ENDOCRINE CHANGES ASSOCIATED WITH THE EFFECT OF NUTRITION ON THE TIMING OF RECONCEPTION AND PUBERTY IN DAIRY CATTLE

BY

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DECLARATION

I declare that this thesis for the

degree of M. Sc. (Agric) - Animal Sciences - at the University of Pretoria

has not been submitted for a degree any other university.

Signature: .....

Date: 25-5-2000

Summary

Reproduction is a major factor limiting the efficiency of dairy cattle production. The mechanism whereby nutrition alters ovarian and uterine functions is unclear, but changes in key hormones and metabolites at the systemic or tissue level or both, may constitute one link between metabolic regulation of reproduction and nutrition.

The aim of the thesis is to review the endocrine factors that control reconception and puberty in dairy cattle and to test whether IGF-1, IGFBP-3, leptin and glucose concentrations are responsible for controlling the onset of these events.

To study the endocrine factors that control reconception in dairy postpartum cows, multiparus Fresian cows were fed one of two diet: 80 % or 120 % of requirements. Blood sample were collected weekly from calving to conception. Concentrations of plasma IGF-1, IGFBP-2, IGFBP-3, leptin and glucose were determined. At conception, cows fed at 80 % of requirements had lower body condition scores, body weights and plasma IGFBP-3 concentrations than cows fed at 120 % of requirements. Within dietary treatments, plasma IGFBP-3 and leptin concentration increased from week 2 postpartum to conception.

Fresian heifers were used to study the endocrine control of compensatory growth and onset of puberty in growing cattle. Treatments were designed to result in two different growth rates (restricted 0.3 kg/d; control 0.6 kg/d) during the first phase of the experiment. During the second phase of the experiment animals received the same amount of food (on a per kg body weight basis). Results from this experiment show that concentrations of glucose, IGF-1 and IGFBP-3 were directly affected by the restriction

program. Delayed puberty in feed-restricted animals was also shown to be mediated by changes in the endocrine system. Early puberty (control animals) achieved by accelerated prepubertal growth resulted in earlier attainment of high concentrations of IGF-1, IGFBP-3 and leptin. Increased concentrations of leptin and IGFBP-3 (and the accompanying change in the ratio of IGF-1 to IGFBP-3) during puberty appear to signal the hypothalamus that body reserves and the nutritional environment are adequate to support reproduction.

Leptin, glucose, IGF-1 or/and IGFBP-3 were all found to be involved with reconception and puberty. In the future, manipulation of one or all of these three factors could have an impact on reproductive efficiency in domestic livestock. Further research is needed to determine the nature of the relationship between leptin, IGF-1, IGFBP-3 and glucose. Furthermore, more studies are needed to understand the metabolic signals affecting reproductive activity at different stages of growth (long term nutritional restriction) or at different stages of follicular growth (short term nutritional restriction). Research on ovarian activity in diverse mammalian species may be a useful way to improve our understanding of the complex reproductive signaling system.

Samevatting

Reproduksie is die hoof faktor wat die doeltreffendheid van melkbeesproduksie beinvloed. Die meganisme waardeur reproduksie deur voeding beinvloed word is nog nie duidelik nie, maar veranderinge in sleutelhormone en metaboliete op sistemiese- of weefselvlakke, of beide, verteenwoordig een skakel tussen metaboliese beheer van reproduksie en voeding.

Die doel van hierdie proefskrif is om die endokriene faktore wat herkonsepsie en puberteit in melkbeeste beinvloed, in oorsig te neem, en om te toets of IGF-1, IGFBP-3, leptin en glukose konsentrasies verantwoordelik is vir die beheer van die aanvang van hierdie verskynsels.

Om die endokriene faktore wat herkonsepsie beinvloed, te bestudeer, is een van twee diete (of 80% of 120% van behoeftes) aan Vrieskoeie gevoer. Bloedmonsters is weekliks vanaf kalwing tot herbevrugting versamel. Konsentrasies van plasma IGF-1, IGFBP-2, IGFBP-3, leptin en glukose is bepaal. Koeie wat teen 80% van behoeftes gevoer is het laer liggaamskondisie tellings, liggaamsmassas, en plasma IGFBP-3 konsentrasies tydens herbevrugting getoon as dié wat teen 120% van behoeftes gevoer is. Binne behandelings, het plasma IGFBP-3 en leptin konsentrasies vanaf week 2 tot bevrugting gestyg.

Vries- verse is gebruik om die endokriene beheer van kompensatoriese groei en puberteit in groeiende beeste te bestudeer. Behandelings is ontwerp om twee verskillende groeitempos gedurende die eerste fase van die proef daar te stel (beperk: 0.3 kg/d; kontrole: 0.6 kg/d). Gedurende die tweede fase van die proef het alle diere dieselfde

hoeveelheid voer ontvang (op 'n per- kg- liggaamsmassa basis). Konsentrasies van glukose, IGF-1 en IFGBP-3 is almal deur die beperking van voerinname beinvloed. Daar is bewys dat die vertraagde puberteit in diere wat aan beperkte voeding onderworpe is, deur veranderinge in die endokriene stelsel bewerkstellig is. Vroeër puberteit in die kontrole behandeling het tot gevolg dat hoë konsentrasies van IGF-1, IGFBP-3 en leptin op 'n vroeër ouderdom bereik is. Dit wil voorkom of verhoogde konsentrasies van leptin en IGFBP-3 (en die samehangende verandering in die verhouding van IGF-1 tot IGFBP-3) gedurende puberteit seine verteenwoordig wat die hipotalamus inlig dat liggaamsreserwes en die voedingsomgewing voldoende is om reproduksie te onderhou.

Daar is gevind dat leptin, glukose, IGF-1 en/of IGFBP-3 almal betrokke is by puberteit en herkonsepsie. In die toekoms kan manipulasie van een of al drie van hierdie faktore 'n impak op die doeltreffendheid van reproduksie in plaasdiere hê. Verdere navorsing word benodig om die presiese aard van die verband tussen leptin, glukose, IGF-1 en IGFBP-3 te bepaal. Navorsing is ook nodig om te verstaan hoe metaboliese seine wat reproduksie beinvloed, verskil teen verskillende groeistadia (langtermyn voedingsbeperking) en teen verskillende stadia van follikelgroei (korttermyn voedingsbeperking). Navorsing oor eierstokaktiwiteit in verskillende spesies soogdiere kan 'n nuttige manier wees om ons kennis van die endokriene beheer van die ingewikkelde reproduksie stelsel te genereer.

Chapter 1

1. Aims and motivation

1.1. Problem statement and hypothesis

Inadequate nutrition impairs reproductive function in many mammalian species. Reproduction is one of the main factors determining productive efficiency of dairy and beef cattle (Dziuk and Bellows, 1983). The duration of postpartum anestrous is of major economic importance in dairy cows. Cows must conceive within 80 to 85 days postpartum if they are to achieve a calving interval of one year. It has been shown that postpartum anestrous periods can be extremely variable and frequently exceed 80 days (Peters and Riley, 1982). Two main factors have been related to the number of days between calving and normal ovarian activity in cows: the first is the amount of nutrients derived from tissue mobilization and the second is the quantity of milk produced (Staples et al., 1990). Both factors affect energy balance. The effect of energy balance on reproduction is postulated to be mediated by the action of metabolic signal(s) that are recognized by the brain (Hoggard et al., 1998), however, the exact nature of these factors is unclear. A better understanding of the endocrine basis of the link between nutrition and postpartum anestrus could facilitate the development of methods to increase reproductive efficiency.

Another major problem in dairy production systems concerns the duration of the period that animals are not producing (eg. prepartum period; growing phase). The controlled growth and development of prepubertal females to conceive at an early age is also important aspect of livestock production. Low growth rates during the growing phase increase the unproductive period, and a high plane of nutrition postweaning will result in early puberty in heifers (Buskirk et al., 1995); but growth rates greater than 700 g/d may compromise later milk performance (Sejrsen et al., 1982). The reason for this reduction in

future performance is that there is a decrease in mammary secretory development because excessive fat tissue is deposit in the mammary gland (Sejrsen et al., 1982). A high growth rate may result in greater weights of internal organs in relation to body weight resulting in greater rates of metabolism and greater nutrient requirements per unit of body weight (Koong et al., 1982). Animals which are fed at a restricted level of nutrition typically exhibit increased growth rates when re-alimented; this phenomenon is known as compensatory growth. Factors that affect the extent of compensatory growth include efficiency of feed utilization, feed intake and maintenance requirements (Lopez Saubidet and Verde, 1976). Although much research has been devated to understanding compensatory gain, several aspects such as the endocrine basis of compensatory growth effects and the effect of compensatory growth on the time to puberty remain unknown. A better understanding of the signal(s) that are involved in regulating the onset of puberty, as in the case of postpartum estrus, could enable reproductive efficiency to be improved.

Dietary energy restriction has been shown to suppress episodic release of luteinizing hormone (LH) secretion in cattle (Imakawa et al., 1987). A high frequency mode of LH secretion is important for the final phase of maturation of ovarian follicles and thus for induction of estrus and ovulation (Hansel and Convey, 1983; Randel, 1990). However, the mechanism by which restricted energy intake suppresses LH secretion has not been clearly determined.

Leptin is a metabolic signal which may regulate gonadotrophin-releasing hormone (GnRH) secretion (Bronson, 1998). Leptin, the product of the recently identified obese gene (*ob/ob*), is a pleiotropic peptide hormone that has been linked to the regulation of energy balance and body composition (Spurlock et al., 1998). Leptin is a peripherally derived signal that is secreted into the blood (Halaas et al., 1995) and actively transported

availability in the developing lamb affected LH secretion by changing the pulse frequency. Muller et al. (1998), summarized evidence indicating that glucose concentration regulates leptin secretion from cultured rat adipocytes, and concluded that glucose is an important regulator of leptin expression and secretion.

The overall hypothesis of this thesis is that the period effects of food restriction on reproduction in dairy cattle during the growth period (prepubertal stage) and postpartum period are mediated by changes in IGF-1, IGFBP-3, leptin and glucose activity. The aim of the thesis is to review the endocrine factors that control reconception and puberty in dairy cattle and to test whether IGF-1, IGFBP-3, leptin and glucose concentrations are responsible for controlling the onset of these events.

1.2. Endocrine factors involved in the control of the interval to first ovulation postpartum and the initiation of puberty in ruminants

Interruption of reproduction is a normal adaptive response when animals are exposed to insufficient nutrition or challenged energetically. Because ovarian function is controlled by gonadotrophin secretion from the pituitary gland, the site of nutritional influences on the ovary is probably located at the hypothalamic-pituitary axis. Undernutrition affects all levels of hypothalamic–pituitary–ovarian axis functioning, but the primary locus of altered function in nutritional infertility in thought to be hypothalamic GnRH secreting neurons (Wade et al., 1996).

The production and release of follicle-stimulating hormone (FSH) and LH is regulated by two specific GnRH, these are FSH-releasing hormone (FSHRH) and LH-releasing hormone (LHRH). Energy restriction impairs pulsatile LH secretion by decreasing the hypothalamic release of LHRH (Kile et al., 1991). These authors found that pulsatile administration of LHRH restored the concentration of LH in energy-restricted ewes. It is important to note that dietary energy restriction does not change the number of pituitary receptors for LHRH (Tatman et al., 1990). Day et al. (1986) showed that administration of GnRH reinstates reproductive function in food-deprived heifers; however, Peters et al. (1985) reported that very few lactating cows ovulated in response to pulsatile administration of LHRH when delivered at a time when animals were in negative energy balance. On the other hand, Canfield and Butler (1991) reported that the patterns of LH in cows in positive or negative balance are similar. The conflicting opinions on the subject may be related to differences in the level of dietary restriction, metabolic status and time of treatment. The re-establishment of a normal LH pulse pattern is the key factor

responsible for ovarian follicular development and the initiation of postpartum ovarian cycles (Butler and Adams, 1989).

LH concentration and LH pulse frequency increase two months before heifers reach puberty (Day et al., 1984). This increased LH secretion is associated with decreased ovarian estradiol negative feedback (Day et al., 1987), but the specific endocrine mechanism responsible for increased LH secretion before puberty in heifers is still unclear. The LH suppression induced by food deprivation involves a steroid dependent mechanism: in rats the negative feedback actions of estradiol and LH are clearly enhanced (Nagatani et al., 1994), but in sheep energetic challenges inhibit LH release by an estradiol-independent mechanism (Foster and Olster, 1985). Unfavorable nutritional conditions can lower LH secretion and delay puberty (Bronson, 1986). The onset of puberty in the growing heifers is, at least in part, a result of decreased estradiol feedback (Day et al., 1984). Furthermore, Schillo et al. (1992) have shown that the LH response to estradiol injections in ovariectomized heifers increased from 4 to 12 month of age. These data together suggest that a reduction in sensitivity to estradiol occurs as heifers approach sexual maturity.

Studies with cyclic cows (Richards et al., 1991) show that estradiol enhances the secretion of IGF-1 in well-nourished cows, but not in under-fed cows. IGF-1 responses to estradiol treatment is also influenced by breed, and differences as high as 40 % have been recorded (Simpson et al., 1997). A possible explanation for this effect is that changes in hepatic receptors for GH can modify the response of IGF-1 under different nutritional conditions or in different breed.

Opiate peptides, acting through specific opiate-binding sites, exert direct inhibitory effects on anterior pituitary LH release in vitro (Blank et al., 1986). β-endorphin is released from the hypothalamus into the pituitary portal system (Koening et al., 1986). Enhanced

β-endorphin release during negative energy balance may be related to the stimulation of appetite and dietary intake that usually occurs in early lactation, and may exert inhibitory effects on hypothalamic GnRH release and LH pituitary release (Butler and Adams, 1989).

FSH promotes follicle growth and estrogen production by granulosa cells in the ovarian follicle. FSH is able to maintain follicular wave patterns in the presence of depressed LH secretion (Crowe et al., 1998). Before the first postpartum follicular wave in dairy cattle, a peak of FSH occurs, which may be required to restart the cycle of ovarian activity (Beam and Butler, 1997). Growth factors play an important role in modulating the actions of gonadotrophins on follicular cells. FSH and LH interact with various growth factors in the ovary. In particular, FSH enhances granullosa cell sensitivity to IGF-1 by increasing the numbers of IGF-1 receptors (Monniaux and Pisselet, 1992). IGF-1 promotes both the replication and differentiation of granulosa cells and affects virtually all aspects of granulosa cell function, including enhancement of gonadotrophin-stimulated production of progesterone, estrogen and proteglycans, as well as LH receptor induction (Simmen et al., 1998).

Another member of the family of reproductive hormones is progesterone, produced mainly by the corpus luteum (CL). An increase in the plasma concentration of progesterone is used to determine the onset of luteal activity in postpartum or pubertal sheep and cattle (Adams et al., 1998). Plasma progesterone concentration was reported to be correlated with plasma iGF-1 concentration in lactating cows (Spicer et al., 1990), and this is caused by an increase in the size of the CL consistent with the action of IGF-1 in the ovary (Lucy et al., 1995). However, Yung et al. (1996) found no difference in progesterone concentration in cows treated with bST, although plasma IGF-1 was increased after the

administration of bST. The reason for the difference between the results of these studies is not clear, but may be related to energy balance of the animals used.

In summary, normal ovarian activity in both pubertal and postpartum cattle is determined by an interaction between various gonadotropic hormones. As will be discussed in more detail in the following sections, the interlay between the gonadotropic hormones is modulated by other factors such as glucose availability, growth factor activity and secretion of other hormones related to body reserve status.

1.2.1. The role of insulin-like growth factor system in regulation of reproductive activity

The IGF system consists of two forms of IGF, designated IGF-1 and IGF-2, and six IGFBP which are associated with different affinities to the IGF. The IGF-1 and IGF-2 are single polypeptides with a structure similar to proinsulin. The IGF are involved in mitogenic reactions and also in cell differentiation (Jones and Clemmonds, 1995), and implicated in many biological processes such as lactation, growth and reproduction. IGF are produced locally in many organs of the body, where they influence both metabolic and proliferative activities (Jones and Clemmonds, 1995). Hepatic IGF-1 synthesis and secretion is stimulated by GH. Most of the circulating IGF and binding proteins in blood are produced by the liver, but IGF have been detected in various biological fluids (McGuiere et al., 1992).

The IGFBP are believed to play a major role in the regulation of IGF action and have been reported to attenuate as well as augment the actions of the IGFs in their target tissues. Cultured granulosa cells from pigs were found to secrete IGFBP-2 and IGFBP-3 (Giudice, 1992). IGFBP-2 mRNA has been detected in the immature, pre-ovulatory and luteal whole ovary, and low expression of IGFBP-3 mRNA has been detected in the immature and preovulatory ovary (Hammond et al., 1985). IGFBP-3 mRNA is present in ovine and bovine oviducts in both the mucosa and muscle layers, with peak in expression occurring during the preovulatory stage of the cycle (Stevenson and Wathes, 1996). In the bovine there is low expression in the endometrial stroma and concentrations are higher in nonpregnant than in pregnant cows (Wathes et al., 1998). Even though the biological actions of these IGFBP remain to be fully elucidated, they have been shown to increase the circulating half-life of IGF (Baxter, 1993).

Energy balance and nutritional status affect IGF-1 concentration, and this may indicate whether adequate energy is available for animals to initiate and maintain ovarian activity (Rutter et al., 1989). The relationship between serum GH and IGF-1 is "uncoupled" by feed restriction (McGuire et al., 1992; Cohick and Clemmons, 1993). Kirby et al. (1993) found that the effect of fed restriction on IGF-1 concentration in cows was greater in serum than in follicular fluid. During negative energy balance, GH pulses are high in frequency and amplitude, and serum concentrations of IGF-1 are suppressed (Butler et al., 1981; Breier et al., 1986). In ruminants, dietary restriction or periods of negative energy balance are associated with increased concentration of circulating IGFBP-2 and decreased concentration of IGFBP-3 (Vicini et al., 1991). In lactating dairy cows, energy balance during the first 6 weeks postpartum has an inverse relationship to days to first postpartum ovulation (Butler et al., 1981). Riis et al. (1998) observed changes in plasma concentration and distribution of IGF-1 and its binding proteins at the beginning of the lactation. This may be an homeorehetic adaptation to lactation because increased IGFBP-3 concentration would limit IGF-1 effects on different tissues other than the mammary gland. Roberts et al. (1997) found that the concentrations of IGF-1, IGFBP-2 and IGFBP-3 during the second week postpartum were indicators of the capacity of energy-retricted cattle to resume cycling after parturition. IGF-1 exerts a negative feedback on GH and a stimulatory effect on type II gonadotrophin at the pituitary cell level in the eel (Huang et al., 1998), suggesting that IGF-1 may extent a stimulatory effect on the induction of the puberty as well reconception. Collectively, these results suggest that IGF-1 could be a potential hormonal mediator of the effects of negative energy balance on luteal function.

It has been proposed that the IGF system can control the local production of various components of the uterus and placenta of several species including the rat (Zhou and Bondy, 1992), human (Han et al., 1996), sheep (Reynols et al., 1997) and cow (Spicer and Stewart, 1996). IGF-1 in the ovary of the cow is thought to originate from the circulatory, IGF-1 pool, although IGF-1 is synthesized by granulosa cells (Spicer et al, 1993). In ewes, IGF-1 concentrations were maximal in both the mucosa and muscle layers of the oviduct wall at estrous (Stevenson and Wathes, 1996), and concentrations gradually decreased during the luteal phase (Wathes et al., 1998). In the porcine and ovine uterus, IGF-1 is secreted into the uterine lumen around the time of estrous, so that maximal concentrations are present during sperm transport but several days before the embryo arrives in the uterus (Geisert et al., 1991; Ko et al., 1991). A similar pattern of secretion of IGF-1 has been reported to occur in cows (Schmidt, 1994). Martikainen et al., (1997) reported that neither the ovary nor the adrenal gland significantly contributes to the circulating pool of IGF-1 or IGFBP-3 in obese, severely hyperandrogenic women.

The IGF system plays an important role in modulating the actions of gonadotrophins on follicular cells. IGF-1 increases the sensitivity of bovine follicular cells to LH and FSH in cattle (Spicer et al., 1993). Furthermore, IGF-1 stimulates granulosa cell proliferation and enhances the action of gonadotrophins in promoting granulosa cell differentiation in several species (Adashi et al., 1985; Erickson et al., 1989; Mariana et al., 1998). Although IGF mRNA has been detected in bovine luteal cells but not bovine granulosa cells (Stirling et al., 1991), Guitierrez et al. (1997a) found that IGF-1 and insulin stimulates proliferation and steroidogenesis in bovine granulosa cells. Gutierrez et al. (1997b) suggest that bovine granulosa cells do not produce IGF-1 *in vivo*, although IGF-1 production by bovine granulosa cells *in vitro* has been reported (Spicer et al., 1993).

It has been shown that prepubertal sensitivity of granulosa cells to FSH differs between breeds with different ovulation rates during adulthood, and it was suggested that these differences are mediated by IGF-1 (Mariana et al., 1998). In other words, differences in sensitivity of granulosa cells to FSH (caused by IGF-1 action) result in different ovulation rates. These findings suggest that the possibility exists of using IGF-1 concentration as an early marker (prepubertal stage) for potential ovulation rate in sheep and goats.

One important mechanism by which energy deficit impairs reproductive activity is by suppressing the LHRH and the LH pulse frequency necessary for ovarian follicles to grow to the preovulatory stage. Mean plasma LH concentration and the number of episodic LH peaks increase after the maximal negative energy balance, and first ovulation occurs soon after for most cows (Canfield and Butler, 1991). Spicer and Stewart (1996) suggest that IGF-1 may play a significant role in basal and LH-modulated thecal cell steroidogenesis and mitogenesis during follicular development in cattle. It has been shown that chronic IGF-1 administration in prepubertal animals accelerates the pubertal decrease in sensitivity of LH to estradiol negative feedback (Wilson, 1995). Effects of IGF-1 on LH secretion were found in castrate male sheep with and without an estradiol implant (Adams et al., 1997), suggesting that IGF-1 could have independent effects on the sensitivity of LH to estradiol. It appears that this represents a physiological role for IGF-1 as a nutritional modulator of reproduction. This possibility is supported by the results of Richards et al. (1991), who found that poor nutrition inhibits the effects of estradiol on IGF-1 secretion in cyclic cows. Furthermore, Simpson et al. (1997), working with gonadectomized Angus and Brahman cattle, found that estradiol treatment increased GH concentration by 70 % and that was caused by an increase in pulse amplitude and not in pulse frequency. The authors

also found that IGF-1 and IGFBP-3 concentration were greater in cows with estradiol implants. Collectively, this data suggests that IGF-1 and IGFBP-3 can act as metabolic signals to modify LH secretory patterns and reproductive activity in cattle. These signals may also operate also in the young prepubertal female because the concentration of IGF-1 increases during puberty in rodents, primates and cattle (Luna et al., 1983; Handelsman et al., 1987; Renaville et al., 1993). The increased IGF-1 concentration during puberty can be attributed to estradiol effects, but suppression of steroid production during puberty failed to reduce serum IGF-1 concentrations to prepubertal levels (Mansfield et al., 1988). Furthermore, Hiney et al. (1991) demonstrated that IGF-1 can stimulate LHRH release in prepubertal rats, and suggested that this growth factor may play a role as a metabolic signal for sexual maturation.

In summary, ovarian function is affected by the IGF system in cattle and other species, and IGF-1 promotes a favorable gonadotrophin environment. Furthermore, IGF-1 may act as a metabolic signal for the establishment (in prepuberal heifers) or re-establishment of ovarian activity. The ratio between IGF-1 and its binding proteins (mainly IGFBP-2 and IGFBP-3) may be involved in delaying the initiation of or sexual maturation in animals during severe feed restriction.

1.2.2. The role of glucose in regulation of reproductive activity:

The mayor energy substrate utilized by the central nervous system during times of adequate nutrition is glucose. As ruminants absorb only limited quantities of glucose from the gastrointestinal tract, endogenous production of glucose from non-carbohydrate precursors (gluconeogenesis) represents a major source of glucose. The primary energy substrates absorbed are volatile fatty acids, of which propionate is the principal gluconeogenic volatile fatty acid. During food deprivation, amino acids and glycerol derived from catabolism of body tissue represent the major glucose precursors.

Previous experiments on glucose metabolism of the ovary have shown a marked stimulatory effects of LH on the rate of glucose uptake (Rabiee et al., 1997). Theses authors, also showed that glucose is the major source of energy for the ovary in sheep. Furthermore, it has been shown that insulin mediates the effect of increased ovulation rate in ewes by increasing glucose uptake by the ovary (Downing et al., 1995). In contrast to these results, Funston et al. (1995), have shown that the direct effect of suppression of LH secretion is not related to the deprivation of glucose supply to the ovary. It is important to bear in mind that glucose is not the only source of energy for the ovary, as acetate and lactate are also utilized, albeit to a lesser extent than glucose. In summary, it can be stated that glucose (and insulin) play an important role the ovarian function, and that the effect of nutrition on ovulation may be mediated, at least partially, by glucose availability in the ovarian environment.

The effect of glucose restriction on LH patterns is different to that of the effects of dietary energy restriction because inadequate nutrition causes a reduction in LH frequency, whereas glucose restriction reduces LH pulse amplitude (Schillo, 1992), suggesting that glucose is not the only factor affecting LH concentration. Furthermore, increasing glucose

availability in nutritionally adequate cows did not alter pulsatile patterns of LH or LHRH-stimulated LH release (McCaughey et al., 1988). Abomasal infusion of propionate enhanced blood glucose concentrations and release of LH following a GnRH challenge in prepuberal heifers (Rutter et al., 1983). Rutter et al. (1989) showed that increasing glucose availability in anestrous suckled cows markedly increased serum concentration of IGF-1. In this experiment, serum concentration of insulin increased in parallel with IGF-1 concentrations in anestrous weaned cows infused with phlorizin (increased glucose loss in the urine) and suckled cows receiving a glucose spike treatment. On the other hand, intravenous infusion of glucose in postpartum beef cows has not established a direct role for increased glucose supply in the enhancement of postpartum rebreeding (Randel, 1990).

In summary, feed restriction or deprivation reduce the plasma glucose concentrations and this reduction mainly affects LH concentration and modulation (pulse and amplitude). The effect of glucose on LH secretion appears to operate within the central nervous system at specific receptor. In addition, glucose has specific effects on ovarian cells, including synergism with the gonadotrophins.

1.2.3. The role of Leptin in regulation of reproductive activity

Leptin is a 16 kDa polypeptide hormone with multiple functions in the animal body (Zang et al., 1997). Leptin is secreted by adipocytes as well as the placenta, stomach and mammary gland (Zang et al., 1997; Bado et al., 1998). The main site of action of leptin is the hypothalamus (Chehab et al., 1996). The role of leptin on reproduction appears to be that of delaying reproductive activity until body reserves are sufficient to maintain pregnancy and lactation (Foster and Nagatani, 1999). Leptin receptors are single membrane-spanning receptors (Schwartz et al, 1996), and are present in the testes, lung, kidney, liver and ovary (Tartaglia et al, 1995; Spicer and Francisco, 1998). Between 5 to 20 % of leptin in blood is bound to a binding protein which may regulates leptin activity (Lin et al., 1998).

Leptin concentration is correlated with adipocyte volume (Chilliard et al, 1998) and body fat percentage (Havel et al., 1996). Several factors are known to increase plasma leptin concentration: body fatness (Houseknecht et al., 1998), insulin (Saladin et al., 1995), glucocorticoids (Dyer et al., 1997) and estrogens (Shimizu et al., 1997). Another factor that affects leptin concentration is nutritional status; animals under poor nutritional conditions have lower leptin concentrations than well-fed animals (Ahima et al., 1996; Chilliard et al., 1998). Furthermore, short-term overfeeding in humans which did not change body weight increased serum leptin concentration by 40 % (Kolaczynski et al., 1996). Taking in consideration the effects of energy balance and level of intake, it is suggested that leptin not only reflects the amount of fat, but can also act as a short-term metabolic signal.

The relationship between insulin and leptin concentration is not clear at present, and much of the information available is contradictory. Chilliard et al (1998) found no relationship between insulin and leptin serum concentration in cattle; on the other hand,

reduced insulin concentrations were found in mice after a single leptin injection (Cohen et al, 1996). A better interpretation could perhaps be made if insulin, leptin and glucose concentration are all taken into account, because glucose is known to play a major role in the secretion of leptin in rat adipocytes (Muller et al, 1997). A five-hour intravenous infusion of leptin into wild-type mice increased glucose turnover and glucose uptake, but decreased hepatic glycogen content (Kamohara et al., 1997). These authors found similar effects after intracerebroventricular infusion of leptin, suggesting that effect of leptin on glucose metabolism is mediated by the central nervous system. Further experiment are need to understand if the correlation found between leptin and insulin has any biological meaning or whether these associations are simply caused by some other independent factor.

Negative energy balance reduces the concentration of IGF-1 and IGFBP-3 and increases the concentration of IGFBP-2 in humans, sheep and cattle (for discussion, see 1.2.1.). Leptin has been reported to regulate growth hormone secretion (LaPaglia et al., 1998), but chronic incubation of isolated adipocytes with either GH or IGF-1 had no effect on leptin expression and secretion (Hardie et al, 1996). Furthermore, LaPaglia et al. (1998) showed that although daily leptin administration was able to fully prevent the fasting-induced fall in serum GH, it failed to restore IGF-1 concentration to control levels. The results of LaPaglia et al. (1998) provide evidence that leptin may function as a neuromodulator, communicating the nutritional status of the animal to the hormonal system.

Animals with higher rates of gain have higher internal organ masses, higher percentages of adipose tissue and reach puberty earlier (Koong et al., 1982). Leptin concentration was direct linked to puberty in mice by Ahima et al. (1997) who established

that the delay in puberty in the obese genotype (*ob/ob*) was caused by the action of leptin. Furthermore, administration of recombinant leptin to *ob/ob* female mice completely restored gonadotrophin secretion, secondary sex organ weight and function as well as fertility (Barash et al., 1996).

Body fatness at calving is inversely correlated with the interval between parturition and return to estrus (Randel, 1990). Animals that have lost body weight during the early postpartum period have lower LH levels than those that are maintaining body weight (Rutter and Randel, 1984). Serum LH and testosterone levels are increased by leptin administration in fasted mice (Ahima et al., 1996). The direct action of leptin on LH and FSH is not clear at present, but it may interact with IGF-1 and IGFBP-3 in decreasing FSH sensitivity to the negative feedback effect of estradiol. Leptin may be implicated in prolonged anestrus in postpartum cows in negative balance. Ahima et al (1996) also showed that leptin concentration was a key factor in the delay of estrus in mice, and treatment with leptin reversed this effect. IGF-1, IGFBP-2 and IGFBP-3 (Roberts et al., 1997) are known to affect the postpartum interval in cattle, but the exact role of leptin in bovine reproduction function has yet to be demonstrated

1.3. Summary of literature review

It is well-established that reproductive activity is delayed by undernutrition, and that the primary cause is related to atypical secretory patterns of LH and FSH. The exact nature of the link between nutritional status and these reproductive hormones is, however, unclear. Various sources of information suggest that IGF-1, IGFBP, glucose and leptin may play important roles in this respect.

1.4. Experimental objectives

In order to address the above-mentioned lack of understanding of the link between nutrition and reproduction, a series of experiments was conducted to study endocrine changes associated with the return to estrous in postpartum dairy cows and the tinitiation of puberty in dairy heifer fed at different planes of nutrition.

The overall aim of these experiments was to determine whether plasma concentration of IGF-1, IGFBP-2, IGFBP-3, leptin and glucose were associated with the initiation of ovarian activity in dairy cattle, and if so, to postulate the nature of their roles.

Chapter 2

2. Endocrine changes associated with the effect of nutrition on postpartum anestrus and reconception in dairy cows

2.1. Objective:

The aim of this study was to determine whether plasma concentrations of IGF-1, IGFBP-2, IGFBP-3, leptin or glucose were associated with postpartum reconception in Fresian cows fed at two different planes of nutrition.

2.2. Material and methods:

All animal protocols were approved by the University of Pretoria Ethics Committee. Eleven multiparous Fresian cows (615 +/- 18 kg body weight and with a body condition score of 4) were fed one of two diets: 80 % or 120 % of requirements (NRC, 1988) from calving until conception. Cows were milked twice daily and fed individually once daily. Diets were adjusted on a weekly basis according to body weight, body condition score, stage of lactation, daily intake and milk yield. The diet consisted of (dry matter basis): 18 % lucerne hay, 9 % corn silage, 8 % cotton seed, 13 % *Eragrostis curvula* hay and a 52 % of a commercial energy-protein supplement. The diet contained 10.9 MJ ME/kg dry matter and 176 g crude protein/kg dry matter. The diet composition was identical for the two dietary groups and treatments were effected by changing the amount of feed offered. Body weight was measured and body condition score (on a scale of 1 to 5) was estimated weekly. Heparinized blood samples were collected weekly by jugular venipuncture from calving until conception. Samples were centrifuged immediately (3000 x *g* for 12 min.), and plasma was stored at – 20 °C until analysis for progesterone, IGF-1,

Statistical analyses:

Data was analyzed by analysis of variance (SAS, 1987). The model took the effect of diet and time to resume estrus into account. Pearson correlation coefficients were calculated between weekly averages of variables. Simple and multiple linear regression analysis were carried out.

2.3. Results:

Body condition score did not differ between treatments at the beginning of the experiment (Fig. 2.1), but differed at the time of conception ($P < 0.01$).

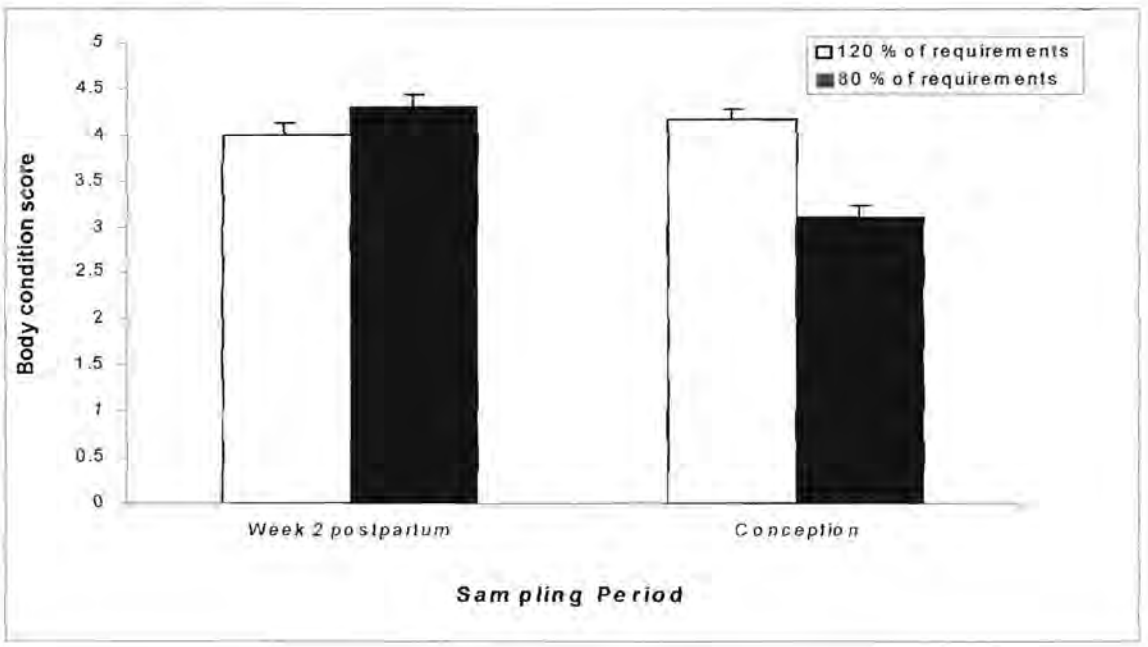

Figure 2.1. Body condition score at week 2 postpartum and at conception in postpartum cows at two level of nutrition.

Body weight was not different ($P > 0.05$) at the beginning of the experiment, but cows fed at 120 % of requirements were heavier ($P < 0.05$) than cows fed at 80 % of requirements at the time of conception (Fig. 2.2).

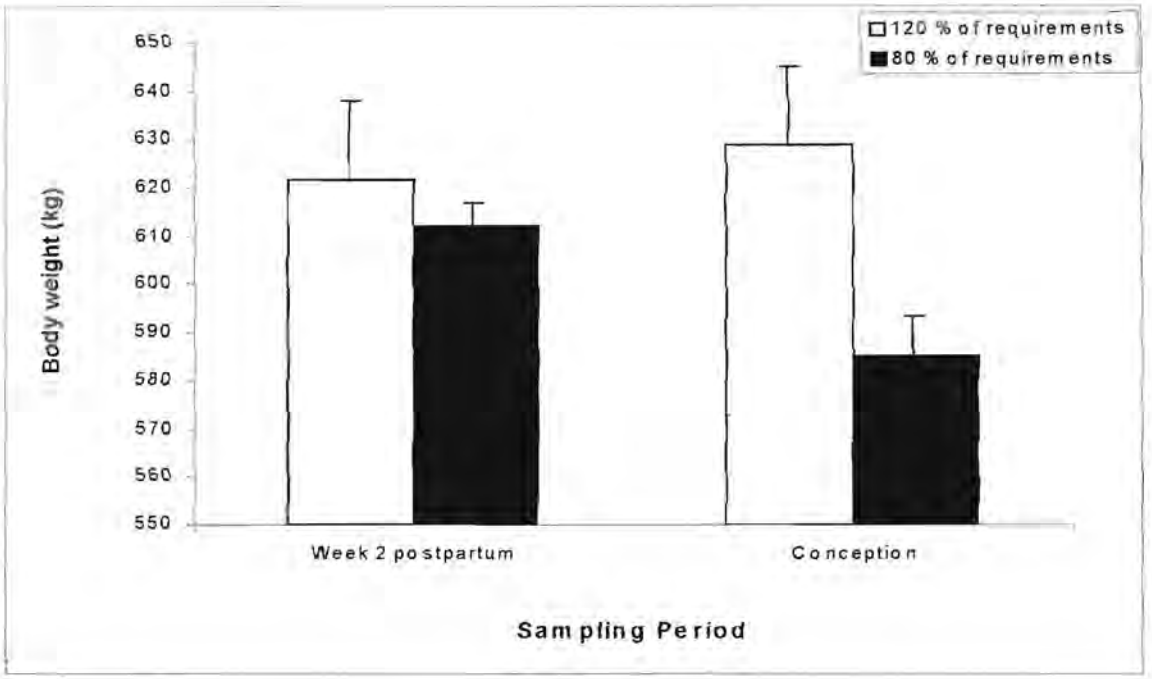

Figure 2.2. Body weight at week 2 and at conception in postpartum cows fed at two levels of nutrition.

There were no differences ($P > 0.05$) between week 2 and day of conception for mean body condition score or body weight in cows fed at 120 % of requirements, but cows fed at 80 % of requirements lost 119 g/d body weight ($P < 0.05$) and 26 % of initial body condition score ($P < 0.01$) from week 2 to conception. Nutritional treatment influenced the interval from calving to conception ($P < 0.05$). Cows fed at 80 % of requirements expressed estrus and became pregnant at 118 +/- 11 d (mean +/- s.e.m.) postpartum and cows fed at 120 % of requirements at 48 +/- 10 d (mean +/- s.e.m.). Neither level of nutrition nor sampling period had any effect ($P > 0.05$) on plasma concentration of IGF-1 (51.1 +/- 6.68 ng/ml), IGFBP-2 (40.1 +/- 6.9 arbitrary units) or glucose (3.8 +/- 0.3 mmol/l). Plasma IGFBP-3 concentration (Fig. 2.3) increased from week 2 to conception

for both treatments ($P < 0.01$), but concentrations in cows fed at 120 % of requirements were higher than those fed at 80 % of requirements in both instances ($P < 0.01$).

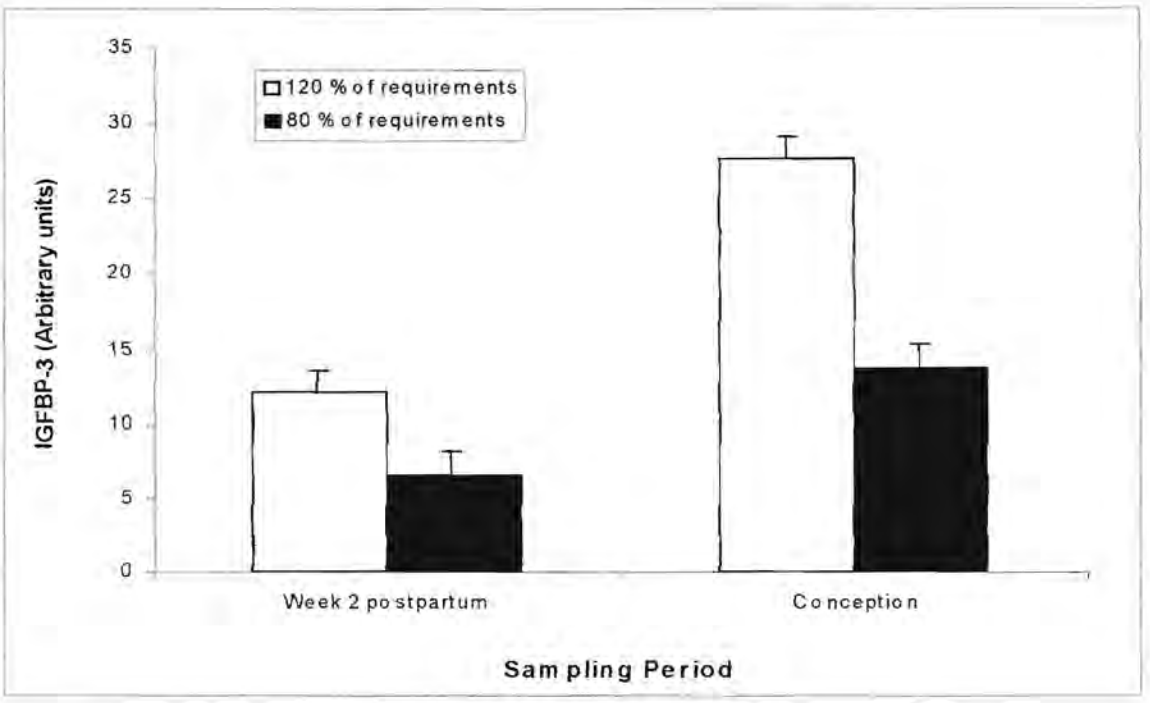

Figure 2.3. Plasma insulin-like growth factor binding protein-3 (IGFBP-3) concentration at week 2 postpartum and at conception in postpartum cows fed at two levels of nutrition.

Plasma IGFBP-3 concentration increased from week 2 to conception by 133 % in cows fed at 120 % of requirements and 85 % in cows fed at 80 % of requirements. At week 2, cows fed at 120 % of requirements had 85 % greater plasma IGFBP-3 concentrations than cows at 80 % of requirements. At conception, plasma IGFBP-3 concentrations were 115 % greater in cows fed at 120 % of requirements than those fed at 80 % of requirements. There were no differences in plasma leptin concentration (Fig. 2.4) between dietary treatments ($P > 0.05$), but concentrations were higher at conception ($P < 0.05$) than at week 2 postpartum.

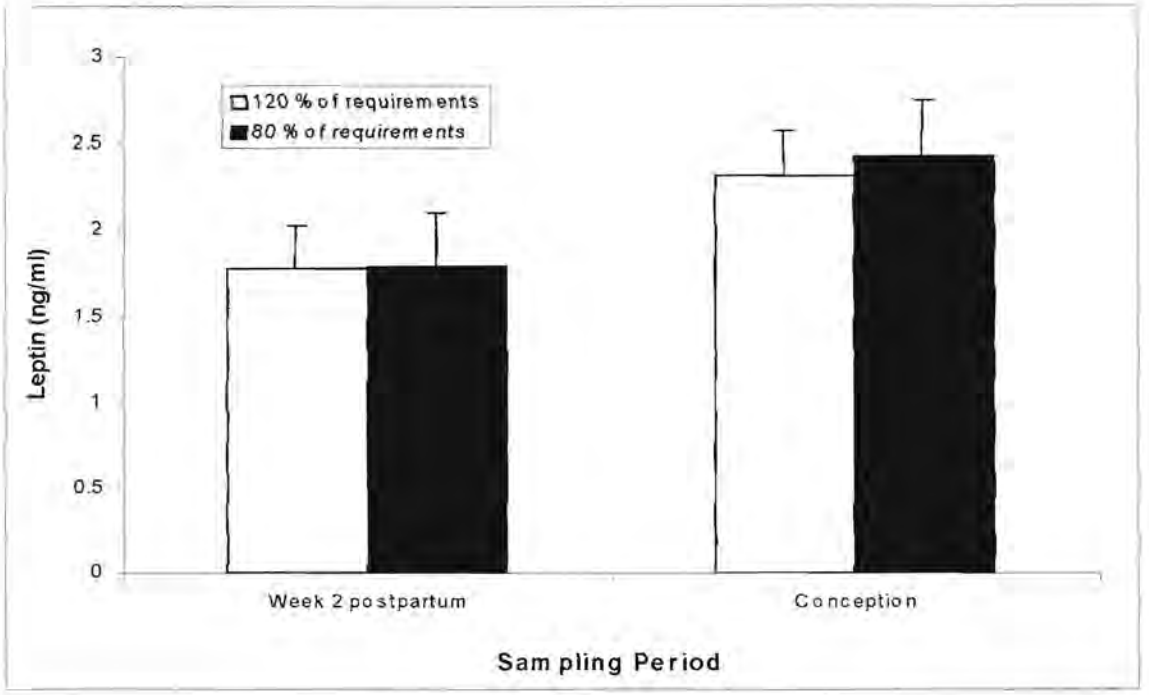

Figure 2.4. Plasma leptin concentration at week 2 postpartum and at conception in postpartum cows fed at two levels of nutrition.

2.4. Discussion:

Several studies have established a link between measures of nutritional status and fertility (Selk et al, 1988; Richards et al., 1989; Bronson and Manning, 1991; Bishop et al., 1994; Hall et al., 1997). In this experiment, cows fed at 80 % of requirements took 35 % longer to conceive than those fed at 120 % of requirements. Similar results were found by Oxenreider and Wagner (1971), who reported that cows fed at 66 % of requirements took 70 % longer form a follicle (10 mm diameter) than cows fed excess nutrients. A similar delay in return to estrous in response to dietary restriction has been reported by others (Dziuk and Bellows, 1983; Rutter and Randell, 1984). The caused of this delay in return to

estrus appears to be mainly due to impaired LH secretion (Bishop et al., 1994; Adams et al., 1997). In the present study, a low body condition score (insufficient adipose reserves) and associated with this, a high correlation ($r = -0.74$; $P < 0.01$) was also observed between body condition score and days open, when data for both treatments was pooled.

Plasma glucose concentration was proposed to be a good index to determinate rate of steroidogenesis and gonadotrophin secretion in cattle (Sen et al., 1979). In this experiment no differences in glucose concentration were observed, suggesting that changes in glucose concentration were not directly involved in mediating the effect of food restriction on days open.

Restriction of feed intake in ruminants increases secretion of GH and reduces IGF-1 concentration (Breier et al., 1986). The stimulation of LH receptors by IGF-1 secreted from thecal cells appears to be critical for the establishment of follicular dominance (Stewart et al., 1996), and it has been suggested (Schams et al., 1999) that changes in IGF-1, IGF-2 and the IGFBPs secretion serve to mediate the process of follicular development and influence life-time of the CL. In human plasma approximately 99 % of the total IGF-1 is bound to IGFBPs (Yu et al., 1999). The biological action of IGFBPs are not fully elucidated, but have been shown to inhibit or potentiate IGF bioavailability depending on experimental conditions (Murphy, 1998). Roberts et al. (1997) found that in cows fed below requirements which failed to resume estrus, IGFBP-2 was elevated and IGFBP-3 decreased, and suggested that IGFBP concentration could be used to identify females capable of returning to estrus under poor nutritional conditions. Jimenez-Krassel et al. (1999) working with primiparous Holstein cows in positive energy balance and injected with rbST, found that IGFBP-3 concentration were correlated with GH and IGF-1 concentrations in intrafollicular fluid, and explained the early and multiple follicular waves

observed in cows treated with rbST on this basis. In the present experiment there were no differences in plasma IGF-1 or IGFBP-2 concentrations between nutritional treatments or over time (week 2 *vs.* conception day), but IGF-1 concentrations were weakly ($r = 0.45$; $P < 0.05$) correlated with IGFBP-3 concentration. Plasma IGFBP-3 concentrations were lower in cows fed at 80 % of requirements. The IGFBP are believed to play a major role in the regulation of IGF action and been reported to attenuate as well as augment the actions of IGF in different tissues (Blum et al., 1989; Giudice, 1992; Funston et al., 1995). Furthermore, when data for both treatment was pooled a notably high correlation ($r = -0.81$; $P < 0.01$) was observed between IGFBP-3 concentration and days open. This indicates that it may be possible to use IGFBP-3 concentration as an early marker to predict time for recovery of reproductive activity in postpartum dairy cows, and to asses nutritional adequacy.

Plasma leptin concentration in humans, rodents and pigs is highly correlated with adipocyte size, fat mass and body mass index (Houseknecht et al., 1998). Furthermore, leptin concentration is affected by factors such as nutritional status (Chilliard et al., 1998) and physiological status (Mukherjea et al., 1999). It has been shown that leptin administration can restore fertility induced by negative energy balance in mice, even when this stimulus continues (Barasch et al., 1996), indicating that a critical level of leptin is required for normal reproduction. In this study, an increase in plasma leptin concentration from week 2 to conception supports the concept that a critical leptin concentration needed to restart luteal activity. Taken together, the information available and the results of this study with regard to plasma leptin concentration indicate that a threshold blood concentration of leptin is necessary to re-initiate reproductive activity in cattle, but this

does not appear to be the only signal to the reproductive system for re-establishment of ovarian activity.

2.5. Conclusion:

Nutritionally induced differences in the interval between calving and conception could be explained in terms of peripheral concentrations of IGFBP-3 and leptin. Furthermore, this study demonstrate that plasma IGFBP-3 concentration can be used as a predictor of days open fed different diets. Nutritionally induced anestrous has been associated with a decrease in the frequency of LH pulses. The mechanism by which nutrient deprivation regulates estrous in postpartum cows presumably involves a metabolic signal that modulates LH secretion. Although IGFBP-3 and leptin concentrations were affected by nutritional status, the exact nature and impact of these factors on reproductive performance remains to be determined. In the future, better understanding of endocrine mechanisms that regulate reconception in postpartum dairy cows may allow the design of diets that do not adversely reduce IGF-1, IGFBP-3 and leptin concentration in plasma.

Chapter 3.

3. The insulin-like growth factor system and leptin: role as possible metabolic signals for regulating puberty and growth in dairy heifers

3.1. Objective

The aim of this experiment was to study how changes in the blood concentrations of IGF-1, IGFBPs, leptin and glucose mediate the effects of food restriction and compensatory growth on the age of puberty in Fresian heifers.

3.2. Material and methods

Twelve Fresian heifers (6 months old; 179.8 +/- 6 kg BW; mean +/- s.e.m.) were allocated to one of two dietary treatments. Treatments were designed to result in two different growth rates during the first 13 weeks of the experiment: 0.3 kg/d (restricted treatment) or 0.6 kg/d (control treatment). From week 14 to 30, the restricted group received the same amount of food (on a per kg BW basis) as was fed to the control group (Fig. 3.1). All animals were fed individually and the diet contained (dry matter basis) lucerne (18 %), corn silage (9 %), cotton seed (8 %), *Eragrostis curvula* hay (13 %) and a commercial energy-protein supplement (52 %). The diet contained 10.9 MJ ME/kg dry matter and 176 g crude protein/kg dry matter. The same diet was used for both treatments but the amount fed differed. Animals were fed daily (8 am), and intake was recorded on a daily basis. Weekly body mass was recorded prior to feeding in the morning, and jugular blood samples were taken at the same time. Feed allocation required to sustain the desired rate of growth (i.e. 0.3 or 0.6 kg/d) was adjusted on a weekly basis, and calculated using the feed intake and body weight gain recorded during the preceding week.

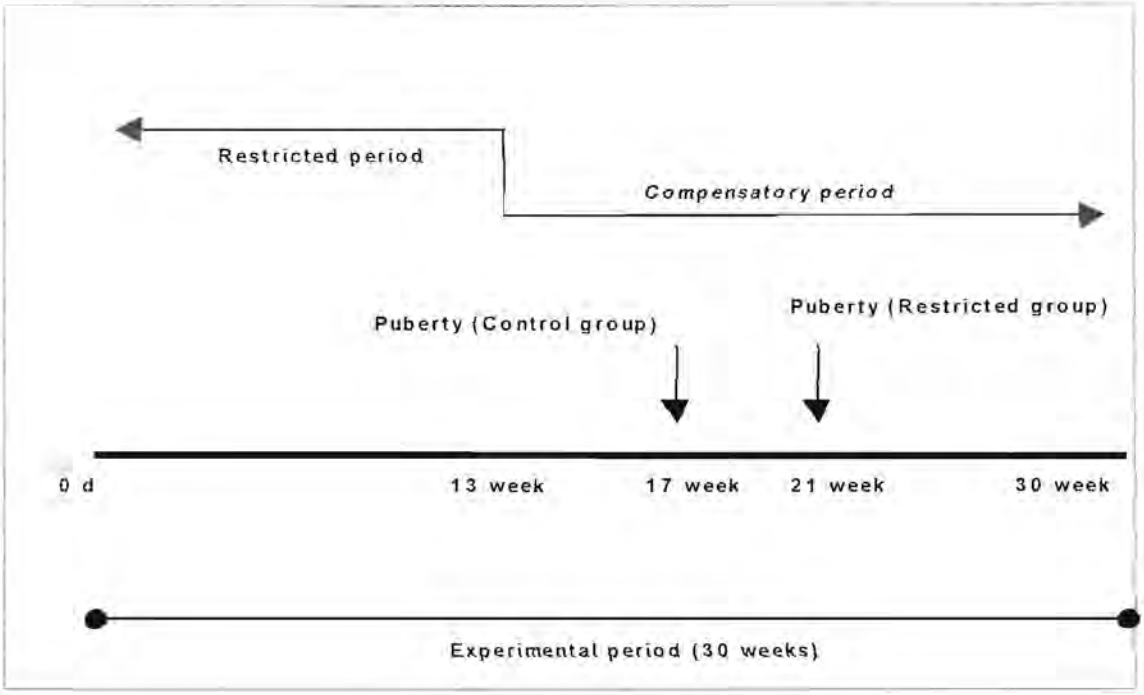

Figure 3.1. The experimental design. The day on which feeding and sampling commenced is designated as day 0 of the experiment. During the following 13 weeks two groups of heifers were fed either to sustain a 0.3 kg/d growth rate (Restricted group) or 0.6 kg/d growth rate (Control group). At week 13 the restriction period was ended and the compensatory period began (week 14 to 30). During the compensatory period the restricted group was offered the same amount of food per unit of body weight (kg) as the control group. The experiment ended at week 30.

Blood samples were collected into heparinised tubes and centrifuged (3000 x g for 12 min) within 20 min of collection, and plasma was stored at –20 °C until analysis. Plasma samples taken from week 13 to 24 were analyzed for progesterone concentrations. Plasma samples collected during weeks 0 , 7, 13, 15, 17, 21 and 30 were analized for leptin and glucose concentration. Plasma samples from weeks 0, 7, 13, 15, 17, 19, 21, 23 and 30 were analyzed for IGFBP-2 and IGFBP-3 concentrations. Plasma samples from weeks 0, 13, 15, 17 and 21 were analyzed for IGF-1 concentrations. For comparative purposes,

analyses were done for the corresponding treatment during the week represented by the mean date of puberty of either treatment (weeks 17 and 21).

Plasma IGF-1 concentration was analyzed by a non-extraction ELISA (Diagnostic Systems Laboratories Inc., USA). The inter and intra assay C.V. was 7.61 % and 3.6 % respectively. Concentrations of plasma glucose were determined by an enzymatic colorometric assay (South African Institute for Medical Research, South Africa). The inter and intra assay C.V. was 3.44 and 1.52 % respectively. Progesterone concentrations were determined using an ELISA (DRG® International, Inc., USA). The inter and intra assay C.V. was 9.1 % and 4.2 % respectively. Relative abundance of IGFBP was determined by SDS-PAGE and Western blotting. Plasma leptin concentrations were determined by ELISA (DRG® International, Inc., USA). The inter and intra assay C.V. was 5.36 % and 4.85 % respectively. Further details of assays used are given in chapter 2.

Progesterone concentrations were used to establish the presence of luteal activity. The beginning of ovulatory cycles was defined as the week prior to the first time circulating concentrations of progesterone exceeded 1 ng/ml for two consecutive weeks. Estrous was defined as the expression of overt behavioral signs such as mounting. Heifers were observed every day for signs of estrous from 7 am to 4 pm, and were equipped with a tail heat detector (Heatmount™ Detectors, Kamar Inc., USA).

Statistical analyses:

Data were analyzed by one-factor repeated measures ANOVA for IGF-1, IGFBP-2, IGFBP-3, glucose, leptin, progesterone, growth rate, intake and feed conversion ratio with dietary treatment as the factor (SAS, 1987). Differences between treatments were analyzed by analysis of variance followed by the Duncan multiple-range test.

3.3. Results:

Heifers in the control treatment reached puberty sooner ($P < 0.01$) than the restricted group. The mean age at puberty was 43.8 +/- 0.75 (mean +/- s.e.m.) weeks for the control group (17 weeks after the beginning of the experimental period), and 47 +/- 0.63 (mean +/- s.e.m.) weeks for the restricted group (21 weeks after the beginning of the experimental period). Mean body weight at puberty was 256.3 +/- 5.22 (mean +/- s.e.m.) kg BW and was not affected by treatment ($P > 0.05$). Body weight was similar ($P > 0.01$) between treatments during the early (weeks 0 to 7) or late (weeks 26 to 30) stage of the experimental period (Fig. 3.2). Growth rate differed between treatments ($P < 0.01$) from week 0 to 25 (Fig. 3.3). Body weight at the end of the trial was 294.1 +/- 9.3 kg BW (mean +/- s.e.m.) and did not differ between the two treatments ($P > 0.05$). Actual growth rates during the restriction period (0.62 kg/d and 0.33 kg/d) were similar to the target rates set for the two treatments (Fig. 3.3). Growth rates were 0.58 kg/d (control treatment) and 0.74 kg/d (restricted treatment) following the restriction period. There were differences ($P < 0.05$) in dry matter intake during the restriction period as a result of the experimental protocol, however, no difference ($P > 0.05$) was observed between the two dietary treatments after the restriction period. There were no differences ($P > 0.05$) between dietary treatments for feed conversion ratio (Fig. 3.4) during the first 13 weeks of the experiment, but during the following 14 weeks, a superior feed conversion ratio was observed for the restricted treatment required ($P < 0.05$). There were no treatment differences ($P > 0.05$) for plasma concentration of leptin, IGF-1, IGFBP-3, IGFBP-2 and glucose at the beginning of the experiment (Figs. 3.5 to 3.8).

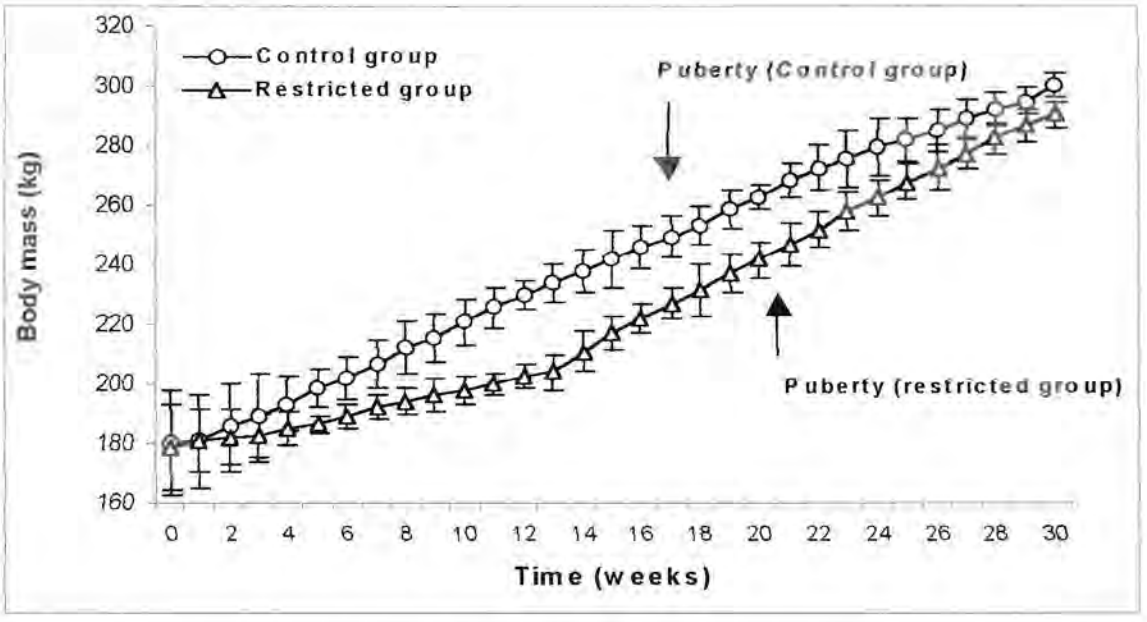

Figure 3.2. Body mass in heifers fed to maintain different growth rates. The control group was fed to sustain a growth rate of 0.6 kg/d throughout the experimental period. The restriction group was fed to sustain a growth rate of 0.3 kg/d during the first 13 weeks and received same amount of food as the control group for the remainder of the experimental period.

By the end of the restriction period (week 13) there were differences ($P < 0.01$) between dietary treatments for plasma leptin, IGF-1, IGFBP-3 and glucose concentrations. There were no differences ($P > 0.05$) in IGFBP-2 concentrations between treatments or sampling periods. Leptin concentration (Fig. 3.5) was higher ($P < 0.01$) in the control group animals than in the restricted animals at weeks 7, 13, 15, 17 and 21. Within the control group, there was a sequential increase ($P < 0.01$) in plasma leptin concentration between weeks 0, 7, 13, 15 and 17 respectively (Fig. 3.5).

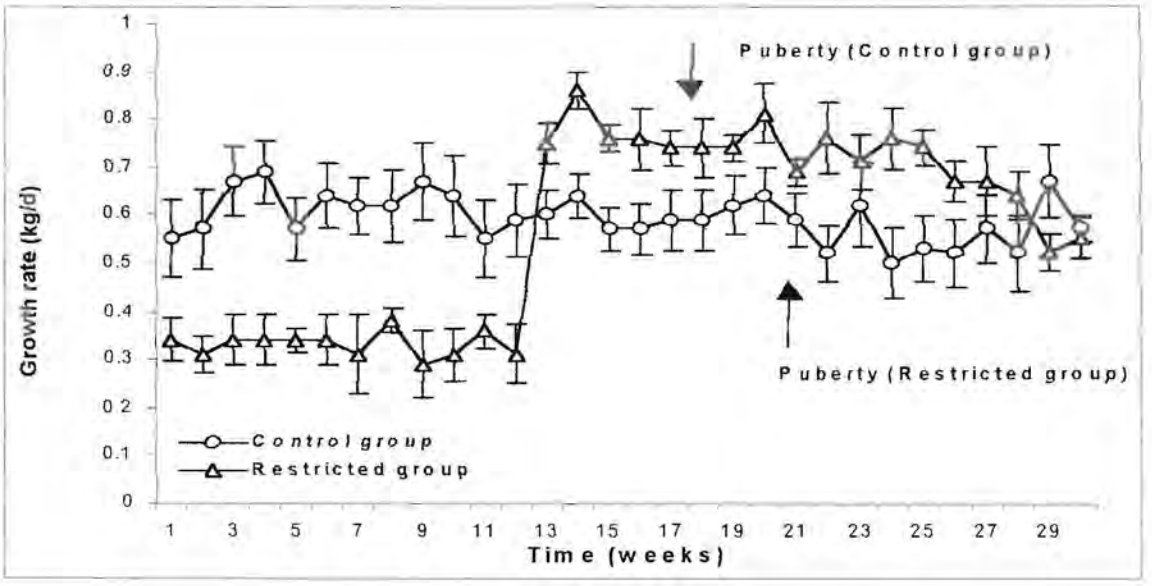

Figure 3.3. Growth rate in heifers fed to maintain different growth rates. The control group was fed to sustain a growth rate of 0.6 kg/d throughout the experimental period. The restriction group was fed to sustain a growth rate of 0.3 kg/d during the first 13 weeks and received same amount of food as the control group for the remainder of the experimental period.

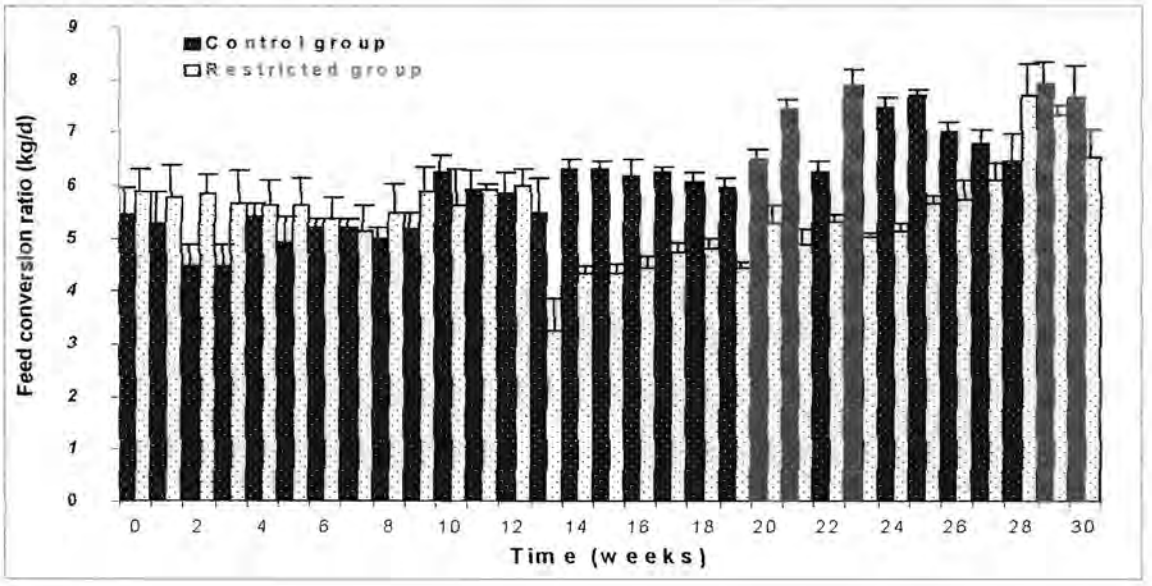

Figure 3.4. Food conversion ratio in heifers fed to maintain different growth rates. The control group was fed to sustain a growth rate of 0.6 kg/d throughout the experimental period. The restriction group was fed to sustain a growth rate of 0.3 kg/d during the first 13 weeks and received same amount of food as the control group for the remainder of the experimental period.

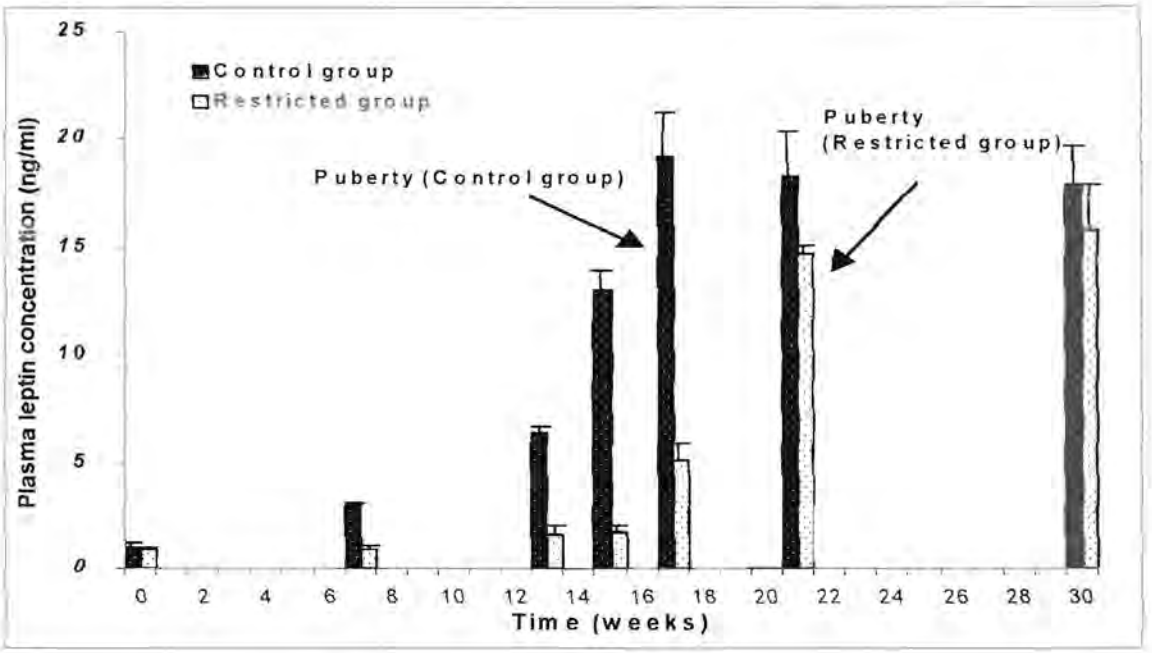

Figure 3.5. Plasma leptin concentration in heifers fed to maintain different growth rates. The control group was fed to sustain a growth rate of 0.6 kg/d throughout the experimental period. The restriction group was fed to sustain a growth rate of 0.3 kg/d during the first 13 weeks and received same amount of food as the control group for the remainder of the experimental period.

In the restricted group, plasma leptin concentration was higher in weeks 17, 21 and 30 than in weeks 0, 7, 13 and 15, but no difference ($P > 0.05$) were found between weeks 21 and 30. Plasma IGF-1 concentration (Fig. 3.6) was higher in the control group than in the restricted group at the end of the restriction phase (week 13). Plasma IGF-1 concentrations during the compensatory growth period (weeks 17 – 30) did not differ ($P > 0.05$) between treatments (Fig. 3.6). Higher IGF-1 concentrations were observed in the restricted group during week 15, when growth rates were at their highest ($P < 0.01$). Within the restricted group, IGF-1 concentration differed ($P < 0.01$) between samples taken from week 13 *vs.* week 15, but not during the restriction period (0 to 13 weeks) or between weeks 15, 17 and 21. Plasma IGF-1 concentration in the control group remained

unchanged ($P > 0.05$) during weeks 13, 15 and 17, but was lower ($P < 0.05$) at week 0 than during the rest of the period.

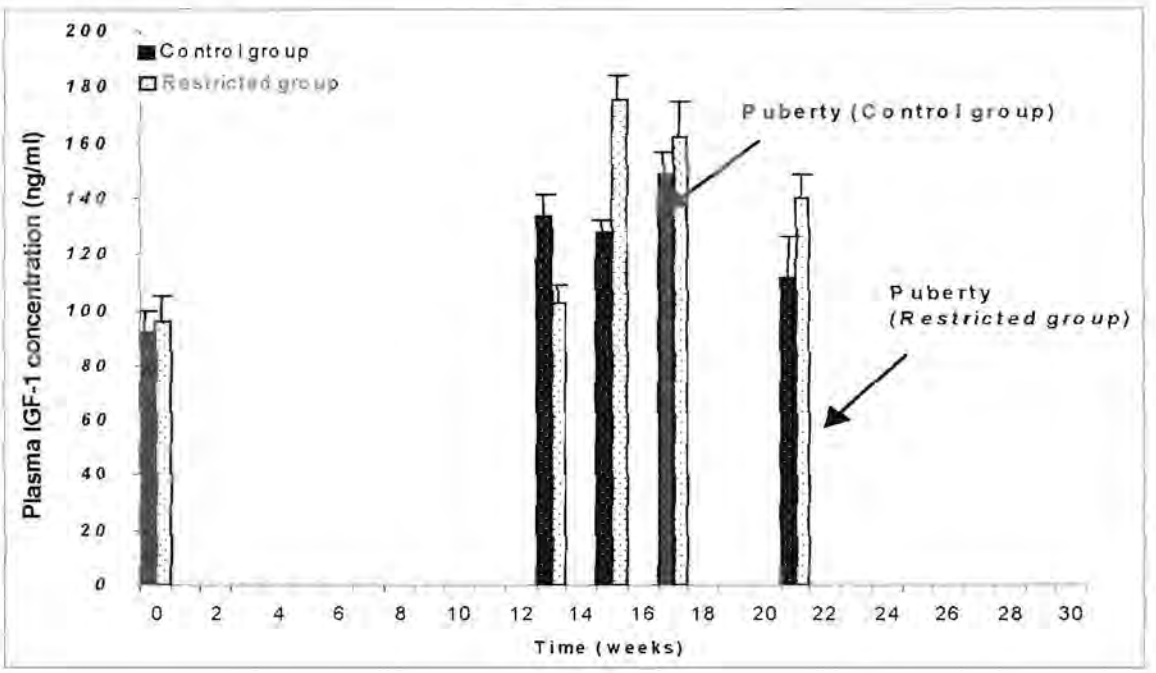

Figure 3.6. Plasma insulin-like growth factor-1 (IGF-1) concentration in heifers fed to maintain different growth rates. The control group was fed to sustain a growth rate of 0.6 kg/d throughout the experimental period. The restriction group was fed to sustain a growth rate of 0.3 kg/d during the first 13 weeks and received same amount of food as the control group for the remainder of the experimental period.

Plasma IGFBP-3 concentrations (Fig. 3.7) differed ($P < 0.05$) between treatments at week 7, 13, 19 and 21. Within the control group, differences were observed between week 0 and weeks 7, 13, 15, 17 and 19. Plasma IGFBP-3 concentrations were higher during weeks 15 and 17 than week 19, 21, 23 and 30 in control group. Within the restricted group, differences ($P < 0.05$) were observed between the restriction period and the compensatory growth period. Plasma IGFBP-3 concentration increased ($P < 0.05$) during compensatory period from week 13 until week 21. After week 21, plasma IGFBP-3 concentration decreased ($P < 0.05$) compared to that of the peripubertal period (weeks 17 to 21).

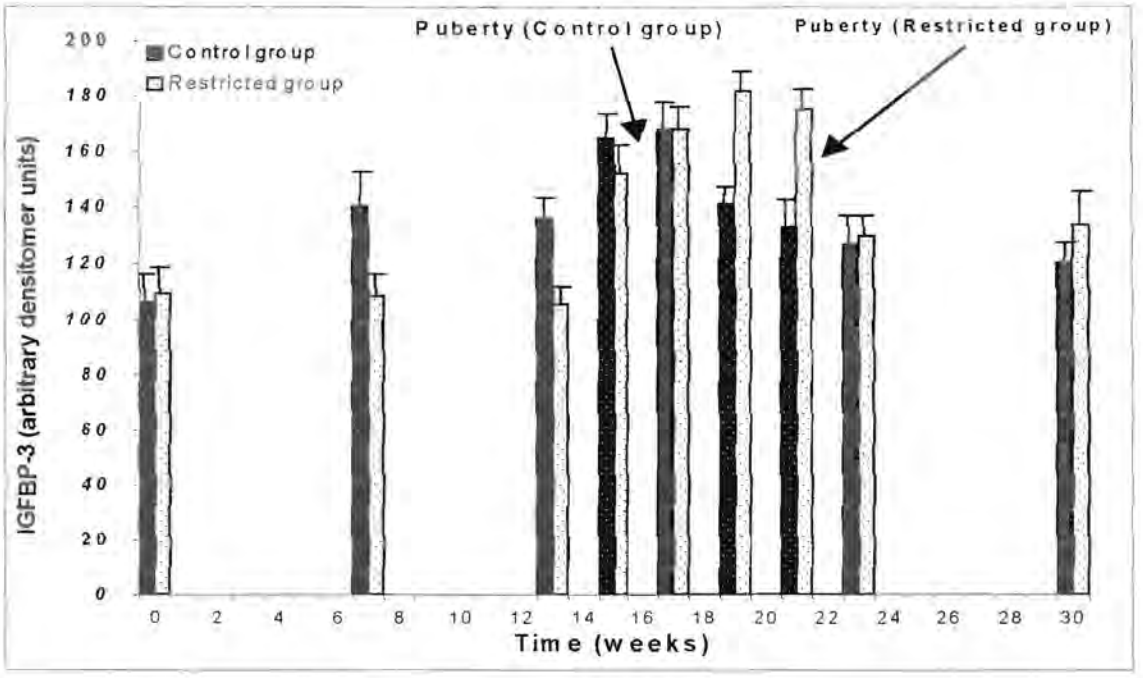

Figure 3.7. Plasma insulin-like growth factor binding protein-3 (IGFBP-3) concentration in heifers fed to maintain different growth rates. The control group was fed to sustain a growth rate of 0.6 kg/d throughout the experimental period. The restriction group was fed to sustain a growth rate of 0.3 kg/d during the first 13 weeks and received same amount of food as the control group for the remainder of the experimental period.

Plasma glucose concentration (Fig. 3.8) was higher ($P < 0.05$) in the control group than the test group during the restriction period. During week 15 (compensatory period) higher ($P < 0.01$) concentrations were observed in the previously restricted group than in the control group. There were no difference between weeks 17, 21 and 30. There were no differences in glucose concentration between weeks in the control group over the entire period, but in the restricted treatment concentrations of glucose during in the compensatory phase were higher than those during the restricted phase ($P < 0.05$). No differences were observed between weeks during the compensatory phase in restricted group animals.

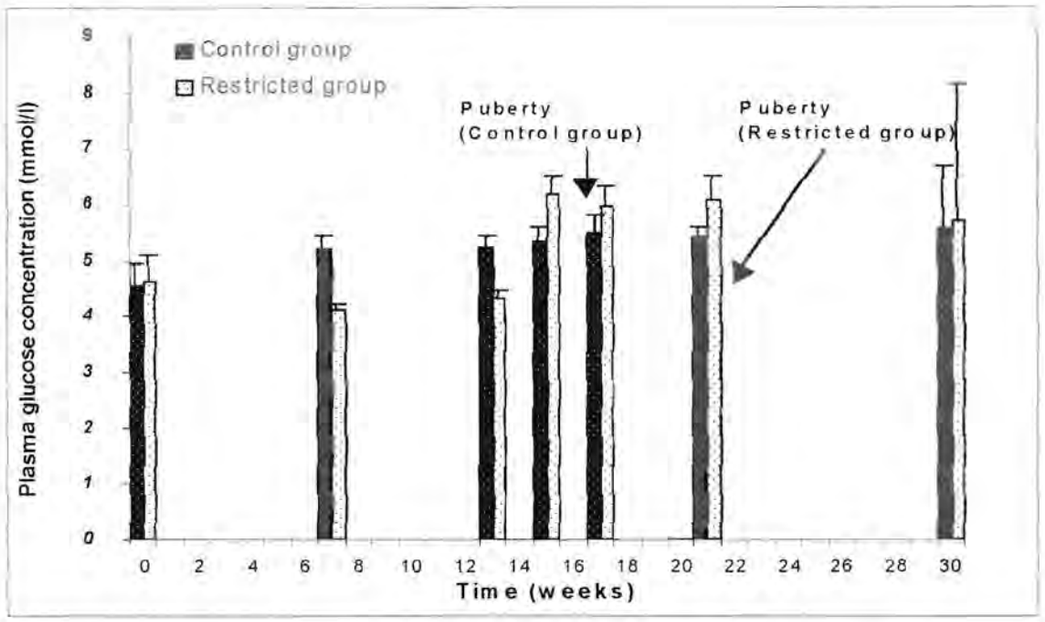

Figure 3.8. Plasma glucose concentration in heifers fed to maintain different growth rates. The control group was fed to sustain a growth rate of 0.6 kg/d throughout the experimental period. The restriction group was fed to sustain a growth rate of 0.3 kg/d during the first 13 weeks and received same amount of food as the control group for the remainder of the experimental period.

At week 17 (when the mean of control group animals reach puberty) difference were found ($P < 0.01$) between treatments for plasma leptin concentration, but no difference were found in plasma IGF-1, IGFBP-2, IGFBP-3 and glucose (Table 3.1). When most of the restricted animals onset puberty (week 21), plasma leptin ($P < 0.01$) and IGFBP-3 concentration differ between treatments, but no difference were found at puberty in plasma IGF-1, IGFBP-2 and glucose concentration (Table 3.1).

(Yambayamba et al., 1996), diet digestibility (Murphy and Loerch, 1994) and nutrient partitioning (Kamalzadeh et al., 1998) may all be changed by feed restriction, and contribute to compensatory growth effects in ruminants. In this experiment, the restricted group had a lower growth rate than the control group during the first 13 weeks (restriction

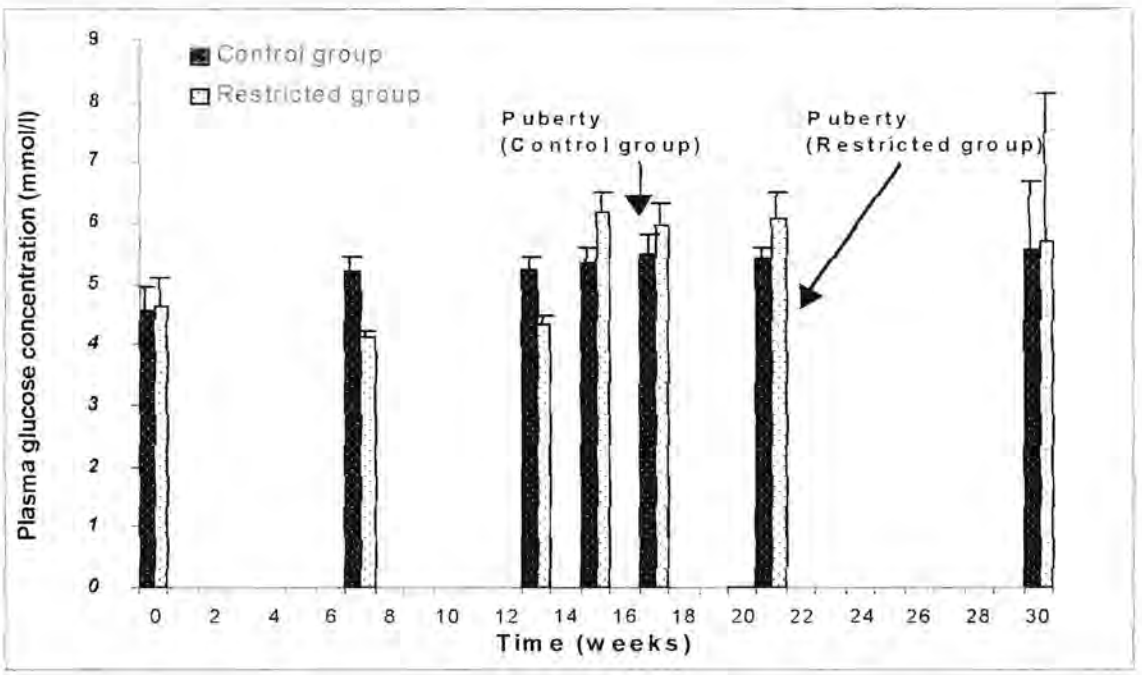

Figure 3.8. Plasma glucose concentration in heifers fed to maintain different growth rates. The control group was fed to sustain a growth rate of 0.6 kg/d throughout the experimental period. The restriction group was fed to sustain a growth rate of 0.3 kg/d during the first 13 weeks and received same amount of food as the control group for the remainder of the experimental period.

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Table 3.1. Mean concentration of hormones and metabolites in plasma during the control and restricted treatment in Fresian growing heifers[A]

		IGF-1 (1)	IGFBP-2 (2)	IGFBP-3 (2)	Leptin (3)	Glucose (4)
Week 0						
	Control	91.4 +/- 9.3	66 +/- 11.3	106 +/- 10.2	0.979 +/- 0.2	4.5 +/- 0.4
	Restricted	95.7 +/- 8.1	58 +/- 9.6	109 +/- 9.6	0.879 +/- 0.2	4.6 +/- 0.5
Week 13						
	Control	133.4 +/- 6.3	69 +/- 4.7	136 +/- 7.7	6.316 +/- 0.4	5.2 +/- 0.2
	Restricted	102.8 +/- 7.8	65 +/- 6.3	105 +/- 6.3	1.624 +/- 0.4	4.3 +/- 0.2
Puberty*						
	Control	148.4 +/- 12.6	73 +/- 9.3	168 +/- 9.3	19.11 +/- 2.1	5.5 +/- 0.3
	Restricted	140 +/- 15.3	75 +/- 7.3	182 +/- 6.2	14.69 +/- 0.4	6.1 +/- 0.4

[A] Values presented are means +/- s.e.m. for concentration of hormones and metabolites
(1) Insulin-like growth factor-1, values are in ng/ml
(2) Insulin-like-growth factor binding protein, value are arbitrary densitometer units
(3) Values are in ng/ml
(4) Values are in mmol/l
(*) Data for the control treatment mean is derived from actual values recorded during the week of puberty for each heifer. Data for the restriction treatment is derived from values recorded during the week which corresponded to the mean week of puberty for the control treatment, i.e. week 17.

3.4. Discussion:

Although much research has been done to elucidate the mechanism of compensatory gain and its effects on mass and puberty, aspects of this phenomenon remain poorly understood. In particular, experiments where different planes of nutrition have been applied have given conflicting results in terms of animal performance, endocrine status and feed intake. Intake (Hornick et al., 1998), nutrient requirements (Sainz et al., 1995), composition of gain, energy utilization (Carstens et al., 1991), endocrine status (Yambayamba et al., 1996), diet digestibility (Murphy and Loerch, 1994) and nutrient partitioning (Kamalzadeh et al., 1998) may all be changed by feed restriction, and contribute to compensatory growth effects in ruminants. In this experiment, the restricted group had a lower growth rate than the control group during the first 13 weeks (restriction

period). During the following 17 weeks, the growth rate of the restricted animals was greater than that of the control group (compensatory growth). Growth rate was greater during the first 4 weeks of the re-alimentation period than during the following 13 weeks in the restricted group animals (Fig. 3.3). Several studies have shown a similar pattern, and this was attributed to differences in the ratio of protein : fat deposition during initial vs. subsequent mass gain (Wright and Russel, 1991; Sainz et al., 1995; Hornick et al., 1998), and to differences in the efficiency of energy and protein utilization (Fox et al., 1972). Growth and development of various organs is affected by feed restriction and subsequent refeeding (e.g. kidney, liver and gastrointestinal tract), and this could also explain much of the differences. Changes in gut-fill and energy content of gain are considered to be the most important cause of compensatory growth (Carstens et al., 1991; Hornick et al., 1998).

Yambayamba et al. (1996) found evidence of an association between GH concentration and growth rate, and this association was strongest during the early compensatory growth period characterized by high growth rates. Working with two growth rates (1.36 kg/d and 0.26 kg/d) in beef heifers, Yelich et al. (1996) found differences in the pattern of GH concentration during the restriction period that were associated with growth rate and level of intake. This difference disappeared when heifers attained similar growth rates. It is probable that the main effect of these changes in GH secretory pattern would be to influence IGF-1 secretion. Hornick et al. (1998) found that growth rate was associated with serum IGF-1 concentration in animals that were restricted at different times and also during the first phase of refeeding. Van den Brande (1986) suggested that rapid growth rate during compensatory growth is caused by a change in tissue sensitivity to IGF-1. On the other hand, plasma IGF-1 concentration during the restricted period could result from enhanced clearance of the IGF-1 fraction which is bound to the binding proteins (Breier

and Gluckman, 1991). In this study, plasma IGF-1 and IGFBP-3 concentrations in both treatments followed a pattern similar to that of growth rate. Plasma IGF-1 and IGFBP-3 concentration was higher in the restricted group than in the control group during the early compensatory phase. Higher concentrations of GH during the early compensatory stage (Yambayamba et al., 1996), changes in GH receptor activity (Davis, 1988) and interactions between IGF-1 and IGFBP-3 (Spicer and Chamberlain, 1999) could explain compensatory growth effects in young heifers. Riedl et al. (1998), working with renal transplanted peripubertal children, found that catch-up weight after surgery was better related to changes in the ratio of IGF-1 to IGFBP-3 than to the total amount of IGF-1 or IGFBP-3.

The higher average daily gain in the control heifers resulted in attainment of puberty at a younger age than in restricted animals, but body weight at puberty was similar for both treatments. Several authors (Menge et al., 1960; Short et al., 1971; Yelich et al. 1996) have been shown that there is a inverse correlation between the level of intake and age at puberty in heifers. Nutritional status affects circulating concentrations of IGF-1, as discussed above, and this may affect not only growth rate, but other physiological functions. IGF-1 stimulates mitogenesis and steroidogenesis in ovarian granulosa and thecal cells (Stewart et al., 1996). Spicer and Chamberlain (1999) found that IGFBP-3 inhibited the mitogenic effects of IGF-1 in granulosa cells in cattle ovaries and inhibited estradiol production, but did not decrease progesterone concentration. Furthermore, a change in the ratio of IGFBP-3:IGF-1 produced different effects on steroidogenesis and cell division, suggesting that IGFBP-3 has regulatory effects on reproduction. Data obtained in this experiment, show that IGFBP-3 concentration changes during the peripubertal period (weeks 17 and 21) but not IGF-1 concentration. This would have resulted in a change in the ratio between IGFBP-3 and IGF-1, and suggests that variation

in IGF-1:IGFBP-3 ratio could possibly mediated the time to reach puberty. Results obtain by Jones et al. (1991), Granger et al. (1989) and Yelich et al. (1996) differ from results obtained in the present experiment in terms of association between puberty and IGF-1 concentration. These authors found that IGF-1 increased before puberty, independent of dietary treatment. Differences in breed, age, duration of restriction, level of intake and sampling protocols could possibly be responsible for these differences. In this experiment, differences in growth rate could be explained by differences in IGF-1 concentration, but not the difference in the time taken to reach puberty. IGFBP-3 concentration differences, however, could possibly be explain the effect on puberty.

Spicer and Chamberlain (1999) have shown that IGFBP-2 is produced in ovarian follicular cells and can modulate the availability of IGF-1 in different ways, acting via endocrine, paracrine and autocrine pathways during follicular development. In this experiment peripheral concentrations of IGFBP-2 were similar between treatments suggesting that IGFBP-2 did not affect the availability of IGF-1 in the peripheral circulation.

Fast-growing heifers had greater LH concentrations than those subject to nutritional restriction (Yelich et al. 1996). Furthermore, Evans et al. (1994) and Day et al. (1986) found that LH concentration and frequency increased at puberty. The endocrine mechanisms responsible for inducing increased LH secretion before the first ovulation are not clear. Pulsatile secretion of LH is necessary to initiate luteal activity in heifers (Day et al., 1984). Furthermore, it has been shown that the circulating LH concentration and pulse frequency increase two month proceeding puberty in cattle (Kinder et al., 1987). Negative energy balance can lower IGF-1 and LH secretion and this can contribute to delayed puberty (Richards et al., 1991). A synergistic effect between IGF-1 and LH on

steroidogenesis in bovine thecal cells that mediated by changes in the concentration of LH receptors has been shown (Stewart et al., 1995). In this study, IGF-1 concentrations were elevated at the time of puberty in both treatments in comparison with the concentrations at the end of the restriction period.

Animals fed restricted diets at different stages and subject to differences in the severity of growth restriction had different amount of adipose tissue (Sainz et al., 1995). In this experiment, feed restriction prolonged the time required to reach puberty. Although adiposity was not measured in this trial, the differences in weight gain would be expected to be related to differences in adiposity. The importance of a critical amount of fat for attainment of puberty is well-known (Frisch and McArthur, 1974; Snow et al., 1989; Frisch, 1994), suggesting that an interaction exits between adipose tissue and the hypothalamic-pituitary-gonadal axis to ensure that sufficient energy supplies for pregnancy and lactation are deposited before commencement of reproductive cycles. Leptin is a recently discovered protein that is mainly synthesized in adipose tissue (Considine and Caro, 1996). The rate of leptin production is directly related to body fat mass in several species (Prolo et al., 1998). Although circulating leptin appears to correlate with the amount of adipose tissue, changes in energy intake and growth rate can alter serum leptin concentration (Foster and Nagatani, 1999). Amstalden et al. (1999), found that fasting (56 h) in prepubertal heifers reduced leptin mRNA expression in adipose tissue by 45 % and

were associated with time required to reach puberty. Foster and Nagatani (1999) suggest that leptin affects the time taken to reach puberty by its interaction with IGF-1 as well through glucose availability. In conclusion, it is possible that leptin could regulate GnRH secretion in young heifers, and that puberty was delayed in the restricted group because this hormone, in conjunction with IGF-1 and IGFBP-3, constitutes a signal indicating that energy reserves were not sufficient to initiate GnRH secretion. Nyomba et al. (1999), working with lean and obese human females, found that leptin was better related to IGFBP-3 concentration and body mass index than to IGF-1 and glucose concentration. Chehab et al., (1996) reported that prepuberal female mice injected with leptin reached puberty 9 days before untreated mice and suggested that leptin acts as signal for initiation of puberty, but concluded that this is not the only signal involved in the onset of puberty.

Glucose availability is also thought to be a metabolic signal involved with the control of GnRH secretion. In sheep (Bucholtz et al., 1996) and cattle (Stewart et al., 1995) glucose availability is associated with LH secretion. This finding suggests that glucose could also serve as a signal for providing information about body composition during development, and may regulate GnRH secretion. Yambayamba et al. (1996) found that plasma glucose concentration in beef heifers was higher after restriction than during restriction. Furthermore, Foster and Nagatani (1999) proposed that glucose serves as a metabolic signal because the concentration of this metabolite increases during puberty, increasing transport into the cell and glucose metabolism. Sano et al. (1999) found that the glucose turnover and insulin sensitivity was altered in feed-restricted sheep. In this experiment, plasma glucose concentration did not change at puberty for either treatment; however, differences between treatments were found after the restriction period. Plasma glucose concentrations change with nutrient supply and this could alter the LH pattern in

heifers growing at different growth rates (Yelich et al., 1996). The relationship between plasma glucose concentration and luteal activity are contradictory (Richards et al., 1989; Rutter and Manns, 1987), and not clear mechanism has been identified. Emilsso et al. (1998) showed that leptin inhibited the stimulatory effects of glucose on insulin secretion in *ob/ob* mice. At present, the mechanism by which leptin regulates insulin secretion is not clear. In this experiment, no association was found between leptin and glucose concentration (Figs. 3.5 and 3.8).

The exact role of leptin and IGFBP-3 in the onset of puberty needs future investigation. It is suggested that the increased concentrations of leptin and IGFBP-3 during pubertal development can act as physiological signals for the onset of puberty. A better understanding of the numerous factors affecting compensatory growth and puberty could contribute to the development of improved nutritional strategies for replacement heifers, reducing the time required to reach puberty.

3.5. Conclusion:

The present results show that concentration of glucose, IGF-1 and IGFBP-3 were directly affected by the restriction program and contribute in same measure to present knowledge of the endocrine basis of compensatory growth.

Delayed puberty in feed-restricted animals was also shown to be mediated by changes in the endocrine system. Early puberty (control animals) achieved by accelerated prepubertal growth results in earlier attainment of high concentrations of IGF-1, IGFBP-3 and leptin. Increase concentrations of leptin and IGFBP-3 (and the accompanying change in the ratio of IGF-1 to IGFBP-3) during puberty appear to signal the hypothalamus that body reserves and the nutritional environment are adequate to support reproduction in

ruminants. The precise mechanism by which leptin, glucose, IGF-1 and IGFBP-3 concentrations are linked remains unknown. The management and manipulation of these endocrine factors may be useful in the future to induce early puberty in younger or/and thinner animals and to reduce the interval between calving and conception in ruminants.

Chapter 4: Integration and conclusion

The early postpartum period in lactating dairy cows and the peripubertal stage in young heifers was characterized in previous chapters as involving many metabolic adjustments and their interplay with complex endocrine mechanisms related to normal ovarian activity. To understand the impact of physiological demands (lactation, pregnancy or growth) on reproductive activity it was necessary to consider the associations among metabolic factors including glucose utilization, adiposity (leptin), tissue growth (IGF-1 and IGFBP) and ovarian activity (Chapter 1). Is important to bear in mind that during periods of energetic insufficiency these functions will compete for nutrients, and ovulation has a relatively low priority.

One of the potential metabolic cues for initiation of reconception and puberty might be the status of body fat reserves. The search for a metabolic signal originating from adipose tissue has led to the identification of leptin. Plasma leptin concentration in cattle and sheep is decreased by undernutrition and increased by refeeding. In the experiment on puberty (Chapter 3) plasma leptin concentration was affected by the adiposity of the animal. An other finding of these experiments is that in dairy cattle it is possible to link peripheral leptin concentration with reproductive activity, but threshold levels differed between peripubertal animals (Chapter 3) and mature cows (Chapter 2). Postpartum cows became pregnant when plasma leptin concentration reached 3 – 4 ng/ml, and young prepubertal heifers at 20 ng/ml.

The results suggest that normal ovulatory activity is subject to regulation not only by leptin, but by multiple signals related to energy balance. During negative energy balance in early lactation, the rapid increase in utilization of glucose for milk lactose production results in lower plasma concentrations of both glucose and insulin compared to

that in later stages of lactation. LH pulse patterns may be depressed directly by low insulin concentration or secondarily by increased production and utilization of ketones as a consequence of fat mobilization. In these experiments glucose concentration appeared to affect growth rate in heifers but no direct effect on reproductive activity was found either in prepuberal heifer or in postpartum cows.

In these experiment it was found that IGF-1/IGFBP-3 could also act as a metabolic signal for the regulation of puberty and reconception in dairy cattle. In sheep the continued exposure of uterus to progesterone induces the production of an inhibitor of IGFBP-3 activity (Ko et al., 1991), which may explain the interaction between IGFBP-3 and IGF-1 relates to reproduction. A change in the concentration of IGFBP-3, and thus the ratio of IGFBP-3 to IGF-1, will change the bioavailability of IGF-1 and its effect on various tissues. In the present experiment, although nutrition during the postpartum and growth period of dairy cattle affected reproductive activity, no conclusion could be made regarding the existence of a single specific nutritional signal controlling the initiation of reproductive activity. Leptin, glucose, IGF-1 or/and IGFBP-3 were all found to be involved with reconception and puberty. In the future, manipulation of one or all of these three factors could have an impact on reproductive efficiency in domestic livestock. Further research is needed to determine the nature of the relationship between leptin, IGF-1, IGFBP-3 and glucose. Bronson (1998), in his review, mentioned that "many experimental studies in animals and large number of correlational studies in humans have failed to find a direct effect of fatness on ovulation in either adult or peripubertal females". This can be perhaps be explained by the concept that the link between normal ovarian activity and nutrition is not determined by a simple or single unique signal, but probably by several factors with overlapping effects and by the interaction. Further research is

needed to understand metabolic signals affect reproductive activity at different stages of growth (long term nutritional restriction) or at different stages of follicular growth (short term nutritional restriction). It has been proposed that follicular growth and development in the bovine (Lussier et al., 1987) and in the rat (Hirshfield, 1991) can occur under basal concentrations of the metabolic hormones and growth factors until a certain stage of granulosa cell generation is reached, after which concentrations of growth factors and hormones have to increase in order to avoid ovarian atresia. Finally, differences between mammalian species in terms of reproductive activity appears to be the result of differences in signaling systems. The study of ovarian activity in diverse mammalian species may be a useful way to improve our understanding of the complex reproductive signaling system.

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