Animal, page 1 of 6 © The Animal Consortium 2011
doi:10.1017/S1751731111002321

Effect of *prepartum* somatotropin injection in late-pregnant Holstein heifers on metabolism, milk production and *postpartum* resumption of ovulation

A. Schneider[1,2†], E. Schwegler[1,2], P. Montagner[1,2], L. T. Hax[1,2], E. Schmitt[1,2], L. F. M. Pfeifer[2,3], F. A. B. Del Pino[2,4], I. Bianchi[2,5], G. R. Paludo[6] and M. N. Corrêa[1,2]

[1]*Department of Veterinary Clinics, Veterinary College, Federal University of Pelotas, Campus Universitário, CEP 96010-900 Pelotas, RS, Brazil;* [2]*Núcleo de Pesquisa, Ensino e Extensão em Pecuária (NUPEEC), Veterinary College, Federal University of Pelotas, Campus Universitário, CEP 96010-900 Pelotas, RS, Brazil;* [3]*Animal Reproduction Laboratory, EMBRAPA CPAFRO, BR 364 – Km 5, 5, CEP 76815-800 Porto Velho, RO, Brazil;* [4]*Department of Biochemistry, Federal University of Pelotas, Campus Universitário, CEP 96010-900, Pelotas, RS, Brazil;* [5]*Department of Pathology, Veterinary College, Federal University of Pelotas, Campus Universitário, CEP 96010-900, Pelotas, RS, Brazil;* [6]*Department of Pathology, Veterinary College, University of Brasília, Campus Universitário Darcy Ribeiro, Asa Norte, CEP 70910-970, Brasília, DF, Brazil*

(Received 28 April 2011; Accepted 28 October 2011)

The aim of this study was to determine the effect of prepartum *somatotropin injection in late-pregnant Holstein heifers on metabolism, milk production and resumption of* postpartum *ovulation. For this study, 31 late-pregnant Holstein heifers were used. The heifers were assigned randomly into two treatments: (1) 500 mg sc injections of somatotropin (somatotropin treatment,* n *= 15) at −35 and −21 days, and, if pertinent, at −7 days from expected calving date and (2) no treatment (control group,* n *= 16). Blood samples were collected weekly from −5 to 7 weeks after calving. Heifers with progesterone concentrations in plasma above 1 ng/ml in two consecutive* postpartum *samples were considered as having resumed ovarian activity. A higher proportion (*P *= 0.04) of heifers treated with somatotropin resumed ovarian activity in the first 7 weeks* post partum *(73.3%; 11/15) compared with the control group (37.5%; 6/16). A higher number (*P *= 0.02) of heifers in the somatotropin treatment group also ovulated during the first* postpartum *follicular wave (53.3%; 8/15) compared with the control group (12.5%; 2/16), as indicated by the number of heifers ovulating in the first 3 weeks* post partum*. Pregnancy rate was not affected by treatments (*P *> 0.10) and averaged 40.0% (6/15) in somatotropin-treated and 25.0% (4/16) in control heifers when evaluated up to 150 days in milk. Somatotropin treatment increased the average daily milk production by 2.8 kg/cow per day (*P *< 0.0001) and reduced the somatic cell count (*P *= 0.009). Plasma IGF-I was higher (*P *< 0.05) for somatotropin-treated heifers in the* prepartum *period. Insulin and body condition score were higher (*P *< 0.05) and non-esterified fatty acids were lower (*P *< 0.05) for somatotropin-treated cows in the early* postpartum *period. In conclusion, somatotropin injection during the* prepartum *period in late-pregnant Holstein heifers was able to increase the proportion of heifers resuming ovarian activity early* post partum*, inspite of higher milk production.*

Keywords: bST, dairy heifers, IGF-I, ovarian activity, primiparous

Implications

Physiological changes during the transition period can have a great impact on health and performance. In our study, *prepartum* injection of somatotropin reduced negative energy balance (NEB) intensity as indicated by several metabolic markers evaluated in the *postpartum* period. A more intense NEB is usually associated with a delay in resumption of normal ovarian function. In this way, we observed a higher proportion of somatotropin-treated heifers ovulating earlier. Moreover, cows treated with somatotropin *prepartum* increased milk production. Along with earlier resumption of estrous cycles, this strategy can have an important impact on farm profitability.

Introduction

During the early *postpartum* period, high-producing cows experience a period of negative energy balance (NEB) that occurs as the requirements for milk production exceed the

† E-mail: augusto.schneider@live.com

energy obtained through feeding (Butler, 2003). In early *postpartum* dairy cows, the duration and intensity of NEB, the level of body condition score (BCS) loss and the milk yield are strongly associated with the timing of the first ovulation (Butler, 2003; Wathes *et al*., 2007). Moreover, cows that ovulated earlier and had more estrous cycles before the first artificial insemination (AI) had a higher conception rate (Thatcher and Wilcox, 1973). As a consequence, strategies that minimize NEB and BCS loss in the early *postpartum* period could have a positive impact on the timing of the first ovulation and conception rate.

Supplementing cows with low doses of somatotropin during the periparturient period has been associated with beneficial effects on the physiological adaptations and liver function (Gulay *et al*., 2003 and 2004). Somatotropin has direct effects on the partitioning of nutrients to target tissues (Bauman and Vernon, 1993), as well as indirect effects expressed in the mammary gland and other tissues that are mediated by IGF-I (Jones and Clemmons, 1995). In this regard, somatotropin injection during the *peripartum* period is able to increase serum IGF-I concentration and milk production (Putnam *et al*., 1999; Gulay *et al*., 2004; Carriquiry *et al*., 2009b). In addition, injection of somatotropin exclusively in the *prepartum* period can also lead to increased dry matter intake (DMI) in the early *postpartum* period (Putnam *et al*., 1999). These studies indicate that *prepartum* injection of somatotropin is a potential tool to improve metabolic adaptation of transition dairy cows, and thus can have a positive impact on early *postpartum* ovulation.

Somatotropin plays a pivotal role in stimulating liver production of IGF-I (Jones and Clemmons, 1995). Circulating IGF-I is highly related to the extent of NEB (Lucy *et al*., 2001; Wathes *et al*., 2007), and concentrations start to decline in the third trimester of lactation, reaching a nadir at calving (Knight, 2001). Elevated plasma concentration of IGF-I is beneficial because it acts as a modulator of gonadotropin action in the ovary, stimulating granulosa and theca cell proliferation and differentiation (Armstrong and Webb, 1997) and preventing follicular atresia (el-Roeiy *et al*., 1994). Dairy cows that ovulated the first *postpartum* dominant follicle had higher serum IGF-I concentration in the *prepartum* and *postpartum* periods (Butler *et al*., 2006; Kawashima *et al*., 2007). However, somatotropin injection starting at 10 days *post partum* had no effect on the interval from calving to first ovulation (Carriquiry *et al*., 2009a). Nevertheless, the number of recruited follicles is increased in dry cows or heifers injected with somatotropin (Gong *et al*., 1991 and 1993; Kirby *et al*., 1997). This effect persisted for at least 21 days after termination of treatment (Kirby *et al*., 1997). These lines of evidence indicate that *prepartum* somatotropin injection can have beneficial effects on follicular development in the early *postpartum* period.

On the basis of previous research, the hypothesis of this study is that the better adaptation provided by *prepartum* somatotropin injection in late-pregnant Holstein heifers would lead to earlier *postpartum* resumption of ovarian activity and increased milk production.

Material and methods

All procedures carried out in this experiment were approved by the Committee for Ethics in Animal Experimentation from the Universidade Federal de Pelotas (Pelotas, RS, Brazil).

Experimental design

For this study, 31 late-pregnant Holstein heifers in a commercial dairy herd in southern Brazil (32° 16′ S, 52° 32′ E) were used. The heifers had a mean BW of 568.8 ± 47.8 kg and BCS of 3.4 ± 0.4 (ranging from 2.5 to 4.0) at the beginning of the experiment. All calvings occurred in a 30-day interval during the winter season.

At −35 days from expected calving, the heifers were assigned randomly into two groups: (1) 500 mg somatotropin (500 mg/2 ml; Boostin®, LG Life Sciences, Seoul, Korea) by sc injections (somatotropin-treated group, $n = 15$) at −35 and −21 days, and, if pertinent, at −7 days (eight heifers that did not anticipate calving) from expected calving date; and (2) no treatment (control group, $n = 16$). All heifers were managed under the same conditions and nutritional regimen (Table 1).

Sample collection, measurement and analyses

Blood samples were collected weekly from −5 to 7 weeks after calving via venipuncture of the jugular vein into two tubes: one containing EDTA and potassium fluoride and the other without an anticoagulant. Samples were collected in the interval after milking and before feeding. In the *postpartum* period, heifers were milked twice on a daily basis at 0330 and 1530 h and production was recorded daily (Alpro®, DeLaval, Kansas City, MO, USA) and combined for weekly averages. BW was estimated weekly using a chest tape and BCS was evaluated weekly by one technician.

Concentrations of glucose and non-esterified fatty acids (NEFA) were measured colorimetrically using commercial kits, Glucose PAP Liquiform (Labtest Diagnostica, Lagoa Santa, SP, Brazil; Tabeleão *et al*., 2008) and NEFA-HR (Wako USA, Richmond, VA, USA) using the micro-method described by Ballou *et al*. (2009). The intra- and inter-assay coefficients of variation (CV) for glucose and NEFA were <10%.

The procedures for IGF-I, insulin and progesterone analysis followed those specified by the manufacturer. Plasma IGF-I and insulin concentrations were measured using sandwich-type immunoassay commercial kits, DSL-10-2800 ACTIVE Non-extraction IGF-I ELISA (Obese *et al*., 2008) and DSL-10-1600 ACTIVE Insulin (Diagnostics Systems Laboratories Inc., Webster, TX, USA; Lee *et al*., 2002), respectively. The intra-assay CV was 4.2% and 4.0% and the inter-assay CV 8.5% and 13.0% for IGF-I and insulin, respectively.

Progesterone concentrations in plasma were measured using a solid-phase ELISA that is based on the principle of competitive binding using a commercial kit, Progesterone IB79105 (Immuno-Biological Laboratories Inc., Minneapolis, MN, USA; Colazo *et al*., 2008). The intra-assay CV was 9.9% and the inter-assay CV was 5.5%. Only the plasma samples collected from weeks 2 to 7 were analyzed. Heifers with progesterone concentrations in plasma above 1 ng/ml in

Table 1 *Ingredient and nutrient composition of* prepartum *and* postpartum *diets*

	Prepartum		*Postpartum*
Ingredients	Up to −21 days to expected calving	−21 to 0 days to expected calving	0 to 60 days in milk
Native pasture	*Ad libitum*	*Ad libitum*	–
Ryegrass pasture	–	–	*Ad libitum*
White clover pasture	–	–	*Ad libitum*
Sorghum grain (kg)	–	1.20	4.60
Soybean meal (kg)	–	1.00	2.30
Soybean hulls (kg)	–	1.50	0.80
Rice bran (kg)	–	–	1.70
Urea (kg)	–	0.05	0.10
Premix (kg)	–	0.25	0.50
Nutrient composition (dry matter basis)			
CP* (%)	12.0	16.3	19.0
Fat* (%)	1.8	3.0	4.1
NDF* (%)	74.2	50.9	26.5
ADF* (%)	37.5	30.8	15.1

*Estimated based on diet analyses in National Research Council (NRC) software (2001).

two consecutive samples were considered as having resumed ovarian activity (Stevenson and Britt, 1979).

Composite milk samples were collected weekly at the afternoon milking. Milk protein, fat and lactose assays were carried out using IR spectrophotometry (Bentley 20000, Bentley Instruments Inc., Chaska, MN, USA). The somatic cell count (SCC) assay was carried out using flow cytometry (Somacount 300, Bentley Instruments Inc.).

Reproductive management

After 50 days in milk (DIM), estrus was detected by visual observation and cows presenting visual signs of estrous behavior (standing when mounted) were artificially inseminated (AI). Visual observation of estrous behavior and AI were carried out by the same technician. Pregnancy diagnosis was carried out using ultrasound 30 days after AI and rechecked 60 days later.

Statistical analyses

The results are presented as means ± standard error of the mean (s.e.m.). All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, NC, USA). Analyses involving repeated measures over time (e.g. BCS, BW, glucose, NEFA, insulin, IGF-I, milk production and composition) were compared between treatments using ANOVA for repeated measures using the MIXED procedure to evaluate the main effects of time, treatment and their interactions (Littell *et al*., 1998). SCC values were log transformed before analysis. The statistical models and data analyses were designed and carried out separately for the *prepartum* and *postpartum* periods. When the interaction between treatment and time was significant ($P < 0.05$), pair-wise comparison of individual means was conducted. Percentage of cows ovulating before 3 and 7 weeks was analyzed using χ^2-test. Pregnancy rate was evaluated using the CAT MOD procedure for repeated measures at 60, 100, 120 and 150 DIM. A value of $P < 0.05$ was considered significant and a value of between 0.05 and ≤0.1 was considered as a tendency.

Results

A higher proportion ($P = 0.04$) of heifers treated with somatotropin resumed ovarian activity in the first 7 weeks *post partum* (73.3%; 11/15) compared with the control group (37.5%; 6/16). A higher number of heifers in the somatotropin treatment group also ovulated ($P = 0.02$) the first *postpartum* dominant follicle (53.3%; 8/15) compared with the control group (12.5%; 2/16), as indicated by the number of heifers ovulating in the first 3 weeks *post partum*. Concentrations of progesterone for ovulatory and non-ovulatory heifers are represented in Figure 1. For non-ovulatory heifers, progesterone concentrations were never higher than 1 ng/ml. Pregnancy rate was not different ($P = 0.30$) between somatotropin-treated (40.0%; 6/15) heifers and control heifers (25.0%; 4/16) when evaluated up to 150 DIM.

Data for milk production and composition are summarized in Table 2. Somatotropin treatment increased the average daily milk production ($P < 0.0001$) and reduced the SCC ($P = 0.009$).

Data for plasma variables are summarized in Figure 2. Briefly, we did not detect an effect of week of study, treatment or treatment × week interaction on *prepartum* plasma glucose concentrations. For *postpartum* glucose, there was only an effect of time ($P = 0.0004$) with plasma glucose being higher ($P < 0.02$) at week 1. For plasma IGF-I concentration, there was a week ($P = 0.002$), treatment ($P = 0.03$) and week × treatment ($P = 0.03$) effect. Plasma IGF-I was higher for somatotropin-treated heifers at weeks −4 and −3. There was only an effect of week ($P = 0.002$) for *postpartum* IGF-I concentrations, being higher ($P < 0.05$)

at weeks 5, 6 and 7 than at weeks 1 and 3. For *prepartum* insulin, there was an effect of week ($P < 0.0001$) and treatment ($P = 0.002$). Insulin concentrations were higher ($P < 0.05$) at weeks −5 and −4 and were higher ($P < 0.05$) for somatotropin-treated heifers. *Postpartum* insulin concentrations were higher ($P = 0.01$) for somatotropin-treated heifers and were higher ($P < 0.05$) at weeks 6 and 7.

We detected an effect of the week for *prepartum* ($P = 0.0006$) and *postpartum* ($P = 0.0006$) BCS. For the *prepartum* period, BCS was higher ($P < 0.05$) at week −5 and there was no effect of treatment ($P = 0.93$). For the *postpartum* period, BCS was lower ($P < 0.05$) at weeks 6 and 7. *Postpartum* BCS was higher for somatotropin-treated heifers (2.7 ± 0.02) than in control heifers (2.6 ± 0.02; $P = 0.02$). Effects for the treatment, week or treatment × week interaction for *prepartum* (560.3 ± 9.2 kg) or *postpartum* (530.3 ± 5.7 kg) BW were not significant.

Discussion

As hypothesized, *prepartum* somatotropin treatment reduced intensity of NEB as observed by increased insulin and reduced NEFA concentrations and BCS loss in the *postpartum* period.

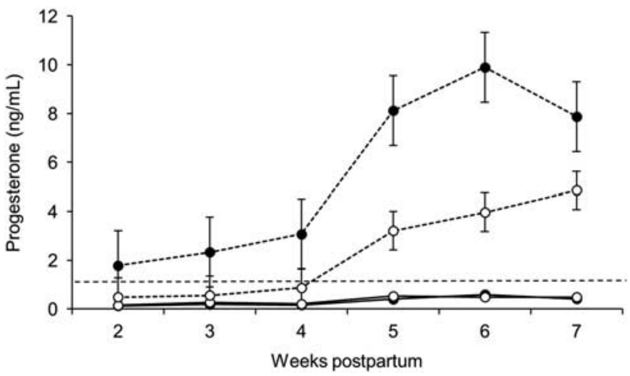

Figure 1 Plasma progesterone concentrations from 2 to 7 weeks after calving for somatrotopin-treated (●) or control heifers (○) that ovulated (- - - -) or not (——). The dashed line (- - - -) indicates the 1 ng/ml limit used to divide cows that were considered as ovulatory or non-ovulatory. Heifers were treated with 500 mg of recombinant bovine somatotropin at weeks −5 and −3 from calving.

Consequently, *prepartum* somatotropin increased milk production and the proportion of heifers ovulating in the first 7 weeks *post partum*. Despite the positive effects in the resumption of ovarian activity, we did not find any significant increase in the pregnancy rate for treated heifers, probably because of low statistical power and number of heifers enrolled in this study.

Somatotropin treatment increased the number of heifers resuming ovarian activity in the first 7 weeks *post partum*. In addition, more heifers in this treatment ovulated the first *postpartum* dominant follicle, as indicated by the rise in progesterone concentration before 21 DIM (Kawashima *et al*., 2006). It is well-established that cows ovulating during the first *postpartum* follicular wave have higher serum IGF-I concentration in the *prepartum* and *postpartum* periods (Butler *et al*., 2006; Kawashima *et al*., 2007). In this study, heifers treated with somatotropin had higher IGF-I concentration in the *prepartum* period, but not in the *postpartum* period. Britt (1992) hypothesized that the developmental competence of the oocyte and the steroidogenic capacity of the follicle, in high yielding dairy cows, is determined by their biochemical environment during the long period (up to 80 days) of follicular growth before ovulation. Thus, primary follicles exposed to adverse conditions associated with the metabolically challenging period of NEB during the early *postpartum* period may be less capable of producing adequate amounts of estrogens. Furthermore, such follicles would be doomed to contain an inferior oocyte, which will then be ovulated approximately 60 to 80 days *post partum* (Leroy *et al*., 2008). Although this hypothesis was never tested with regard to the NEB challenge, it was found to be true for cows undergoing a short period of heat stress (Roth *et al*., 2001a and 2001b) and can explain the effects of *prepartum* IGF-I on *postpartum* follicle development observed in this study.

Another interesting point is that somatotropin-treated heifers had higher insulin concentrations in the *postpartum* period. Circulating insulin concentration is proposed as a key factor in mediating the effect of dietary intake on ovarian function (Armstrong *et al*., 2002). In this manner, dairy cows fed diets designed to increase circulating insulin concentrations demonstrated increased ovarian function (Armstrong *et al*., 2001) and earlier onset of estrous cycles (Gong *et al*., 2002). In addition, cows that ovulated the first *postpartum*

Table 2 *Milk production and composition in control and somatotropin-treated groups*

	Treatment			*P*-values		
Variable	Control	Somatotropin	s.e.m.	Treatment	Time	Treatment × time
Milk[1] (kg/cow per day)	22.3	25.1	0.4	<0.0001	0.01	0.71
Lactose[2] (%)	4.7	4.7	0.01	0.42	0.60	0.57
Fat[2] (%)	3.1	3.1	0.04	0.46	0.16	0.13
Protein[2] (%)	3.0	3.0	0.01	0.34	0.27	0.58
SCC (×1000 cells/ml)	123.5	64.4	9.7	0.0009	0.31	0.63

SSC = somatic cell count.
[1]Weekly average from daily measurements taken from 8 to 45 days *post partum.*
[2]Average of samples collected weekly from 8 to 45 days *post partum.*

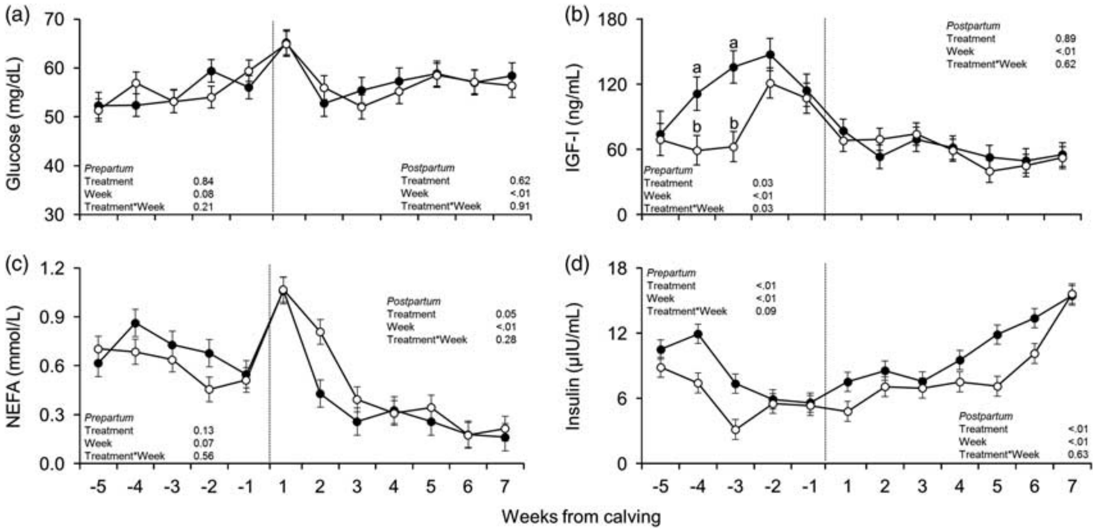

Figure 2 Plasma concentrations of glucose (mg/dl) (a), IGF-I (ng/ml) (b), non-esterified fatty acids (NEFA; mmol/l) (c) and insulin (µIU/ml) (d) from −5 to 7 weeks from calving for somatotropin-treated (●) or control heifers (○). Heifers were treated with 500 mg of recombinant bovine somatotropin at weeks −5 and −3 from calving.

dominant follicle had higher circulating insulin concentrations than anovulatory cows (Butler *et al.*, 2006). The increased ovulation rate is probably due to positive effects of insulin on steroidogenesis (Butler *et al.*, 2004) via amplification of gonadotropin action (Spicer and Echternkamp, 1995).

Milk production was increased by *prepartum* somatotropin treatment, in agreement with a previous observation (Putnam *et al.*, 1999). In the same way, *prepartum* injection of somatotropin in ewes led to higher milk production and mammary cell proliferation (Stelwagen *et al.*, 1993), probably mediated by IGF-I (Baumrucker and Stemberger, 1989). Furthermore, according to the SCC data, the health of the mammary gland was also improved in heifers injected with somatotropin. The decreased SCC in dairy cows receiving somatotropin has already been shown elsewhere (Putnam *et al.*, 1999; Gulay *et al.*, 2004), and can also have contributed to increased milk production (Hortet *et al.*, 1999).

Previous studies have shown that cows treated with a low dose of somatotropin in the *peripartum* period had a better recovery of BCS during early lactation, even though they produced more milk (Putnam *et al.*, 1999; Gulay *et al.*, 2003). Studies indicate that the extra energy to support increased milk yield in somatotropin-treated cows arose from greater DMI rather than from greater mobilization of body reserves (Putnam *et al.*, 1999; Gulay *et al.*, 2004). In agreement with these observations, in this study, somatotropin-treated heifers had lower BCS loss and better energy balance related parameters, such as higher insulin and lower NEFA concentrations in the early *postpartum* period, even though they produced more milk. Vallimont *et al.* (2001) also found a reduction in *postpartum* NEFA concentration after injecting cows with somatotropin exclusively in the *prepartum* period, in concert with our current findings.

In conclusion, somatotropin injection during the *prepartum* period in late-pregnant Holstein heifers increased proportion of heifers resuming ovarian activity during the first 7 weeks *post partum*, inspite of higher milk production. In addition, *prepartum* somatotropin also improved parameters that indicate that these heifers had a less severe NEB during the early *postpartum* period.

Acknowledgments

The authors thank the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support. The authors thank Granja 4 Irmãos S/A that provided cows and farm facilities.

References

Armstrong DG and Webb R 1997. Ovarian follicular dominance: the role of intraovarian growth factors and novel proteins. Reviews on Reproduction 2, 139–146.

Armstrong DG, Gong JG, Gardner JO, Baxter G, Hogg CO and Webb R 2002. Steroidogenesis in bovine granulosa cells: the effect of short-term changes in dietary intake. Reproduction 123, 371–378.

Armstrong DG, McEvoy TG, Baxter G, Robinson JJ, Hogg CO, Woad KJ, Webb R and 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. Biology of Reproduction 64, 1624–1632.

Ballou MA, Gomes RC, Juchem SO and DePeters EJ 2009. Effects of dietary supplemental fish oil during the peripartum period on blood metabolites and hepatic fatty acid compositions and total triacylglycerol concentrations of multiparous Holstein cows. Journal of Dairy Science 92, 657–669.

Bauman DE and Vernon RG 1993. Effects of exogenous bovine somatotropin on lactation. Annual Reviews on Nutrition 13, 437–461.

Baumrucker CR and Stemberger BH 1989. Insulin and insulin-like growth factor-I stimulate DNA synthesis in bovine mammary tissue in vitro. Journal of Animal Science 67, 3503–3514.

Britt JH 1992. Impacts of early *postpartum* metabolism on follicular development and fertility. Proccedings of the Annual Convention of the American Association of Bovine Practitioners 24, 39–43.

Butler WR 2003. Energy balance relationships with follicular development, ovulation and fertility in *postpartum* dairy cows. Livestock Science 83, 211–218.

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

Butler ST, Pelton SH and Butler WR 2006. Energy balance, metabolic status, and the first *postpartum* ovarian follicle wave in cows administered propylene glycol. Journal of Dairy Science 89, 2938–2951.

Carriquiry M, Dahlen CR, Weber WJ, Lamb GC and Crooker BA 2009a. *Postpartum* ovarian activity in multiparous Holstein cows treated with bovine somatotropin and fed n-3 fatty acids in early lactation. Journal of Dairy Science 92, 4876–4888.

Carriquiry M, Weber WJ, Dahlen CR, Lamb GC, Baumgard LH and Crooker BA 2009b. Production response of multiparous Holstein cows treated with bovine somatotropin and fed diets enriched with n-3 or n-6 fatty acids. Journal of Dairy Science 92, 4852–4864.

Colazo MG, Ambrose DJ, Kastelic JP and Small JA 2008. Comparison of 2 enzyme immunoassays and a radioimmunoassay for measurement of progesterone concentrations in bovine plasma, skim milk, and whole milk. Canadian Journal of Veterinary Research 72, 32–36.

el-Roeiy A, Chen X, Roberts VJ, Shimasakai S, Ling N, LeRoith D, Roberts CT Jr and Yen SS 1994. Expression of the genes encoding the insulin-like growth factors (IGF-I and II), the IGF and insulin receptors, and IGF-binding proteins-1–6 and the localization of their gene products in normal and polycystic ovary syndrome ovaries. Journal of Clinical Endocrinology and Metabolism 78, 1488–1496.

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

Gong JG, Bramley TA, Wilmut I and Webb R 1993. Effect of recombinant bovine somatotropin on the superovulatory response to pregnant mare serum gonadotropin in heifers. Biology of Reproduction 48, 1141–1149.

Gong JG, Lee WJ, Garnsworthy PC and Webb R 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early *postpartum* period on reproductive function in dairy cows. Reproduction 123, 419–427.

Gulay MS, Hayen MJ, Teixeira LC, Wilcox CJ and Head HH 2003. Responses of Holstein cows to a low dose of somatotropin (bST) *prepartum* and *postpartum*. Journal of Dairy Science 86, 3195–3205.

Gulay MS, Hayen MJ, Liboni M, Belloso TI, Wilcox CJ and Head HH 2004. Low doses of bovine somatotropin during the transition period and early lactation improves milk yield, efficiency of production, and other physiological responses of Holstein cows. Journal of Dairy Science 87, 948–960.

Hortet P, Beaudeau F, Seegers H and Fourichon C 1999. Reduction in milk yield associated with somatic cell counts up to 600 000 cells/ml in French Holstein cows without clinical mastitis. Livestock Production Science 61, 33–42.

Jones JI and Clemmons DR 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocrine Reviews 16, 3–34.

Kawashima C, Kaneko E, Amaya Montoya C, Matsui M, Yamagishi N, Matsunaga N, Ishii M, Kida K, Miyake Y and Miyamoto A 2006. Relationship between the first ovulation within three weeks *postpartum* and subsequent ovarian cycles and fertility in high producing dairy cows. Journal of Reproduction and Development 52, 479–486.

Kawashima C, Fukihara S, Maeda M, Kaneko E, Montoya CA, Matsui M, Shimizu T, Matsunaga N, Kida K, Miyake Y, Schams D and Miyamoto A 2007. Relationship between metabolic hormones and ovulation of dominant follicle during the first follicular wave post-partum in high-producing dairy cows. Reproduction 133, 155–163.

Kirby CJ, Smith MF, Keisler DH and Lucy MC 1997. Follicular function in lactating dairy cows treated with sustained-release bovine somatotropin. Journal of Dairy Science 80, 273–285.

Knight CH 2001. Lactation and gestation in dairy cows: flexibility avoids nutritional extremes. Proccedings of the Nutrition Society 60, 527–537.

Lee SH, Engle TE and Hossner KL 2002. Effects of dietary copper on the expression of lipogenic genes and metabolic hormones in steers. Journal of Animal Science 80, 1999–2005.

Leroy JL, Opsomer G, Van Soom A, Goovaerts IG and 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. Reproduction in Domestic Animals 43, 612–622.

Littell RC, Henry PR and Ammerman CB 1998. Statistical analysis of repeated measures data using SAS procedures. Journal of Animal Science 76, 1216–1231.

Lucy MC, Jiang H and Kobayashi Y 2001. Changes in the somatotrophic axis associated with the initiation of lactation. Journal of Dairy Science 89, E113–E119.

National Research Council (NRC) 2001. Nutrient requirements of dairy cattle, 7th edition. National Academy Press, Washington, DC, USA.

Obese FY, Humphrys S, Macmillan KL and Egan AR 2008. Measuring concentrations of insulin-like growth factor-I with an enzyme-linked immuno-sorbent antibody assay in plasma samples from Holstein cows. Journal of Dairy Science 91, 160–168.

Putnam DE, Varga GA and Dann HM 1999. Metabolic and production responses to dietary protein and exogenous somatotropin in late gestation dairy cows. Journal of Dairy Science 82, 982–995.

Roth Z, Meidan R, Shaham-Albalancy A, Braw-Tal R and Wolfenson D 2001a. Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles. Reproduction 121, 745–751.

Roth Z, Arav A, Bor A, Zeron Y, Braw-Tal R and Wolfenson D 2001b. Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. Reproduction 122, 737–744.

Spicer LJ and Echternkamp SE 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Domestic Animal Endocrinology 12, 223–245.

Stelwagen K, Grieve DG, Walton JS, Ball JL and Mcbride BW 1993. Effect of *prepartum* bovine somatotropin in primigravid ewes on mammogenesis, milk-production, and hormone concentrations. Journal of Dairy Science 76, 992–1001.

Stevenson JS and Britt JH 1979. Relationships among luteinizing hormone, estradiol, progesterone, glucocorticoids, milk yield, body weight and *postpartum* ovarian activity in Holstein cows. Journal of Animal Science 48, 570–577.

Tabeleão VC, Goulart MA, Schwegler E, Weiser MA, Moura SV, Silva VM, Pereira VS, Del Pino FAB and Corrêa MN 2008. Ruminal and metabolic evaluation of bovines maintained in semi-confinement system. Archivos de Zootecnia 57, 147–154.

Thatcher WW and Wilcox CJ 1973. *Postpartum* estrus as an indicator of reproductive status in the dairy cow. Journal of Dairy Science 56, 608–610.

Vallimont JE, Varga GA, Arieli A, Cassidy TW and Cummins KA 2001. Effects of *prepartum* somatotropin and monensin on metabolism and production of periparturient Holstein dairy cows. Journal of Dairy Science 84, 2607–2621.

Wathes DC, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris DG, Kenny D, Murphy J and Fitzpatrick R 2007. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology 68 (suppl. 1), S232–S241.