

# Interactions between nutrition and ovarian activity in cattle: physiological, cellular and molecular mechanisms

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The effects of acute changes in dietary intake on ovarian activity can be correlated with changes in circulating concentrations of metabolic hormones including insulin, insulin-like growth factor I (IGF-I), growth hormone and leptin. There is no corresponding change in circulating gonadotrophin concentrations and it is proposed that the dietary induced changes in ovarian activity, resulting from acute changes in dietary intake, are a result of direct actions of these metabolic hormones on the ovary. Changes in the peripheral concentrations of insulin, IGF-I and leptin were also associated with the initiation of a synchronized wave of follicle growth and it is hypothesized that oestrogen secreted by the developing follicle is involved in regulating the secretion of these metabolic hormones. At the cellular level, physiological concentrations of insulin and IGF-I interact to stimulate oestradiol production by granulosa cells. In contrast, leptin inhibits FSH-stimulated oestradiol production by granulosa cells and LH-stimulated androstenedione production by theca cells. At the molecular level, dietary energy intake affects the expression of mRNA encoding components of the ovarian IGF system and these changes can directly influence the bioavailability of intrafollicular IGF. This, in turn, can increase the sensitivity or response of follicles to FSH and is one mechanism through which nutrition can directly affect follicle recruitment. Dietary induced increases in intrafollicular IGF bioavailability also have a negative effect on oocyte quality, and diets that are optimal for follicle growth may not necessarily be optimal for oocyte maturation.

## Introduction

Nutritional status is a major factor influencing the ability of an animal to reproduce (Robinson, 1990; O'Callaghan and Boland, 1999; Robinson *et al.*, 1999; Webb *et al.*, 1999a,b; O'Callaghan *et al.*, 2000). In adult females, dietary intake acts at various levels within the hypothalamus–pituitary–ovarian axis to influence ovarian activity and is a key factor regulating

embryo survival during pregnancy. However, the detailed physiological mechanisms through which nutrition exerts many of these effects remain to be fully characterized.

A large number of studies have described the effect of nutrition on follicle development. Dietary intake has been positively correlated with the growth rate of the ovulatory follicle (Murphy *et al.*, 1991; Bergfeld *et al.*, 1994; Rhodes *et al.*, 1995; Mackey *et al.*, 1999) and the growth of small ovarian follicles in heifers (Gutierrez *et al.*, 1997a). During lactation, the extent of the negative energy balance deficit is a major factor affecting follicle growth (Beam and Butler, 1999). As well as regulating follicle dynamics, an increasing number of studies are highlighting the link between dietary intake and developmental competence of oocytes (O'Callaghan and Boland, 1999; Boland *et al.*, 2001). Improved nutritional status is positively correlated with embryo survival and is a major factor influencing efficiency in assisted reproduction technologies.

This brief review will concentrate on mechanisms through which acute changes in nutritional status directly regulate ovarian activity. The aim is threefold: first, to summarize the effects of dietary intake on metabolic hormones, particularly insulin, insulin-like growth factor I (IGF-I), leptin and growth hormone in cattle; second to describe some of the cellular and molecular mechanisms through which these hormones act to regulate ovarian function; and third to discuss how these changes influence oocyte developmental competence.

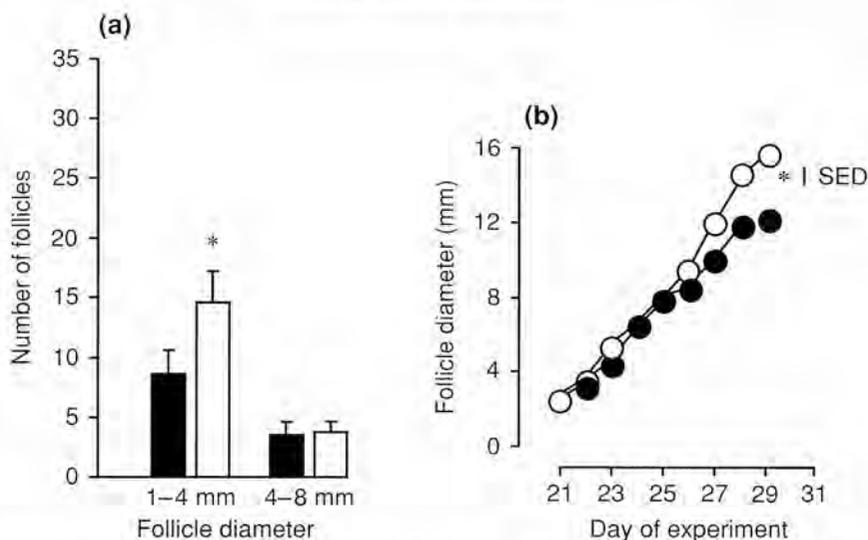
### Nutrition: metabolic hormone–ovarian interactions

Recent studies have shown that short-term changes in the plane of nutrition regulate follicle recruitment without affecting circulating concentrations of FSH (Gutierrez *et al.*, 1997a; Armstrong *et al.*, 2001, 2002a; Gong *et al.*, 2002a). For example, more small (1–4 mm in diameter) but not medium-sized (4–8 mm in diameter) follicles were recorded in cattle offered twice the amount of the maintenance diet compared with cattle offered the maintenance diet (Fig. 1a) and resulted in a larger number of ovulations after a superovulatory protocol (Gong *et al.*, 2002a). The size of the preovulatory follicle was also greater (Fig. 1b) in cattle offered high energy diets compared with those offered low energy diets (Armstrong *et al.*, 2001) and it was hypothesized that metabolic hormones are directly involved in mediating these nutritionally induced changes in follicle dynamics. Some of the evidence for the involvement of growth hormone, insulin, IGF-I and leptin in regulating ovarian activity is summarized below.

#### *Growth hormone*

Recent studies have shown that treatment with exogenous growth hormone has a significant effect on ovarian follicle development (Gong *et al.*, 1991, 1993) and corpus luteum function (Lucy *et al.*, 1999) in cattle. Therefore, it is possible that growth hormone is involved in mediating the interactions between nutrition and ovarian activity. However, in a recent experiment, mRNA encoding growth hormone receptor was not detected in bovine follicles (Lucy *et al.*, 1999) and early experiments *in vitro* (Gong *et al.*, 1994) showed that growth hormone does not affect the proliferation and steroidogenesis of bovine granulosa cells in serum-free culture. In contrast, large luteal cells of bovine corpus luteum express the growth hormone receptor and respond to growth hormone treatment (Lucy *et al.*, 1999).

A dose–response study *in vivo* has indicated that the effect of growth hormone treatment on increasing the number of small follicles in heifers is acting through increased peripheral concentrations of insulin and IGF-I, rather than a direct effect of growth hormone (Gong



**Fig. 1.** (a) The number of small (1–4 mm in diameter) and medium-sized (4–8 mm in diameter) follicles during a superovulatory protocol in cattle offered either a maintenance (■) or twice maintenance diet (□); and (b) the diameter of the ovulatory follicle in cattle offered high energy (○) and low energy diets (●). The high and low energy diets were equivalent to 1.6 and 0.8 times maintenance for metabolizable energy requirements, respectively. \* $P < 0.05$  between diets. Data adapted from Gong *et al.* (2002a) and Armstrong *et al.* (2001).

*et al.*, 1997). Furthermore, the association between acute changes in dietary intake and follicle recruitment (Gutierrez *et al.*, 1997a; Armstrong *et al.*, 2001) was associated with decreased circulating growth hormone concentration (Gutierrez *et al.*, 1997a). In lactating dairy cattle, circulating growth hormone concentrations are positively correlated with milk yield, and cattle selected for increased milk yield have a delayed first ovulation when compared with cattle of lower genetic merit (Webb *et al.*, 1999b; Gong *et al.*, 2002b).

Taken together these results indicate that growth hormone may not be directly involved in the physiological mechanism underlying the nutritional influence on ovarian function in cattle, but an interaction with other metabolic hormones, such as insulin and IGF-I, is more probable (also see discussion in the following sections).

### Insulin

Results from a number of studies indicate the importance of insulin as a signal mediating the effects of acute changes in nutrient intake on follicle dynamics in cattle. For example, the infusion of insulin into beef heifers increased both the diameter of the dominant follicle (Simpson *et al.*, 1994) and ovulation rate in energy-deprived beef heifers (Harrison and Randel, 1986). The initiation of the first ovulation and, therefore, the resumption of normal oestrous cycles after parturition, is delayed in dairy cows selected for high genetic merit for milk yield. This finding has also been shown to be associated with a lower circulating insulin concentration (Webb *et al.*, 1999a) and feeding diets specifically designed to increase circulating insulin concentrations, during early lactation, can advance the time of first ovulation after parturition (Gong *et al.*, 2002b). In addition, cell culture studies have shown that bovine granulosa cells

are critically dependent on the presence of physiological concentrations of insulin (Gutierrez *et al.*, 1997b; Glistler *et al.*, 2001).

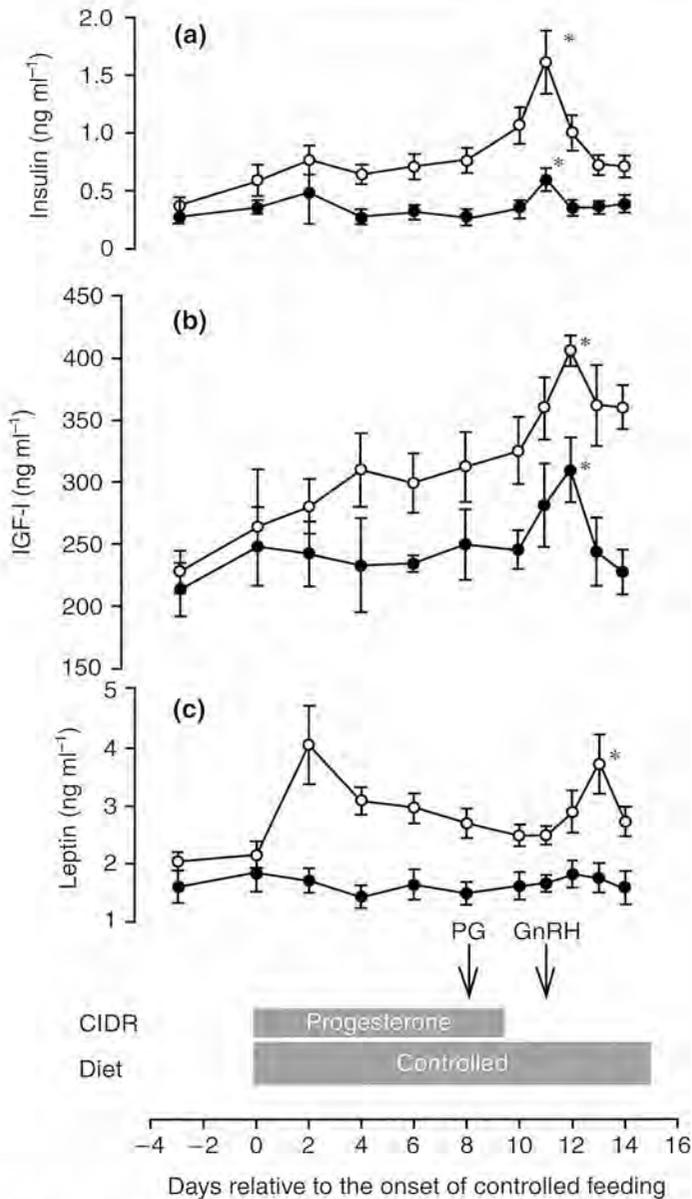
Circulating insulin concentrations, as well as being dependent on dietary intake, change during the oestrous cycle, and significantly increased concentrations are associated with ovulation (Armstrong *et al.*, 2001). The changes in circulating insulin concentrations during an oestrous synchronization procedure in cattle that were offered either maintenance diet or twice the maintenance diet are described (Fig. 2a). Maximum concentrations of insulin occurred on the day of GnRH treatment, and animals offered twice the amount of the maintenance diets produced a significantly higher peak of insulin than those offered maintenance diets. The precise mechanisms that regulate the magnitude of the ovulatory increase in insulin concentrations are not known. However, oestrogen is a prime candidate as the ovulatory associated increase in serum insulin concentrations parallels the increase in oestrogen associated with the development of the dominant follicle. Oestradiol (and other steroids) has also been shown to stimulate both the expression of mRNA encoding insulin and its secretion from the pancreas in a number of species (Morimoto *et al.*, 2001). However, to our knowledge, there is no evidence that dietary manipulation of the growth of the dominant follicle affects circulating oestradiol concentrations, so other mechanisms must also be operating, perhaps through dietary induced changes in the sensitivity of the pancreas to oestrogen, or the interaction with other metabolic hormones (see following sections), to regulate the magnitude of the ovulatory increase in circulating insulin concentrations.

### *IGF and IGF-binding proteins (IGFBPs)*

Dietary induced changes in circulating concentrations of components of the IGF-I system have been described (Clemmons and Underwood, 1991; McGuire *et al.*, 1992; Thissen *et al.*, 1994; Monget and Martin 1997), and circulating IGF-I concentrations are positively correlated with the level of feeding (Vandeharr *et al.*, 1995; Armstrong *et al.*, 2001; Rausch *et al.*, 2002). The effect of acute changes in feed intake on circulating IGF-I concentrations after an artificially induced ovulation in oestrus-synchronized heifers is described (Fig. 2b). Animals fed with twice the amount of the maintenance diet showed higher circulating IGF-I concentrations than those fed the maintenance diet. As with insulin, there is increasing evidence linking nutritionally induced changes in systemic IGF-I concentrations and ovarian activity (Webb *et al.*, 1999b) and maximum concentrations of circulating IGF-I were measured on the day after GnRH treatment in the experiment described (Fig. 2b).

The liver is the main source of systemic IGF-I and growth hormone is the primary regulator of hepatic IGF-I gene expression and secretion (Etherton and Bauman 1998). The results presented here (Fig. 2b), when combined with earlier studies (Gutierrez *et al.*, 1997a; Armstrong *et al.*, 2001, 2002a), show considerable variation between experiments in the magnitude of the changes in IGF-I concentrations associated with changes in nutritional status. This finding indicates that a number of other additional endocrine systems are probably interacting with growth hormone to regulate hepatic IGF-I secretion during periods of acute change in dietary intake. For example, oestrogen, as well as increasing mean concentrations of growth hormone (Grigsby and Trenkle, 1986), stimulates hepatic IGF-I mRNA expression (Richards *et al.*, 1991) and increases circulating concentrations of IGF-I in ovariectomized cattle (Simpson *et al.*, 1997). Insulin has also been shown to increase plasma IGF-I concentrations in dairy cows (McGuire *et al.*, 1995) and to interact with growth hormone to control hepatic IGF-I production (Molento *et al.*, 2002).

In dairy cattle, reduced circulating concentrations of IGF-I are associated with both the periparturient period and acute feed restriction (Kobayashi *et al.*, 1999, 2002). This reduction



**Fig. 2.** Circulating concentrations of (a) insulin, (b) insulin-like growth factor I (IGF-I) and (c) leptin during an artificially induced ovulatory protocol in heifers offered either a maintenance (●) or twice maintenance diet (○). \*Significantly different ( $P < 0.05$ ) from preceding time point, within diets. PG and GnRH represent the time of treatment with prostaglandin  $F_{2\alpha}$  and gonadotrophin-releasing hormone, respectively. Controlled, refers to the period during which animals were fed either maintenance or twice maintenance diets. Animals offered twice the maintenance diet were fed at 1.5 times maintenance diet for 3 days before feeding at twice maintenance diet. CIDR: controlled internal drug releasing devices.

in IGF-I is associated with decreased growth hormone receptor expression in the liver during the periparturient period, but not during acute feed restriction and it was concluded that a number of metabolic and hormonal events interact to control IGF-I gene expression.

The bioavailability of circulating IGF-I and its clearance rate from serum is controlled by IGF-binding proteins (IGFBPs) (Thissen *et al.*, 1994; Rausch *et al.*, 2002) and circulating concentrations of binding proteins are also regulated by feed intake. For example, IGFBP-3 is positively correlated with dietary intake (Rausch *et al.*, 2002) and increased growth rate in cattle is associated with an increased concentration of circulating IGFBP-3 (Vestergaard *et al.*, 1995). In dairy cattle, insulin has been shown to decrease circulating IGFBP-2 concentrations but does not affect IGFBP-3 concentrations (McGuire *et al.*, 1995). Oestrogen treatment has also been shown to increase IGFBP-3 concentrations in ovariectomized cows (Simpson *et al.*, 1997).

The endocrine role for IGF-I in controlling ovarian activity cannot be viewed in isolation and must be combined with factors affecting its production by the liver along with the nutritional induced changes in the serum IGFBP profile.

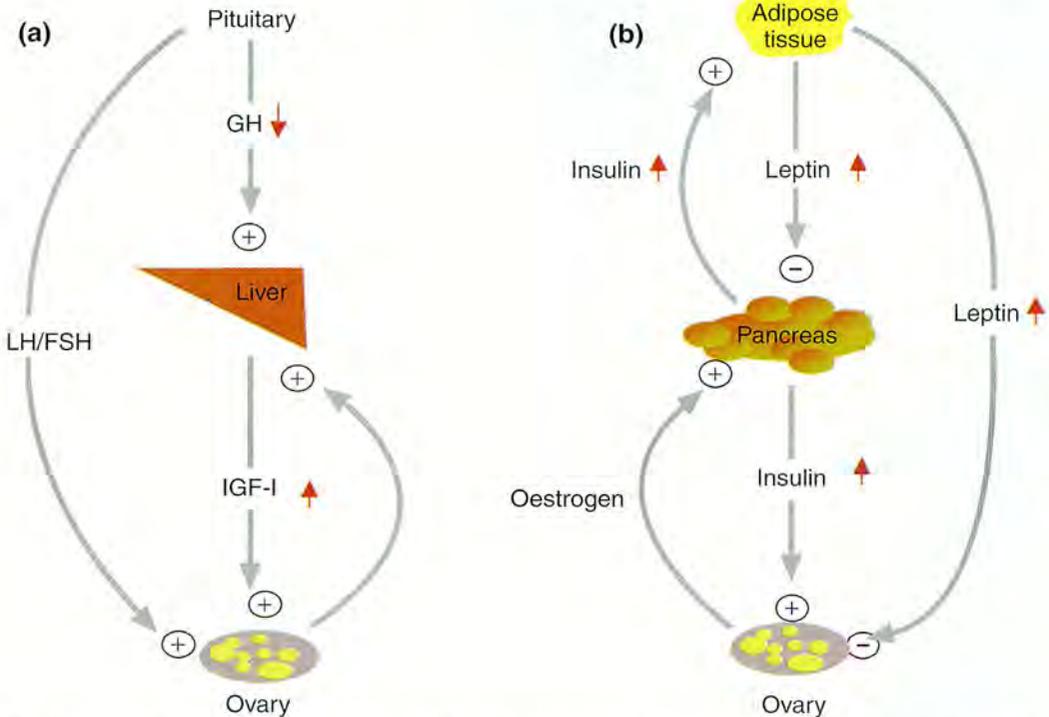
### Leptin

Leptin is produced primarily by adipocytes and increasing evidence supports a role for this hormone as a signal linking nutritional status with reproductive performance (Kiesler *et al.*, 1999; Spicer, 2001). The development of a specific immunoassay for ruminant leptin has confirmed that circulating concentrations are dependent on body condition in lactating cows (Ehrhardt *et al.*, 2000) and to the level of feeding in non-lactating cows (Delavaud *et al.*, 2002). Short-term fasting was shown to reduce expression of mRNA encoding leptin in adipocytes (Amstalden *et al.*, 2000) and was correlated with similar decreases in serum concentrations of insulin and IGF-I. The effect of acute changes in feed intake on circulating leptin concentrations after an artificially induced ovulation in oestrus-synchronized heifers is described (Fig. 2c). Maximum concentrations of leptin were observed within 2 days of the onset of feeding to twice the maintenance diet. A peak in peripheral concentration of leptin was also observed 2 days after GnRH treatment in cattle fed at twice the maintenance diet but not in animals fed the maintenance diet. The reasons for this increase are not known; however, in this respect it is interesting to compare the temporal relationship between the peak concentrations of insulin, IGF-I and leptin after GnRH treatment (Fig. 2). The increase in insulin concentrations precede the increase in IGF-I concentrations, which in turn precede the increase in leptin concentrations, implying that both insulin and IGF-I are involved in regulating the increase in leptin observed 2 days after GnRH treatment.

In summary, in the intact animal, nutritionally induced changes in growth hormone, insulin, IGF-I and leptin can be correlated with corresponding changes in ovarian activity. Steroids produced by the ovary can also regulate the production of these metabolic hormones resulting in a number of interacting positive and negative feedback loops. The complexity of the system is highlighted (Fig. 3) and some of the possible direct interactions between the ovary, somatotrophic axis and adipoinular axis are described. The above discussion clearly indicates that there are a number of mechanisms through which nutrition can act to regulate follicle growth. Some of these mechanisms are discussed in the next section.

### Cellular and molecular mechanisms

Metabolic hormones have potent effects on theca and granulosa cell differentiation in culture and both insulin and IGF-I interact synergistically with gonadotrophins to stimulate

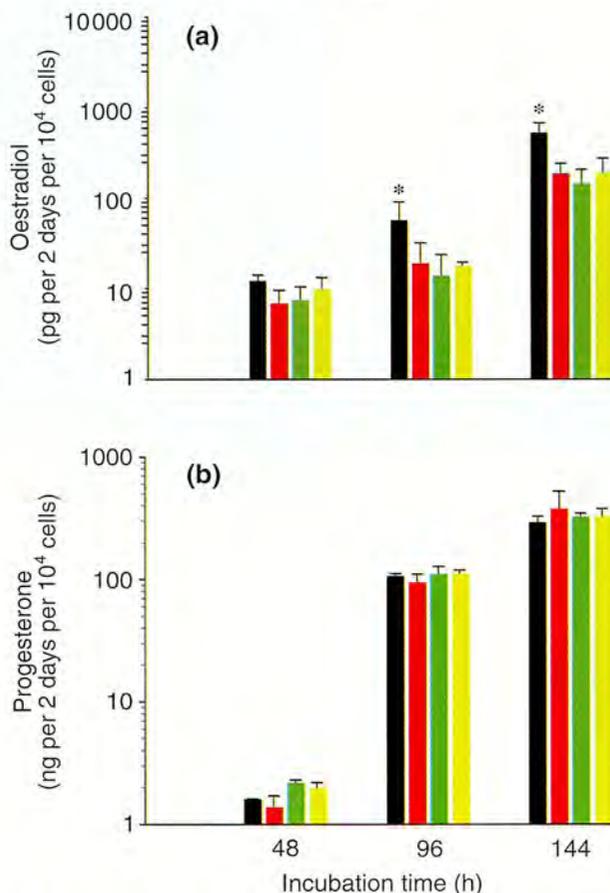


**Fig. 3.** Interactions between the ovary and (a) the somatotrophic axis and (b) the adipoinsular axis in cattle (Kieffer and Habener, 2000). The red arrows indicate the effect of increased dietary intake on the circulating concentrations of growth hormone (GH), insulin-like growth hormone I (IGF-I), insulin and leptin. (+) and (-) represent stimulatory and inhibitory actions, respectively, on target tissues.

oestradiol production by granulosa cells and androstenedione production by theca cells (Gutierrez *et al.*, 1997b; Glistler *et al.*, 2001). In contrast, leptin inhibits the synergistic interaction between gonadotrophins and insulin (Spicer, 2001). Thus, nutritionally induced changes in the concentration of these metabolic hormones have the potential to interact directly with gonadotrophins to regulate follicle growth and steroidogenesis.

We have recently correlated dietary induced increases in circulating concentrations of insulin and IGF-I with increased oestradiol production in cultured granulosa cells from small follicles (Armstrong *et al.*, 2002a). In the intact follicle, the production of oestradiol is controlled by the number of granulosa cells in the follicle, the supply of androgen precursors from thecal cells and the regulation of aromatase activity by FSH. The granulosa cell cultures in the study described by Armstrong *et al.* (2002a) contained testosterone and it was proposed that changes in the amount of oestradiol produced by granulosa cells, resulting from acute changes in feed intake, must be due to changes in the amount or activity of aromatase.

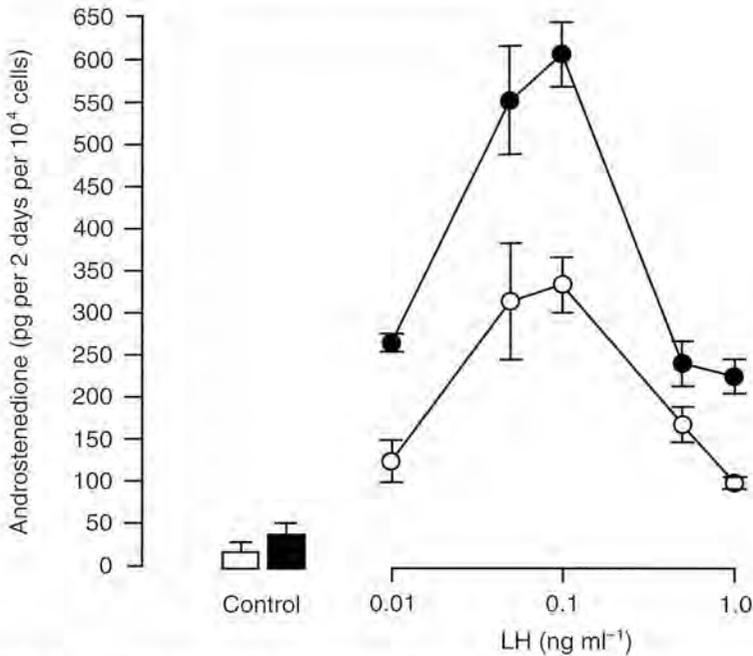
It was suggested that a possible mechanism for this increase in aromatase activity was metabolic hormones directly affecting the follicular IGF system, which in turn increase the response of granulosa cells from small follicles to FSH (Armstrong *et al.*, 2001). Specifically, increased dietary energy was shown to decrease the steady-state concentration of mRNA encoding IGFBP-2 and -4 in small antral follicles. It was hypothesized that the dietary induced decrease in the steady-state concentration of mRNA encoding IGFBP-2 and -4 in small antral



**Fig. 4.** (a) Oestradiol and (b) progesterone production by bovine granulosa cells in serum-free cultures. Cells were cultured for 6 days in serum-free conditions in the presence of 1 ng FSH ml<sup>-1</sup> and either 0, 5, 10 or 20 ng leptin ml<sup>-1</sup>, represented by black, red, green and yellow bars, respectively. Significantly different (\**P* < 0.05) from other treatments within that time period.

follicles will increase the bioavailability of intrafollicular IGF (both locally produced IGF-II and systemically derived IGF-I) in these follicles. The consequent increase in the sensitivity or response of granulosa cells towards FSH would be expected to result in an increase in aromatase activity and result in the observed changes in dietary induced follicle dynamics (Armstrong *et al.*, 2001). Mechanisms involving decreased expression of mRNA encoding IGFBP-2 have also been implicated in regulating the sensitivity of the dominant follicle to FSH (Armstrong *et al.*, 1998) and provides further evidence supporting a key role for IGFBPs in mediating signals controlling folliculogenesis.

The effect of leptin on oestradiol and androstenedione production by granulosa and theca cells in culture is shown (Figs 4 and 5, respectively). The results are similar to those described by Spicer and Francisco (1997, 1998), who reported that leptin inhibited the action of insulin on steroidogenesis. In addition, leptin inhibited only LH-stimulated androgen production by thecal cells and had no effect on the secretion of androgen in the absence of LH. The



**Fig. 5.** LH stimulation of androstenedione production by cultured thecal cells from bovine follicles (4–8 mm in diameter) in the presence (○) and absence (●) of recombinant ovine leptin (20 ng ml<sup>-1</sup>). Controls represent androstenedione production in the absence of LH. Cells were cultured for 6 days in serum-free conditions and production represents the amount of androstenedione in the culture medium during the 4–6 day culture period.

concentrations of leptin used in these studies *in vitro* were within the physiological range of circulating leptin concentrations (described in Fig. 2), further strengthening the hypothesis that leptin is an endocrine signal linking ovarian activity to nutritional status.

### Developmental competence of oocytes

Nutritionally induced changes in endocrine and metabolic signals that regulate follicular growth also influence oocyte maturation (Armstrong *et al.*, 2001; Boland *et al.*, 2001). Acute changes in dietary energy intake influence both the morphology and developmental competence of the oocyte (McEvoy *et al.*, 1995; O'Callaghan *et al.*, 2000). Increased concentrations of ammonia in follicular fluid, as a result of increased intake of highly degradable protein, as well as being associated with altered follicular growth patterns reduce both the number of ova that cleave after insemination and the proportion that develop to the blastocyst stage (Sinclair *et al.*, 2000). Armstrong *et al.* (2001) showed that the quality of oocytes taken from small follicles was negatively correlated with plasma concentrations of urea. However, variation between experiments has been reported in relation to the size category of the follicle that is most susceptible to the interaction between urea and oocyte developmental competence. Energy status may also interact with protein intake to influence these results. In this respect, insulin and IGF-I concentrations were also affected by the different dietary regimens described in this study and an interaction between these metabolic hormones and

ammonia in regulating oocyte developmental competence cannot be excluded (Sinclair *et al.*, 2000).

The ovarian IGF system has the potential to interact directly with the oocyte through the type 1 IGF receptor (Armstrong *et al.*, 2001, 2002a,b). Small follicles from heifers offered high energy diets had significantly reduced amounts of mRNA encoding IGFBP-2 and -4 (Armstrong *et al.*, 2001). As discussed above, we expect this to result in increased bioavailability of IGF in these follicles, which is probably a critical factor controlling oocyte developmental capacity (Armstrong *et al.*, 2002b). Indeed, our results indicate that, in contrast to follicle growth, over-stimulation by IGF and probably insulin (R. Webb, P. C. Garnsworthy and K. D. Sinclair, unpublished) may be detrimental to oocyte development (Armstrong *et al.*, 2001). Recent studies have supported these conclusions by showing that concentrations of IGF-I that are optimal for the growth of preantral follicles *in vitro* may be detrimental to oocyte maturation (McCaffery *et al.*, 2001). We hypothesize that nutritionally induced changes in the ovarian IGF system, coupled with changes in circulating concentrations of insulin and IGF-I that maximize follicle recruitment, may be detrimental to the maturation of the oocyte within the growing follicle.

## Conclusions

The data reviewed in the present study indicate the importance of dietary induced changes in metabolic hormones in controlling ovarian activity. The ovarian IGF system was highlighted as a key component of the mechanisms mediating the effects of insulin and IGF-I on follicle growth. Finally, it is clear that concentrations of IGF-I that are optimal for follicle growth may not necessarily be optimal for oocyte maturation. This is a factor that must be considered when developing nutrition-based solutions to improving fertility in cattle production systems.

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