo development.

■ Effects of bST on Fertility

The use of sustained-release formulations of recombinant bovine somatotropin to increase production in cows is part of a management practice in some dairy herds; this will increase milk production by 10-20% (46). However, there are both positive and negative reports of bST on reproductive function. It has been reported that bST increased the interval from calving to conception (35),

reduced the expression of oestrous behaviour (17) and reduced the concentration of LH (6).

Other studies indicate a beneficial effect on follicular dynamics (14), such as an increase in the numbers of small follicles and earlier development of the preovulatory follicle. Higher pregnancy rates have also been observed in cows treated with bST (36,37). The data suggest that the effects of bST are related to the beneficial effect of IGF-1 on the preovulatory follicle and on the oocyte with a possible positive effect on subsequent CL function. Moreira et al. (37) demonstrated that bST decreased the incidence of unfertilized ova, increased the percentage of transferable embryos in donors, and increased pregnancy rates in recipients.

In conclusion, there are many factors that influence pregnancy in dairy cows and in beef animals. Nutrition seems to have a major effect, but its precise role in relation to follicle growth and oocyte quality is still not fully understood; this will require further attention in future to address the problem of inferior oocyte quality.

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