

Influence of Maternal Nutritional Factors on Ovarian Folliculogenesis in Cattle

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Abstract

Assisted reproductive technologies, such as artificial insemination and embryo transfer, have produced significant outcomes in cattle. These technologies are useful not only for improving fertility but also for increasing the rate of genetic change in beef and dairy livestock. Today, embryo transfer in cattle is both practical and commercially viable. However, bovine embryo transplantation still remains suboptimal under on-farm conditions, one of the main reasons for which may be inefficient nutritional management. The role of nutrition in modulating reproduction and particularly in ovarian function remains unclear. Considerable effort has been made to elucidate the nutritional factors regulating the development of follicles in either a direct or an indirect manner. Moreover, evidence indicates that bovine folliculogenesis is modulated by gonadotropins as well as by metabolic hormones, including growth hormone, leptin, insulin and insulin-like growth factor-1. In addition, metabolites – such as glucose, urea and non-esterified fatty acids – are considered to play a role in regulating ovarian follicular development. Nutrition affects folliculogenesis indirectly by altering the activity of reproductive hormones. Furthermore, nutrition has local impact on the development of follicles at the ovarian level. As a result, nutrition is possibly decisive to the fate of bovine oocyte development, since oocyte quality influences reproductive outcomes such as fertilization and pregnancy rates. Although numerous reviews in the published literature describe nutritional roles in reproduction, the primary focus of this paper is the effect of nutritional factors on folliculogenesis in cattle. A greater understanding of this influence may facilitate nutritional manipulation in order to enhance fertility in cattle in a cost-effective yet very efficient way.

Keywords: Nutrition; Folliculogenesis; Ovary; Fertility; Bovine; Cattle; Hormone; Gonadotropin; Leptin; Insulin; IGF-1

Introduction

The majority of cattle across the world are reared through pastured-base systems, so the availability and quality of grass affects livestock performances, including growth, reproduction and yields of beef and milk. Nutrition provides a basic foundation for reproductive performance; therefore, it is apparent that nutrition plays an important role in enhancing fertility in cattle [1]. However, the effect of nutritional strategies on reproduction is open to debate. Fertility can be improved through nutritional management [2]; nonetheless, the study of Mousavi *et al.* [3] on the effect of fish meal or n-3 fatty acids on ovarian and uterine dynamics, indicates no effect of such diets on ovarian function. Consistent with this, Velazquez *et al.* [4] reported that nutritional strategies do not increase the efficiency of bovine superovulation. Robinson *et al.* [5], however, contended that high-plane feed does enhance oocyte and embryo quality. Moreover, undernutrition has a negative impact on fertility [6,7], suggesting that an adequate supply of nutrients is very important for normal reproduction performance in livestock. A good example is dairy cattle during the transition period, when postpartum cows may suffer from a negative energy balance (NEB). This results particularly in deleterious effects on reproduction in normally high-yielding animals since all nutrients are required prior to milk production. Although the roles of nutrition in reproduction are often complicated and experimental results have been inconsistent [8], numerous studies have attempted to elucidate the functions of metabolites and metabolic hormones in regulation of folliculogenesis. For instance, Silva *et al.* [9] demonstrated clearly the role of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) systems in controlling ovarian folliculogenesis. Furthermore, the influence of nutrition is either direct or indirect [6]. Published research covers primarily the role of gonadotropin hormones (GnRH) in regulating the development of follicles; here, we describe the interrelationship between nutrition and follicular development in cattle. An appreciation of this complex relationship would facilitate enhanced manipulation of reproduction through improved nutritional strategies.

Ovarian folliculogenesis in cattle

Folliculogenesis is defined as “an intricate process that involves the proliferation and differentiation of both somatic and germ cells” [10]. It is regulated by GnRH, metabolic hormones and local factors like IGF-1 systems. Primordial follicles form initially

during the foetal life of a mammal [10]; however, it is important to note that early folliculogenesis and the start of primordial follicle growth are not influenced by GnRH [11]. Monniaux *et al.* [12] maintain that anti-Müllerian hormone (AMH), derived from the granulosa cells of developing follicles, reflects the ovarian follicular reservoir. For example, Gamarra *et al.* [13] reported significantly more small follicles (ranging from 2-7 mm in diameter) in heifers with medium and high plasma AMH than in those with low plasma AMH. Each primordial follicle passes through a continuous series of progressively more developed stages (primary, secondary, pre-antral and antral) towards either ovulation or, in most cases, atresia – the process by which immature ovarian follicles degenerate and are subsequently re-absorbed.

Jaiswal *et al.* [14] contend that a two-wave pattern or three-wave pattern of follicular development is predictable and repeatable within a single oestrous cycle in cattle. During a follicle wave, each cohort of follicles passes through three stages: growing; static; and regressing. In the growing phase, recruitment of follicles is controlled by follicle-stimulating hormone (FSH) [15]. In this stage, the growth rates of recruited follicles around 4 days are similar [14], and the difference occurs during the following static stage when growing follicles progress through deviation. It is well established that the first follicle requiring luteinizing hormone (LH) receptors becomes the dominant follicle while the others depending on FSH regress in response to decreasing concentrations of circulating FSH [11]. Ginther *et al.* [16] showed that the difference in growth rates between the largest subordinate follicle and the future dominant follicle is accounted for by the time taken for deviation or early static stage. They found that the decisive stage occurs at approximately 8 mm and that the dominant follicle produces inhibin hormone which suppresses development of subordinate follicles, resulting in their apoptosis. Moreover, a developing dominant follicle secretes oestrogen that is involved in initiation of luteolysis at the end of the luteal phase. Consequently, the frequency of LH pulses leads to the ovulation of a selected dominant follicle. It is apparent that GnRH play a key role in modulation of folliculogenesis [17,18]; however, follicular growth is monitored by sophisticated interactions between extra-ovarian factors, such as gonadotropins and metabolic factors, and intra-ovarian factors [18] that are described later.

Indirect effects of nutrition on ovarian folliculogenesis

It is evident that metabolic hormones interact with GnRH to regulate ovarian follicular development. Diskin *et al.* [6] pointed out that nutrition indirectly controls folliculogenesis by altering levels of GnRH. It has been shown that a range of metabolic hormones, such as leptin, insulin and IGF-1, may also mediate reproduction. Leptin is a peptide secreted by adipose tissues [19,20], which performs crucial roles in control of feed intake and body weight [20]. In addition, leptin is considered a mediator that is responsive to nutritional stress [21].

In a study of goats, Batista *et al.* [19] demonstrated that leptin and its receptor expressed at mRNA and protein levels are present in all constituents of antral follicles, with particularly high levels in granulosa cells. Williams *et al.* [22] revealed that leptin can stimulate GnRH secretion in cattle. However, Amstalden *et al.* [7], using *in vitro* approaches, suggested that in fully fed cattle leptin does not stimulate release of GnRH from the hypothalamus or adenohipophyseal explants and cells. They further noted that in well-fed animals the effect of leptin on the release of GnRH is limited. In corroboration, Zieba *et al.* [21] claimed that leptin does not increase the frequency of LH pulses in peripubertal heifers regardless of nutritional status and that in fully fed cattle leptin has no impact on the secretion of GnRH and LH. Also, supplementation of leptin in *in vitro* maturation medium does not confer beneficial effects on the developmental competence of prepubertal bovine oocytes [23]. Nevertheless, Block *et al.* [24] demonstrated that leptin is regulated by insulin and GH, and that undernutrition leads to reduction of insulin plasma concentration.

Similarly to leptin, insulin acts as an indirect regulator of development of follicles. In the case of high-producing dairy cows, Garnsworthy *et al.* [25] reported that both rumen-digestible starch and rumen-bypass starch are effective at maintaining plasma insulin and ovarian function. Lucy *et al.* [26] noted that in dairy cows nutrition partitioning causes liver failure in response to GH and to a low production of IGF-1. Interestingly, IGF-1 in association with GH and insulin also plays an indirect role in folliculogenesis in cattle. Chase *et al.* [27], working with Brahman cattle, showed that administration of GH or GH plus insulin elevates significantly circulatory concentrations of IGF-1. However, it appears that IGF-1 has a local influence in follicular development, and this is discussed in detail below. In summary, orchestrated by gonadotropins, metabolic hormones regulate bovine folliculogenesis. It is in this context that the question can be posed of whether nutritional factors can directly modulate folliculogenesis.

Direct effects of nutrition on ovarian folliculogenesis

Nutrients are indispensable for cell maintenance, growth and reproduction. Metabolic hormones exert a direct influence on folliculogenesis at the ovarian level. In intensive-based feeding systems, the addition of either rumen-digestible starch or, less so, bypass starch affects the number and size of follicles [25]. Diskin *et al.* [6] contended that nutrition directly regulates ovarian development via the hepatic system. In agreement with this proposal, Dawuda *et al.* [28] noted that feed intake may affect the hepatic clearance rate of steroid hormones and that consequently circulatory levels of these hormones may influence folliculogenesis.

Metabolic hormones such as leptin, insulin and IGF-1 either suppress or stimulate developing follicles in a concentration-dependent manner. Chagas *et al.* [8] highlighted the local effect of IGF-1 on follicles. Shahiduzzaman *et al.* [29] experimented with inoculation of IGF-1 into dominant follicles. Interestingly, they found that treatment of the second follicle led to an inhibitory effect on the development of the largest follicle by decreasing its growth rate, and thereby measured diameter, suggesting that IGF-1 enhances

development of the follicle during the deviation period. This may explain why reductions in insulin and IGF-1 delay the oestrus cycle and impair the quality of oocytes and corpus luteum function. This is consistent with the report of Cavestany *et al.* [30] showing that the size of the dominant follicle is correlated with IGF-1 concentrations in peripheral blood, and that IGF-1 stimulates development of a larger dominant follicle. Furthermore, Spicer *et al.* [31] revealed that IGF-1 stimulates bovine granulosa cell proliferation. In postpartum cattle, severe NEB decreases hepatic expression of insulin-like growth factor binding proteins (IGFBP)-3, -4, -5 and -6, but increases levels of IGFBP-2 [32]. Synthetic IGF-1 is depressed by severe NEB [32]. A dynamic activity of the IGF system is necessary for follicular selection and advancement before the preovulatory stage [33].

Consistent with the action of IGF-1, insulin plays a role in regulation of ovarian function. Robinson *et al.* [5] showed that resumption of ovarian activity in postpartum cattle is stimulated mainly by insulin. Castañeda-Gutiérrez *et al.* [34] noted that in dairy cows glucose is depressed and insulin levels decrease sharply during the transition period. Moreover, Garnsworthy *et al.* [35] reported that raised plasma insulin concentrations are capable of restoring ovarian activity; nonetheless, they concluded that alteration of levels of this metabolic hormone by introduction of metabolisable protein such as leucine does not have a significant effect on ovarian function in lactating dairy cows. Starch supplements may also not affect plasma insulin concentration and ovarian function since both the products of rumen-digestible starch, propionate, which is absorbed across the rumen wall, and glucose from bypass starch absorbed from the small intestine, affect levels of plasma insulin [25].

It is important to note that metabolites are also involved in regulation of ovarian function. Oliveira Filho *et al.* [2] showed that blood cholesterol, triglycerols and total lipid pattern each correlate with the development of follicles. These authors further suggested that nutritional supplementation before the parturition period is beneficial to maintaining body condition score (BCS) since this strategy efficiently increases availability of cholesterol for assuring ovarian function as well as for improving resumption of ovarian activity. In cattle, BCS is a criterion that is used to determine the nutritional status of an animal. Postpartum cattle in particular experience a NEB due to mobilisation of body reserves for maintenance and lactation [6,13]. A deep NEB reduces the LH pulse, which thereby influences dominant follicles [36]. As a consequence of a NEB there is an increase in plasma non-esterified fatty acids (NEFA). Burke and Roche [37] found that plasma concentrations of NEFA are accelerated when cows have low pasture intakes. However, Burke *et al.* [38] claimed that the increase in nonstructural carbohydrate content of the transition diet did not affect postpartum anovulatory intervals in grazing cattle.

In accordance with the influence of glucose and lipid, protein may also play a role in follicular development. Cattle that are fed regularly with high levels of dietary urea are thought to tolerate the toxicity of urea due either to rumenal microflora increasing methane production through their ammonia digestion or to a change in the cows' hepatic metabolism [39]. Dawuda *et al.* [40], who investigated the effect of excess intake of rapidly degradable nitrogen on metabolism and reproduction in lactating cows, reported that addition of 250 g urea per cow per day neither significantly increases milk yield nor elevates plasma concentrations of insulin and IGF-1. In agreement with this study, Moallem *et al.* [41] concluded that although supplementation of high dietary crude protein for non-lactating heifers results in penetration of urea into pre-ovulatory follicles, this does not impair the features of such follicles.

In extensive farming systems, in spring an imbalance in protein digestion may have a potentially profound impact on the quality of bovine oocytes. However, excess protein supplementation would appear to cause a detrimental impact on the developmental competence of oocytes. Velazquez [4] noted that urea impairs the maturity of oocytes and decreases their quality. This is characterised by a gradual decrease in dry matter intake by cattle and their mobilisation of body fat reserves, resulting in an increase in NEFA but with frequently observed insulin reduction [34].

Nutritional strategies to improve reproductive performance in cattle

Further to consideration of the direct and indirect effects of metabolites and nutrients on fertility in cattle, it is clear that nutritional strategies should be sought to improve follicular quantity and quality. Childs *et al.* [42] showed that supplements of dietary n-3 polyunsaturated fatty acids increase IGF-1 concentrations, leading to improvement in fertility. Incorporation of short-term nutritional supplementation into artificial reproductive technologies such as artificial insemination and embryo transfer may improve outcomes. Gamarra *et al.* [13] reported that for dairy heifers addition of short-term dietary propylene glycol results in a series of changes in metabolites, metabolic hormones, plasma progesterone and small follicle population, causing stimulation of follicular growth. In pasture-based systems, it is beneficial to supplement a highly nutritious diet for multiparous cows in the prepartum period as this strategy enhances ovarian resumption and stimulates the onset of ovulation after calving [25]. Increased provision of dietary starch leads to a rise in plasma insulin, producing a change in oocyte quality. Under pastoral conditions of low quality grass, and availability of a cereal-based supplement equivalent to a 0.9% of live weight concentration is crucial to supply energy, which promotes elevated insulin concentrations in blood [36]. Administration of both GH and insulin significantly improves plasma IGF-1, IGFBP-3, -4 and -5, but decreases IGFBP-2 and urea-nitrogen [27].

In contrast, it appears that in cattle with a high nutritional status, represented by a high BCS, the effects of nutritional supplementation on reproductive performance remain unclear. Santos *et al.* [43] noted that in moderately underfed non-lactating cows nutritional supplementation can enhance the quality of oocytes and embryos. Nevertheless, considerable research on nutritional additions for well-fed cattle has shown that such approaches may not be effective to enhance superovulatory yields [4].

Conclusion

It is evident that nutrition affects bovine ovarian function. In addition, the effects of nutrients as well as of metabolic hormones on folliculogenesis occur both by direct and indirect means. Hence, the diet of cattle probably changes the progress of follicular development, resulting in fluctuations in the turnover of follicles. The mechanism of ovarian regulation of metabolic hormones remains unclear, suggesting that further investigations are required to elucidate this underlying metabolic pathway. Dietary improvements to pasture and forage plants should be considered since they are a very cost-effective way to enhance animal reproductive performance.

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