Anim. Reprod., v.10, n.3, p.199-205, Jul./Sept. 2013

Metabolic hormones and reproductive function in cattle

R. Sartori[1], M.M. Guardieiro, R.S. Surjus, L.F. Melo, A.B. Prata, M. Ishiguro, M.R. Bastos, A.B. Nascimento

Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, Brazil.

Abstract

Diets can alter the concentrations of circulating hormones such as insulin and IGF-I. Such responsive hormones are related directly to nutritional status and moreover, directly or indirectly, associated with reproductive function and fertility. Metabolic hormones are involved in follicular development, number and size of ovarian structures, circulating concentrations of steroid hormones, duration of estrus, steroidogenesis, ovulation and embryonic development. However, circulating metabolic hormones in excess, resulting from high dry matter/energy intake can also contribute to the reduction of oocyte and embryo quality. Although changes in dietary intake affect ovarian function in *Bos taurus* and *Bos indicus* cattle, it seems that overfeeding influences more profoundly oocytes/embryos from heifers and cows of *Bos taurus* than of *Bos indicus* breeds. There is also a distinct effect of nutrition on *in vitro vs*. *in vivo* embryo production, in which metabolic hormones seem to affect more the later stages of follicle development. Thus, this paper presents and discusses the results of some relevant studies on the role of feed intake and its association with metabolic hormones in bovine reproduction.

Keywords: bovine, embryo, IGF-I, insulin, ovary, physiology.

Introduction

Metabolic hormones are associated with diets provided to animals and have an important role in the regulation of reproductive activity from oogenesis or spermatogenesis, embryo development, and fetal growth until parturition. These hormones, mainly insulin and IGF-I are associated with aspects of physiology and reproductive performance in ruminants. Due to confounding factors related to hormones or other substances that change due to dietary alterations, it is often difficult to discern which factor is responsible for timely responses under different moments or feeding amounts.

This article aims to discuss findings from key studies that evaluated the influence of these metabolic hormones in ruminants, especially in the cow. In addition, we will present studies performed in our laboratory, where we observed the association of metabolic hormones and reproductive aspects in cattle, often comparing animals of different genetic groups.

Metabolic hormones

Growth Hormone (GH)

Growth hormone plays an important role in the regulation of ovarian function, however the exact mechanisms of its action are not well understood. It is known that nutritional deficiency leads to increased plasma GH, and its main effect appears to be in regulating the synthesis and release of IGF-I in the liver. However, there is a possibility that GH has a direct effect on the ovary due to the presence of mRNA for GH receptors (Gong *et al*., 1991).

Chase *et al*. (1998) and Bossis *et al*. (1999) have shown that GH release is controlled by nutrition and IGF-I concentration by a negative feedback. However, the GH action on the IGF-I production is dependent on insulin. Underfed animals show high circulating GH and low insulin and IGF-I concentrations. This is due to a lower concentration of liver GH receptors (rGH), which may be due to lower induction of rGH and/or reduction in second-messenger signaling. Insulin induces active rGH and its absence leads to decreased responsiveness to GH (Chase *et al*., 1998).

Butler *et al*. (2003) showed that insulin restores responsiveness to GH in dairy cows with negative energy balance (NEB) induced by lactation, by affecting the expression of IGF-I and rGH. IGF-I continuously increased during the infusion of insulin. Furthermore, higher expression of mRNAs for liver GH and IGF-I receptors were detected in cows infused with insulin compared to the control group. It was concluded that hypoinsulinemia in the postpartum period of dairy cows is responsible for the lower liver rGH expression, resulting in no GH binding and no subsequent secretion of IGF-I.

IGF system

Insulin-like growth factors (IGFs) are produced and secreted primarily by the liver in response to GH stimulus and may or may not be associated with nutrition but mainly due to insulin concentrations. The GHr is found in numerous tissues but it is plentiful in the

[1]Corresponding author: robertosartori@usp.br
Phone: +55(19)3429-4134.
Received: May 15, 2013
Accepted: July 2, 2013

liver. IGFs act as mediators of most growth-promoting actions of GH and are single-chain polypeptides with structural homology to proinsulin. IGFs regulate the proliferation and differentiation of many cell types and have insulin-like metabolic effects.

The IGF system is complex and consists of IGF-I and IGF-II binding to two types of receptors (type I and II), six IGF binding proteins, which are IGFBP-1, -2, -3, -4, , -5 and -6, and an enzyme of inactivation (IGFBPase) of the IGFBPs. The low molecular weight (IGFBP-2, -4, and -5) binding proteins show greater affinities to IGF, which prevent IGFs to bind to their receptors. The IGFBPases degrade IGFBPs providing greater bioavailability of IGF (free) to bind to the receptors (Fortune *et al*., 2004). The IGF-I stimulates cell proliferation and differentiation and acts synergistically with FSH on steroidogenesis by increasing the activity of P450 aromatase (Echternkamp *et al*., 1994).

Webb *et al*. (2004) indicated that these factors play an important role in the initial stages of follicle development acting on granulosa cells of preantral follicles such that mRNA of both IGFBP-2 and IGF type I receptor are expressed. Thus, any change in the components of the IGF system can potentially affect follicular development. Moreover, several authors (Ginther *et al*., 2001; Fortune *et al*., 2004) showed that there is a strong involvement of the IGF system in the selection of the dominant follicle and changes in the IGF system are critical to the establishment of follicular dominance.

Insulin

Insulin is secreted by pancreatic β cells and plays a central role in body metabolism. Besides its anabolic action, it also acts as a marker of energy status to the central nervous system. Besides acting as a potent stimulator of mitosis, physiological concentrations of insulin are probably necessary for normal follicle steroidogenesis in concert with the action of FSH (Bossis *et al*., 1999; Buttler *et al*., 2004). Insulin concentrations of approximately 100 ng/ml stimulate mRNA expression, P450 aromatase activity, and increase the secretion of estradiol (E2; Silva and Price, 2002).

Insulin acts as a signal to mediate the effects of acute changes in the diet on follicle dynamics in cattle. The reduction in fertility of dairy cows under NEB postpartum was associated with decreases in IGF-I and insulin. Insulin concentrations vary throughout the day as well as during the estrous cycle, with a significant increase during the preovulatory period. Estradiol is a strong candidate for mediating these changes, because the increase of insulin occurs in parallel to the increase of E2 associated with the development of the dominant follicle. Estradiol has been shown to stimulate the expression of both insulin mRNA and insulin secretion by the pancreas (Webb *et al*., 2004). We observed (Bastos *et al*., 2010) that pre-prandial insulin concentrations were higher in the estrogenic than the progestational phase of the estrous cycle in both Nelore (11.9 ± 2.05 *vs*. 8.3 ± 1.47 μIU/ml) and Holstein (4.2 ± 2.2 *vs*. 1.05 ± 0.64 μIU/ml) cows.

Glucose

Glucose appears to be a metabolic signal generating information for controlling GnRH secretion. According to its bioavailability, glucose acts within the central nervous system for detecting peripheral glucose status to modulate GnRH and, indirectly, LH secretion. Glucose bioavailability influences both tonic and preovulatory centers to regulate GnRH release and thus LH secretion. Animals with hypoglycemia had a delayed LH release and glucose infusion restored the normal time of LH release induced by E2 (Diskin *et al*., 2003).

Nutrition and metabolic hormones in *Bos taurus vs. Bos indicus*

With the purpose of comparing nutritional and metabolic aspects between *Bos taurus* and *Bos indicus*, a study was conducted with non-lactating Nelore (n = 7) *vs*. Holstein (n = 8) cows to evaluate the profile of feed consumption and circulating concentrations of glucose and insulin pre- and post-prandially (Ishiguro and Sartori, 2013; ESALQ/USP, Piracicaba; unpublished observations). The diet was calculated for maintenance by the National Research Council - NRC (2001). Holstein cows spent more time (135.0 ± 7.0 min) ingesting food than Nelore cows (104.4 ± 5.9 min). There were differences in circulating glucose and insulin which were higher ($P < 0.05$) in Nelore than Holstein cows, as shown in Fig. 1. The substantial difference in circulating insulin, but a discrete difference in glucose between Nelore and Holstein cows are very interesting observations and suggest distinct mechanisms for control of insulin increase after feeding as well as for regulation of glucose turnover between the two breeds.

Influence of nutrition / metabolic hormones on the number of antral follicles

The nutritional flushing prior to ovarian superstimulation may increase follicular population and superovulatory response in cows, which may be associated with increased insulin and IGF-I concentrations in response to higher propionate concentrations. Studies with beef heifers showed that overfeeding for a short period (up to 3 weeks) increased circulating insulin and the number of follicles (Gutierrez *et al*., 1997), improving superovulatory response (Gong *et al*., 2002). However, other studies found no effect or described a negative relationship between feed intake

and follicular population, including those developed in our laboratory when crossbred cows (Bastos *et al*., 2009) or *Bos indicus* heifers (Mollo *et al*., 2007a) were used. Due to the fact that BCS at the beginning of the nutritional flushing may influence embryo production in *Bos taurus* cattle (Adamiak *et al*., 2005), we designed a study to investigate whether differences in the BCS were associated with the nutritional flushing influence to the superovulatory response in Nelore heifers (Bastos *et al*., 2007). Thirty-six pubertal heifers with lower (2.7 ± 0.1, n = 18) or higher (3.7 ± 0.1, n = 18) BCS (scale 1-5) were divided into two groups which were subdivided according to the nutritional requirements in maintenance (M) or Flushing (1.8M). This design resulted in four subgroups: <BCS + Maintenance (<M); <BCS + Flushing (<F); >BCS + Maintenance (>M) and >BCS + Flushing (>F). The nutritional flushing was done during 14 days before the first FSH injection for superovulation, when heifers returned to the maintenance diet. Each heifer was superovulated twice and the interval between procedures was 35 days. The number of follicles ≥3 mm at the time of the first FSH injection did not differ among groups >M, >F, <M, <F, respectively (56.4 ± 6.0, 55.1 ± 4.5, 54.3 ± 6.9 and 48.2 ± 4.8; P > 0.10). There was also no difference in superovulatory response among groups (discussed below).

One of the studies showing negative relationship between nutritional intake and follicular population used 39 *Bos indicus* heifers that were fed with 40.8% of coast-cross hay, 51.9% corn silage and 7.3% mix (energy, urea, minerals, and vitamins) for 9 weeks (Mollo *et al*., 2007a). The heifers were divided into two groups according to the dietary levels based on the maintenance: 1.7 M and 0.7 M. At the end of the seventh week, heifers were subjected to superovulatory treatments. Despite the fact that the heifers in group 1.7 M had higher BCS, higher body weight and more blood insulin concentrations (14.3 ± 1.7 *vs*. 3.0 ± 0.8 µIU/ml), they had lower number of follicles ≥3 mm at the first FSH treatment (32.6 ± 2.5 *vs*. 42.6 ± 6.6; P = 0.10). Superovulatory data of this study will be ahead.

A stimulatory effect of bovine somatotropin (bST) on follicle population has been described in *Bos taurus* and *Bos indicus* cattle. Treatment with recombinant bST increased the number of follicles (6 to 15 mm) in lactating Holstein cows and size of second largest ovarian follicles in both lactating and non-lactating cows (De la Sota *et al*., 1993). Moreover, bST-treated lactating dairy cows before day 12 (first follicular wave, estrus = day 0), had more ovarian follicles between 3 and 9 mm than saline-treated cows (Lucy *et al*., 1993). Also, Buratini *et al*. (2000) observed a significant increase in plasma IGF-I concentration and a 36% increase in number of small follicles (<5 mm) when Nelore heifers (n = 8) were treated with bST on day 3 of the estrous cycle. However, there was no effect on the number of medium (5-9 mm) or large (>9 mm) follicles.

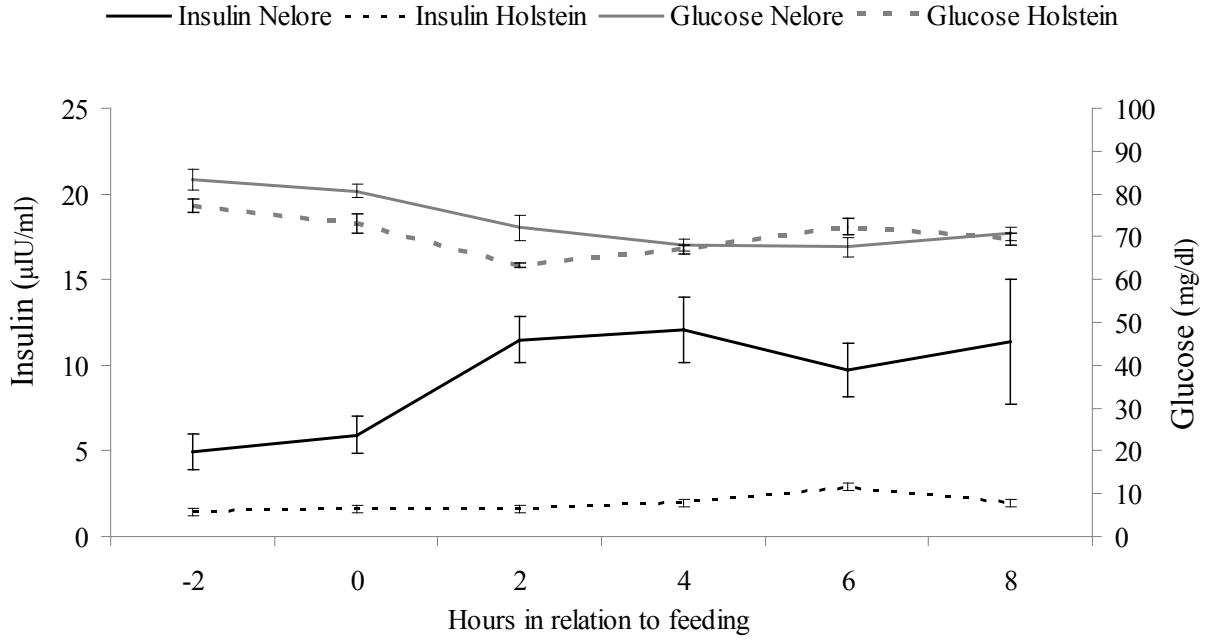

Figure 1. Circulating concentrations of glucose and insulin in Nelore (n = 7) vs. Holstein cows (n = 8) pre- and post-prandially. Breed; day; breed x day (P < 0.05) for glucose and insulin.

Influence of feed intake on ovarian structures and steroid hormones

High feed intake can alter ovarian physiology and hormonal concentrations. In lactating dairy cows there is a high positive correlation (r = 0.88) between DMI and milk production (Harrison *et al*., 1990). Even though high producing dairy cows have larger follicles, serum E2 concentrations were lower when compared to heifers and dry cows (De la Sota *et al*., 1993; Sartori *et al*., 2002; Wolfenson *et al*., 2004). The reasons for lower circulating steroid hormone concentrations in cows with higher DMI are probably related to an increased metabolism of these hormones as discussed by Wiltbank *et al*. (2006).

We compared the ovarian function of pubertal heifers (n = 27) and lactating dairy cows producing 45.7 ± 1.3 kg/d of milk (n = 14) during an estrous cycle (Sartori *et al*., 2004). Likewise, we followed the estrous cycle of pubertal heifers under high (1.7 M) or low (0.7 M) feed intake (Mollo *et al*., 2007b). In these and other studies, there was an association of high DMI with larger sizes of ovarian structures, and lower circulating steroid hormones concentrations. For example, in a recent study (Bastos and Sartori, 2013; ESALQ/USP, Piracicaba; unpublished data), non-lactating Nelore and Holstein cows, were exposed to high or low feed intake. Regardless of the genetic group, overfed cows had higher CL volume (5,146 ± 287 *vs*. 3,964 ± 306 mm^3; P < 0.01), and lower plasma P4 concentrations (2.3 ± 0.2 *vs*. 3.0 ± 0.2 ng/ml; P < 0.03) than those with low feed intake. Likewise, the cows exposed to high DMI had larger ovulatory follicles (15.1 ± 0.8 *vs*. 13.8 ± 0.8 mm; P < 0.01) and lower plasma E2 preovulatory peak (12.6 ± 1.0 *vs*. 16.0 ± 1.0 pg/ml; P < 0.01). Interestingly, regardless of genetic group, overfed cows had follicle deviation occurring later in the cycle (2.9 ± 0.2 *vs*. 2.5 ± 0.2 days after ovulation; P < 0.05) and the diameter when the largest follicle reached deviation was also greater (8.1 ± 0.2 *vs*. 7.4 ± 0.2 mm; P < 0.05).

Influence of dry matter or energy intake associated with high insulin on yield and quality of embryos

Most researchers who have studied the effect of feed intake on embryo production reported negative results on the reproductive function of cattle overfed compared with those fed restricted diets (see details in Sartori *et al*., 2012). The causes of impaired embryo production related to feed intake are still not well understood. However, changes in liver blood flow, in local and circulating metabolites (glucose and IGF-I), in hormone concentrations (insulin and steroids), and in different sources of volatile fatty acids may be involved in these processes. Moreover, we hypothesized that the effects of dietary intake on embryo quality may differ between *Bos taurus* and *Bos indicus* cattle, which consistently show different concentrations of circulating insulin and IGF-I concentrations (Sartori *et al*., 2010). As shown below, it is tempting to speculate that Zebu cattle may be more resistant than European breeds to the effects of changes in feed intake on embryo production and quality.

Changes in DMI as discussed above, may affect the blood concentrations of steroid hormones, IGF-I and insulin, and affect oocyte quality, fertilization or embryo/oocyte transport and early embryonic development (Folman *et al*., 1973, Fonseca *et al*., 1983; Mann *et al*., 1998; Inskeep, 2004), resulting in reduced fertility. In addition to the effects of IGF-I and insulin in steroidogenesis (Gutierrez *et al*., 1997; Armstrong *et al*., 2002; Gong *et al*., 2002) and the sensitivity of the follicle to gonadotropic hormones (Webb *et al*., 2004), as already described, hyperinsulinemia and increased plasma and intrafollicular IGF-I concentrations impair oocyte quality and subsequent embryo development of *Bos taurus* cattle (Armstrong *et al*., 2001; Adamiak *et al*., 2005). In our studies, we investigated how the DM or energy affects *in vivo* and *in vitro* embryo production in Nelore cattle.

In the study by Mollo *et al*. (2007a), after feeding treatment diets for 9 weeks, overfed heifers (1.7 M, n = 20) presented lower superstimulatory (24.0 ± 1.1 *vs*. 48.4 ± 1.6 follicles ≥6 mm; P < 0.001) and superovulatory (15.7 ± 0.9 *vs*. 33.6 ± 1.4 corpora lutea; P < 0.0001) responses in comparison to those in restricted diet (0.7 M, n = 19). Moreover, the number of recovered embryos/ova (6.7 ± 0.9 *vs*. 10.5 ± 0.6; P < 0.0003) and transferable embryos (3.8 ± 0.4 *vs*. 5.7 ± 0.6; P < 0.01) were also lower for the high feed intake heifers. The superstimulatory and superovulatory responses and the number of total and transferable embryos seemed to be compromised by higher circulating insulin concentrations at the first day of FSH treatment (14.3 ± 1.7 *vs*. 3.0 ± 0.8 μIU/ml; P < 0.001). Regardless of treatment, there was a negative correlation (-0.61; P < 0.05) between circulating insulin concentration and the difference in the number of follicles in the ovaries between the last and first day of FSH treatment.

Bastos *et al*. (2007) did not detect any effect on the superovulatory response, embryo production, or embryo quality in heifers (n = 36) with higher or lower BCS fed maintenance (M) or flushing (1.8 M) diets for 14 days before a superovulation treatment. In the study of Surjus *et al*. (2012), little variation of the superstimulatory response (14.6 ± 1.6^{a} *vs*. 12.6 ± 1.4^{b} *vs*. 13.6 ± 1.5^{ab} number of follicles >6 mm; P < 0.05) was reported in non-lactating cows (n = 32) fed maintenance (M, 1.2% of DM/kg of BW), 0.7 M (0.84% of DM/kg of BW) or 1.5 M (1.8% of DM/kg of BW) diets after 42 days of feeding, in a latin-square design. There was no difference for the superovulatory response (11.0 ± 1.4 *vs*. 9.8 ± 1.3 *vs*. 10.2 ± 1.3 corpora lutea; P > 0.10), fertilization rate (P = 0.71) or percentage of viable embryos (P = 0.98) among

experimental groups (Surjus *et al*., 2012). Regardless of treatment, circulating insulin at the beginning of superovulation, was negatively correlated with superovulatory response (r = -0.32) and number of viable embryos (r = -0.22). Pregnancy rates at 23 and 53 days after embryo transfer did not differ between treatments; however, the circulating concentrations of insulin in donors had a low, but significant negative correlation with pregnancy of recipients at 60 days of gestation (r = -0.16; P < 0.05).

Guardieiro *et al*. (2013) supplied concentrate with or without rumen-protected fat (Megalac-E, rich in linoleic acid) to 40 heifers starting 50 days before superovulation, in a cross-over experimental design. Supplemental diets were isocaloric and isonitrogenous. The embryos recovered were cryopreserved and subsequently evaluated for *in vitro* embryo development. The superstimulatory response, number of total embryos/ova, viable embryos, degenerate embryos, or unfertilized oocytes recovered were similar between groups. However, there was negative effect of unsaturated fatty acids on the superovulatory response (15.7 ± 1.2 *vs*. 18.0 ± 1.3 corpora lutea; P = 0.06), hatching rate at 48 hours (17.3 ± 3.3%; n = 137 *vs*. 33.1 ± 4.0%, n = 148, P < 0.009) and at 72 h (30.9 ± 4.0%, n = 137 *vs*. 44.3 ± 4.2%; n = 148; P < 0.04) of *in vitro* culture. This negative effect associated with a rumen bypass fat diet may have been influenced by lower plasma concentrations of IGF-I observed in this experimental group compared to control (374.3 ± 27.2 *vs*. 483.8 ± 26.5 ng/ml, respectively; P < 0.0001).

Our group also performed two studies to investigate the effects of feed intake on *in vitro* embryo production (Martins *et al*., 2006; Prata *et al*., 2011). At the first study, overfed cows (1.7 M, n = 10) in comparison to those underfed (0.7 M, n = 10) had a small but significant increase in number of follicles ≥3 mm in diameter at the time of ovum pick-up (OPU) and associated with this effect was a tendency for a greater circulating concentration of insulin in the 1.7 M group (5.6 ± 0.8 *vs*. 3.5 ± 0.7 µIU/ml; P = 0.06, unpublished). Moreover, the diet with higher energy content tended to reduce the percentage of viable oocytes (44.0%, n = 732 *vs*. 48.6%, n = 623; P = 0.08). In despite of similar numbers of cleaved oocytes and blastocysts between groups, there was a higher expression of the BAX gene and global expression of all evaluated genes on embryos from the lower feed intake group, which may be related to better embryo quality (Martins *et al*., 2006). A study by Prata *et al*. (2011) used the same design as described by Surjus *et al*. (2012), however cows underwent OPU 30 days after the dietary treatments had started. More recovered oocytes (20.2 ± 2.0^{b}, 23.0 ± 2.3^{a}, and 21.5 ± 2.2^{ab}; P < 0.02) and viable oocytes (14.4 ± 1.6^{b}, 17.0 ± 1.9^{a}, and 15.7 ± 1.7^{ab}; P < 0.006) were detected in the 0.7 M diet in relation to the M diet. Surprisingly, cows receiving the 1.5 M diet did not differ from the other groups. Although the number of cleaved oocytes was also higher in 0.7 M cows as compared to M cows (10.7 ± 1.4^{b}, 13.4 ± 1.7^{a}, and 12.6 ± 1.6^{ab} for M, 0.7 M, and 1.5 M; P < 0.04), we did not detect influence of diet on the number (5.4 ± 0.8, 6.9 ± 0.9, and 5.9 ± 0.8; P = 0.15) or percentage of blastocysts produced *in vitro* (31.9, 30.6, and 31.1%; P = 0.67). Moreover, regardless of treatment, cows with lower plasma insulin concentration (3.1 ± 0.75 µIU/ml) showed similar results (P > 0.10) as compared to cows with higher circulating insulin (9.72 ± 0.21 µIU/ml) in all of the variables analyzed, such as blastocyst rate (24.6 *vs*. 25.9%) and conception rate [31.2% (69/221) *vs*. 33.3% (71/213)] at 30 days.

Conclusions

Metabolic hormones are key elements to the reproductive performance, once they can affect different aspects of the physiology of the cow. The IGF system, as well as insulin, associated with reproductive hormones, interacts with ovarian activity, from the initial stages of follicular growth, to the process of selection of the dominant follicle, as well as final development and ovulation. When considering the effects of high DMI / energy on bovine fertility, the negative effects are highlighted, because they are associated with a high metabolism of steroid hormones and with an increase in circulating insulin and IGF-I concentrations, potentially compromising oocyte / embryo quality and lowering conception rates. Interestingly, although controversial, negative effects of hyperinsulinemia seem to be more pronounced in embryos produced *in vivo* than *in vitro*. Thus, we may speculate that in small follicles (<7 mm), this impairment on oocyte quality caused by higher circulating insulin and IGF-I concentrations is less intense, especially in *Bos indicus* cattle. In contrast, low circulating IGF-I may compromise follicle number and development as well as embryo production and cryotolerance.

Acknowledgments

The authors would like to acknowledge the support from EMBRAPA, FAPESP, CNPq and CAPES of Brazil.

References

Adamiak SJ, Mackie K, Watt RG, Webb R, Sinclair KD. 2005. Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. *Biol Reprod*, 73:918-926.

Armstrong DG, McEvoy TG, Baxter G, Robinson JJ, Hogg CO, Woad KJ, Webb R, Sinclair KD. 2001. Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: associations with the ovarian insulin-like growth factor system. *Biol Reprod*, 64:1624-1632.

Armstrong DG, Baxter G, Hogg CO, Woad KJ.

2002. Insulin-like growth factor (IGF) system in the oocyte and somatic cells of bovine pre-antral follicles. *Reproduction*, 123:789-797.

Bastos MR, Martins AC, Melo L, Carrijo LHD, Rumpf R, Sartori R. 2007. Effect of body condition score and feed intake on the superovulatory response and embryo production in Nelore heifers. *Acta Sci Vet*, 35(suppl. 3):1242. (abstract).

Bastos MR, Ramos AF, Driessen K, Martins AC, Rumpf R, Sartori R. 2009. Influência da alta ingestão de matéria seca na resposta superovulatória em vacas mestiças. *Ciênc Anim Bras*, 10:1066-1073.

Bastos MR, Mattos MCC, Meschiatti MAP, Surjus RS, Guardieiro MM, Ferreira JCP, Mourão GB, Pires AV, Biehl MV, Pedroso AM, Santos FAP, Sartori R. 2010. Ovarian function and circulating hormones in nonlactating Nelore versus Holstein cows. *Acta Sci Vet*, 38(suppl. 2):776. (abstract).

Beam SW, Butler WR. 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. *Biol Reprod*, 56:133-142.

Bossis I, Wettemann RP, Welty SD, Vizcarra JA, Spicer LJ, Diskin MG. 1999. Nutritionally induced anovulation in beef heifers: ovarian and endocrine function preceding cessation of ovulation. *J Anim Sci*, 77:1536-1546.

Brockman RP, Laarveld B. 1986. Hormonal regulation of metabolism in ruminants: a review. *Livest Prod Sci*, 14:313-334.

Buratini Jr J, Price CA, Visintin JA, Bó GA. 2000. Effects of dominant follicle aspiration and treatment with recombinant bovine somatotropin (BST) on ovarian follicular development in Nelore (*Bos indicus*) heifers. *Theriogenology*, 54:421-432.

Butler WR. 2000. Nutritional interactions with reproductive performance in dairy cattle. *Anim Reprod Sci*, 60/61:449-457.

Butler WR, Marr AL, Pelton SH, Radcliff RP, Lucy MC. 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. *J Endocrinol*, 176:205-217.

Butler ST, Pelton SH, Butler WR. 2004. Insulin increases 17β-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. *Reproduction*, 127:537-545.

Chase CC, Kirby CJ, Hammond AC, Olson AC, Lucy MC. 1998. Patterns of ovarian growth and development in cattle with a growth hormone receptor deficiency. *J Anim Sci*, 76:212-219.

Chilliard Y, Delavaud C, Bonnet M. 2005. Leptin expression in ruminants: nutritional and physiological regulations in relation with energy metabolism. *Domest Anim Endocrinol*, 29:3-22.

De la Sota RL, Lucy MC, Staples CR, Thatcher WW. 1993. Effects of recombinant bovine somatotropin (Sometribove) on ovarian function in lactating and nonlactating dairy cows. *J Dairy Sci*, 76:1002-1013.

Diskin MG, Mackey DR, Roche JF, Sreenan JM. 2003. Effects of nutrition and metabolic status on circulation hormones and ovarian follicle development in cattle. *Anim Reprod Sci*, 78:345-370.

Echternkamp SE, Howard HJ, Roberts AJ. 1994. Relationships among concentrations of steroids, insulin-like growth factor-I and insulin-like binding proteins in ovarian follicular fluid of beef cattle. *Biol Reprod*, 51:971-981.

Ferreira RM, Ayres H, Chiaratti MR, Ferraz ML, Araújo AB, Rodrigues CA, Watanabe YF, Vireque AA, Joaquim DC, Smith LC, Meirelles FV, Baruselli PS. 2011. The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *J Dairy Sci*, 94:2383-2392.

Folman Y, Rosenber M, Herz Z, Davidson M. 1973. Relationship between plasma progesterone concentration and conception in postpartum dairy-cows maintained on two levels of nutrition. *J Reprod Fertil*, 34:267-278.

Fonseca FA, Britt JH, McDaniel BT, Wilk JC, Rakes AH. 1983. Reproductive traits of Holsteins and Jerseys - effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection on estrus, conception rate, and days open. *J Dairy Sci*, 66:1128-1147.

Fortune JE, Rivera GM, Yang MY. 2004. Follicular development: the role of the follicular microenvironment in selection of the dominant follicle. *Anim Reprod Sci*, 82/83:109-126.

Ginther OJ, Beg MA, Bergfelt DR, Donadeu FX, Kot K. 2001. Follicle seletion in monovular species. *Biol Reprod*, 65:638-647.

Gong JG, Bramley T, Webb R. 1991. The effect of recombinant bovine somatotropin on ovarian function in heifers: follicular populations and peripheral hormones. *Biol Reprod*, 45:941-949.

Gong JG, Armstrong DG, Baxter G, Hogg CO, Garnsworthy PC, Webb R. 2002. The effect of dietary intake on superovulatory response to FSH in heifers. *Theriogenology*, 57:1591-1602.

Guardieiro MM, Machado GM, Bastos MR, Mourão GB, Carrijo LHD, Dode MAN, Leroy JLMR, Sartori R. 2013. A diet enriched in linoleic acid compromises the cryotolerance of embryos from superovulated beef heifers. *Reprod Fertil Dev*. doi.org/10.1071/RD12403.

Gutierrez CG, Oldham J, Bramley TA, Gong JG, Campbell BK, Webb R. 1997. The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. *J Anim Sci*, 75:1876-1884.

Harrison RO, Ford SP, Young JW, Conley AJ, Freeman AE. 1990. Increased milk production versus reproductive and energy status of high producing dairy cows. *J Dairy Sci*, 73:2749-2758.

Inskeep EK. 2004. Preovulatory, postovulatory, and post-maternal recognition effects of concentrations of

progesterone on embryonic survival in the cow. *J Anim Sci*, 82:24-39.

Jungheim ES, Moley KH. 2008. The impact of type 1 and type 2 diabetes mellitus on the oocyte and the preimplantation embryo. *Semin Reprod Med*, 26:186-195.

Landau S, Braw-tal R, Kaim M, Bor A, Bruckental I. 2000. Preovulatory follicular status and diet affect the insulin and glucose content of follicles in high yielding dairy cows. *Anim Reprod Sci*, 64:181-197.

Lawrence MC, Mckern NM, Ward CW. 2007. Insulin receptor structure and its implications for the IGF-1 receptor. *Curr Op Struct Biol*, 17:699-705.

Leroy JL, Opsomer G, Van Soom A, Goovaerts IG, Bols PE. 2008. Reduced fertility in high-yielding dairy cows: are the oocyte and embryo in danger? Part I. The importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high-yielding dairy cows. *Reprod Domest Anim*, 43:612-622.

Lucy MC, De La Sota RL, Staples CR, Thatcher WW. 1993. Ovarian follicular populations in lactating dairy cows treated with recombinant bovine somatotropin (Sometribove) or saline and fed diets differing in fat content and energy. *J Dairy Sci*, 76:1014-1027.

Mann GE, Lamming GE, Robinson RS, Wathes DC. 1998. The regulation of interferon-t production and uterine hormone receptors during early pregnancy. *J Reprod Fertil*, 54:317-328.

Martins AC, Ramos AF, Mollo MR, Pivato I, Camara JU, Carrijo LHD, Driessen K, Rumpf R, Sartori R. 2006. Influência da alta ou baixa ingestão alimentar na produção in vitro de embriões bovinos. *Acta Sci Vet*, 34(suppl. 1):290. (abstract).

Mollo MR, Rumpf R, Martins AC, Carrijo LHD, Saueressig MG, Sartori R. 2007a. Produção de embriões em novilhas Nelore superovuladas submetidas à baixa ou alta ingestão alimentar. *Acta Sci Vet*, 35(suppl. 3):945. (abstract).

Mollo MR, Rumpf R, Martins AC, Mattos MCC, Lopes Jr C, Carrijo LHD, Sartori R. 2007b. Função ovariana em novilhas Nelore submetidas a alta ou baixa ingestão alimentar. *Acta Sci Vet*, 35(suppl. 3):958. (abstract).

National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th ed. Washington, DC: National Academy of Sciences Press. 381 pp.

Pinto AB, Schlein AL, Moley KH. 2002. Preimplantation exposure to high insulin-like growth factor I concentrations results in increased resorption rates in vivo. *Hum Reprod*, 17:457-462.

Prata AB, Surjus RS, Borsato M, Mattos MCC, Basso A, Pontes JH, Mourão GB, Sartori R. 2011. Influência da alta ou baixa ingestão de matéria seca e/ou energia na produção in vitro de embriões bovinos. *Acta Sci Vet*, 39(suppl. 1):338. (abstract).

Saltiel AR, Kahn CR. 2001. Insulin signaling and the regulation of glucose and lipid metabolism. *Nature*, 13:799-806.

Sales JN. 2011. *Efeito da dieta com alta energia nos parâmetros metabólicos, endócrinos e reprodutivos de vacas Bos indicus e Bos taurus* [in Portuguese]. São Paulo, SP: Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo. Thesis.

Sartori R, Rosa GJM, Wiltbank MC. 2002. Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. *J Dairy Sci*, 85:2813-2822.

Sartori R, Haughian JM, Shaver RD, Rosa GJ, Wiltbank MC. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J Dairy Sci*, 87:905-920.

Sartori R, Bastos MR, Baruselli PS, Gimenes LU, Ereno RL, Barros CM. 2010. Physiological differences and implications to reproductive management of *Bos taurus* and *Bos indicus* cattle in a tropical environment. *In*: Lucy MC, Pate JL, Smith MF, Spencer TE (Org.). *Reproduction in Domestic Ruminants VII*. Nottingham, UK: Nottingham University Press. v.1, pp. 357-375.

Sartori R, Guardieiro MM, Mollo MR, Surjus RS. 2012. Feed intake and reproduction in cattle. *In*: Garnsworthy PC, Wiseman J (Org.). *Recent Advances in Animal Nutrition*. Nottingham, UK: Nottingham University Press. v.1, pp. 21-36.

Sasaki S. 2002. Mechanism of insulin action on glucose metabolism in ruminants. *Anim Sci J*, 73:423-433.

Silva JM, Price CA. 2002. Insulin and IGF-I are necessary for FSH-induced cytochrome P450 aromatase but not cytochrome P450 side-chain cleavage gene expression in oestrogenic bovine granulosa cells in vitro. *J Endocrinol*, 174:499-508.

Sinclair KD. 2010. Declining fertility, insulin resistance and fatty acid metabolism in dairy cows: developmental consequences for the oocyte and pre-implantation embryo. *Acta Sci Vet,* 38(suppl. 2):545-557.

Smith TR, Hippen AR, Beitz DC, Young JW. 1997. Metabolic characteristics of induced ketosis in normal and obese dairy cows. *J Anim Sci*, 80:1569-1581.

Surjus RS, Prata AB, Borsato M, Martins da Silveira MC, Mattos MCC, Mattos FCSZ, Monteiro Jr PLG, Mourao GB, Santos FAP, Sartori R. 2012. Influence of high or low intake of dry matter and energy on in vivo production of bovine embryos. *Reprod Fertil Dev*, 24:227.

Webb R, Garnsworthy PC, Gong JG, Armstrong DG. 2004. Control of follicular interactions and nutritional influences. *J Anim Sci*, 82:63-74.

Wiltbank M, Lopez H, Sartori R, Sangsritavong S, Gümen A. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology*, 65:17-29.

Wolfenson D, Inbara G, Rotha Z, Kaimb M, Blocha A, Braw-Tal R. 2004. Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nulliparous heifers. *Theriogenology*, 62:1042-1055.