Relationship between metabolic hormones and ovulation of dominant follicle during the first follicular wave post-partum in high-producing dairy cows

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Abstract

Recent studies suggest that IGF-I is a crucial regulatory factor in follicular growth during early post-partum period. The aim of the present study was to determine in detail the changing profiles of metabolic and reproductive hormones in relation to ovulation of the dominant follicle (DF) of the first follicular wave post-partum in high-producing dairy cows. Plasma concentrations of related hormones in 22 multiparous Holstein cows were measured from 4 weeks pre-partum to 3 weeks post-partum, and the development of DF was observed with colour Doppler ultrasound. Thirteen cows showed ovulation by 15.2 days post-partum. Anovulatory cows showed higher GH and lower IGF-I levels than those in ovulatory cows during the peri-partum period. Each DF developed similarly, and a clear blood flow in the follicle wall was observed despite ovulation or anovulation. In addition, detailed endocrine profiles were analyzed in 9 out of the 22 cows. Five cows showed an increase in plasma oestradiol-17β (E2) with follicular growth followed by E2 peak, LH surge and ovulation. In these cows, plasma IGF-I concentrations remained high until 10 days post-partum followed by a gradual decrease. Subsequently, the insulin level increased together with the E2 peak towards ovulation. These profiles were not observed in anovulatory cows. In conclusion, our data strongly support the concept that IGF-I and insulin represent ‘metabolic signals’ of the resumption of ovarian function post-partum in high-producing dairy cows. Moreover, we provide the first visual evidence that both ovulatory and anovulatory DFs of the first follicular wave post-partum are similarly supplied with active blood flow.


Introduction

The negative energy balance early post-partum that is characterized by the loss of body weight and mobilization of body fat stores does not affect the first follicular wave post-partum (Beam & Butler 1999). In fact, the medium-sized follicles appear 5 days after calving and grow into large follicles at 10 days post-partum in most dairy cows (Savio et al. 1990a, 1990b). However, almost a half of these follicles are not able to ovulate and become atretic or cystic. Most ovulations are observed within 3 weeks postpartum (Lamming & Bulman 1976, Lucy et al. 1992b, Darwash et al. 1997). Our recent study showed that the recovery of ovarian function and subsequent reproductive performance in ovulatory cows within 3 weeks post-partum was superior to that of anovulatory cows (Kawashima et al. 2006). It is generally accepted that the cow with early resumption of the ovarian function has higher fertility (Staples et al. 1990, Senatore et al. 1996, Darwash et al. 1997). However, the factors that control the first ovulation post-partum have not been fully elucidated.

Insulin and insulin-like growth factor-I (IGF-I), metabolic hormones that are changed by feed intake, stimulate oestradiol-17β (E2) production in the granulosa cells (Gutierrez et al. 1997, Glister et al. 2001, Armstrong et al. 2003, Butler et al. 2004) and proliferation of follicular cells (Spicer et al. 1993, Spicer & Stewart 1996). The growth of ovulatory follicles is regulated by metabolic hormones such as IGF-I or insulin and is susceptible to the endocrine and metabolic status during early lactation (Spicer et al. 1990, Lucy
et al. 1992b). The liver-derived IGF-I is a factor that regulates the final maturation of the dominant follicle (DF) during the first follicular wave post-partum (Beam & Butler 1998). The circulating IGF-I in ovulatory cows at the first follicular wave post-partum was higher than that in anovulatory cows (Beam & Butler 1998).

Most of the above studies investigated a few metabolic factors during the first follicular wave post-partum. Therefore, the aim of the present study was to investigate the time-dependent relationship in detail among metabolic hormones, including IGF-I, growth hormone (GH) and insulin, and metabolites such as glucose, non-esterified fatty acids (NEFA), total cholesterol (T-cho) and aspartate aminotransferase (AST), as well as the body condition score (BCS) and ovulation during the first follicular wave post-partum in a homogeneous herd at an early lactation period in high-producing dairy cows. In addition, we used colour Doppler ultrasonography to monitor local blood flow during the growth of individual follicles.

Materials and Methods

Experimental procedures complied with the Guide for Care and Use of Agricultural Animals of Obihiro University.

Animals

The experiment was carried out in the Field Centre of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine. We used 22 multiparous Holstein cows that calved between October 2004 and April 2005. We collected samples from 4 weeks pre-partum to 3 weeks post-partum. Blood was drawn 2 times/day for detection of P4 and metabolic hormones. For measurement of BCS, we used colour Doppler ultrasonography to monitor local blood flow during the growth of individual follicles.

Measurement of P4 and E2

The P4 determination in plasma was performed by enzyme immunoassay (EIA) after extraction by diethyl ether, as described previously (Miyamoto et al. 1992), and the extraction efficiency was 93%. The standard curve ranged from 0.05 to 50 ng/ml, and the 50% of effective dose (ED50) of assay was 3.2 ng/ml. The intra- and interassay coefficient of variation (CV) values averaged 6.7 and 7.2% respectively. The E2 determination in plasma was performed by EIA after extraction by diethyl ether as described previously (Acosta et al. 2000) and the extraction efficiency was 85%. The standard

Blood samples (serum) for biochemical analysis were obtained once a week by caudal venipuncture after measurement of BCS using unheparinized, silicone-coated 9 ml tubes (Venoject, Autosep, Gel+Clot. Act., VP-AS109K; Terumo Corporation, Tokyo, Japan) and the tubes were coagulated for 30 min at 38 °C in an incubator. Blood samples (plasma) for hormonal analysis were obtained by caudal venipuncture twice weekly during the same period using sterile 10 ml tubes containing 200 µl stabilizer solution (0.3 M EDTA (pH 7.4), 1% acetyl salicylic acid). In addition, in 9 out of the 22 cows, we obtained blood samples 4 times/day at 6-h intervals for detection (presence or absence) of luteinizing hormone (LH) surge. To minimize the stress of blood sampling, blood was drawn 2 times/day for detection of E2 peak and 1 time/day for profile of P4 and metabolic hormones. These tubes were centrifuged at 3000 r.p.m. for 20 min at 4 °C, and the serum or plasma samples were kept at −30 °C until biochemical and hormonal analyses.

Ovaries of all cows were scanned twice weekly from 7 days to 30 days after calving by transrectal ultrasonography using a colour Doppler ultrasound scanner (SSD-5500; Aloka Co., Tokyo, Japan) equipped with a 7.5 MHz convex transducer (UST-995-7.5; Aloka Co.). Follicles ≥5 mm were measured for maximum diameter and follicles ≥8.5 mm were regarded as DFs (Ginther et al. 2003). After morphological evaluation, the flow mode was activated for blood flow mapping and the presence or absence of blood flow was assessed for each follicle. Colour signals were used to generate images in which blood flow with a velocity higher than 2 mm/s could be located as areas of colour within the follicle wall.

Identification of ovulation during the first follicular wave post-partum

When the P4 concentration in plasma was increased to more than 1 ng/ml, the cow was confirmed as having returned to luteal activity (Stevenson & Britt 1979). Furthermore, the first ovulation and luteal formation were identified using an ultrasound scanner equipped with a 7.5 MHz convex transducer.

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curve ranged from 1.95 to 2000 pg/ml, and the ED50 of assay was 5.2 pg/ml. The intra- and interassay CV values averaged 6.7 and 8.7% respectively.

Measurement of FSH, LH, GH, IGF-I and insulin

The determination of plasma follicle-stimulating hormone (FSH), LH, GH, IGF-I and insulin was performed by EIA using the biotin–streptavidin amplification technique.

The FSH assay was performed as described previously (Watanabe et al. 1997). The standard curve for FSH ranged from 0.18 to 12 ng/ml, and the ED50 of the assay was 1.7 ng/ml. The intra- and interassay CV values averaged 8.3 and 14.6% respectively.

The LH assay was described previously in detail (Mutayoba et al. 1990). The standard curve for LH ranged from 0.09 to 50 ng/ml, and the ED50 of the assay was 3.1 ng/ml. The intra- and interassay CV values averaged 8.3 and 9.4% respectively.

The GH concentration was measured by the EIA as described by Roh et al. (1997), but with slight modification. A diluted rabbit antibody added to bovine GH (100 µl, ×150 000, D Schams, TU-Munich, Weihenstephan, Germany) was distributed in all wells of a microplate coated with anti-rabbit γ-globulin antiserum, then incubated for 24 h at room temperature and the plate was decanted. After chicken serum diluted in assay buffer (100 µl, 1%) was added to each well, 15 µl GH standard (0.78–100 ng/ml, NIDDK-bGH, AFP-9984C) dissolved in assay buffer or plasma were incubated in wells for 24 h. After decanting the plate, biotin-labelled GH was distributed in all wells and then incubated for 3 h. Finally, colorimetric treatments were carried out. Intra- and interassay CV values were 8.1 and 8.5% respectively, and the ED50 in this assay system was 6.2 ng/ml.

The total IGF-I determination in plasma was performed by the EIA after protein extraction by an acid–ethanol mixture (87.5% ethanol and 12.5% 2 N hydrochloric acid; Daughaday et al. 1980) to obtain free IGF-I from binding protein. Thirty microlitres of human IGF-I standard (Roche; 0.39–50 ng/ml) dissolved in assay buffer or plasma were added to each well coated with anti-rabbit γ-globulin antiserum. In addition, 100 µl bovine guinea pig insulin (×150 000, D Schams) dissolved in assay buffer were distributed in all wells and then incubated for 24 h at 4 °C. After decanting the plates, 100 µl biotin-labelled bovine insulin (×50 000) were distributed in all wells and then incubated for 2 h at 4 °C. Finally, colorimetric treatments were carried out. Intra- and interassay CV values were 9.7 and 14.5% respectively, and the ED50 in this assay system was 800 pg/ml.

Biochemical analyses

In each sample, the concentrations of glucose, NEFA, T-cho and AST were measured using a clinical chemistry automated analyser (TBA120FR; Toshiba Medical Systems Co., Tochigi, Japan).

Statistical analyses

All data from 22 cows was analyzed by repeated ANOVA. There was a tendency for a group×time interaction on plasma concentrations of E2 (P=0.09) and plasma E2 concentrations changed over time in the ovulatory cows (time effect; P<0.05). Mean concentrations of E2 were calculated for each group and each sampling period, and significant differences were detected by Fisher’s protected least significance difference test.

For other data, no significant interaction between group and time was observed. For all metabolites, statistical analyses were performed with two-way ANOVA. Post hoc analyses were performed with Student’s t-test.

Metabolites, BCS and metabolic hormones during the peri-partum period related to the ovulation of the DF at the first follicular wave post-partum

The numbers of cows showing ovulation and anovulation of a DF during the first post-partum follicular wave were

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13 (59.1%) and 9 (40.9%) respectively. In ovulatory cows, the interval between calving and ovulation was 15.2 ± 0.8 days, and after the first ovulation, cows had an ovarian cycle followed by more than 7 days of a luteal phase. The first month milk yield was 45.5 ± 0.4 kg/day in ovulatory cows and 45.1 ± 0.5 kg/day in anovulatory cows.

Figure 1 shows the change of metabolites, BCS and metabolic hormones during the peri-partum period. The serum concentration of glucose was not significantly different between the ovulatory and anovulatory cows during the peri-partum period. Thereafter, the serum glucose concentrations of the anovulatory cows were lower than those of the ovulatory cows during the post-partum period (ovulatory, 59.2 ± 1.3 vs anovulatory, 52.7 ± 1.3; P < 0.001). For BCS, there was no significant difference between ovulatory and anovulatory cows during the pre-partum period; however, BCS in ovulatory cows tended to be higher than that in anovulatory cows during the post-partum period (P = 0.08; Fig. 1). The serum concentrations of NEFA, T-cho and AST were similar between the ovulatory and the anovulatory cows during the period of study (Fig. 1).

The plasma concentration of insulin was not significantly different between the ovulatory and anovulatory cows during the pre- and post-partum periods. The plasma concentration of GH in anovulatory cows was higher than that of ovulatory cows during both pre- and post-partum periods (pre-partum, P < 0.01; post-partum, P < 0.0001; Fig. 1). On the other hand, the plasma concentration of IGF-I in ovulatory cows was higher than that of anovulatory cows during both pre- and post-partum periods (pre-partum, P < 0.05; post-partum, P < 0.01; Fig. 1).

The function and morphology of DF during the first follicular wave post-partum, and metabolic status either ovulatory or anovulatory cows

The DF of the first follicular wave appeared on day 7 post-partum, and the diameter of the DF was approximately 10 mm and blood flow in each follicle was observed by 14 days post-partum despite ovulation or anovulation (Fig. 2). In the ovulatory cows, a corpus luteum with blood flow was observed by 17 days post-partum (Fig. 2). On the other hand, the DF of anovulatory cows continued to grow maintaining identifiable blood flow (Fig. 2).

The plasma concentration of FSH did not differ between the ovulatory and anovulatory cows until 14 days post-partum (ovulation, 2.18 ± 0.09 ng/ml vs anovulation, 2.05 ± 0.09 ng/ml). The ratio of the existence of the DF at the first scanning was 61.5% (8/13) in the ovulatory cows and 44.4% (4/9) in the anovulatory cows, and the diameter of the follicle at that time was not significantly different between the ovulatory and anovulatory cows (ovulation, 9.9 ± 0.7 mm vs anovulation, 9.9 ± 1.0 mm).

The diameter of the DF had similar growth in both groups (time effect; P < 0.01, Fig. 3) and did not differ between the ovulatory and the anovulatory cows during the period of study. The plasma concentrations of E2 in ovulatory cows was higher at 1.5 weeks post-partum than those at 1 and 2 weeks post-partum (P < 0.05; Fig. 3), and higher than those in anovulatory cows at 1.5 weeks post-partum (P < 0.01; Fig. 3). However, there was no significant difference between the ovulatory and anovulatory cows at 2 weeks post-partum, because of the decrease of preovulatory E2 concentrations. In the ovulatory cows, plasma GH concentrations were lower (P < 0.01) and IGF-I concentrations were higher (P < 0.05) when compared with anovulatory cows, while the plasma insulin concentrations did not differ between the ovulatory and anovulatory cows during this period (Fig. 3).
The metabolic endocrine factors related to ovulation of the DF during the first follicular wave post-partum

To observe the relationship between the growth of the DF and the endocrine and metabolic factors (E2, insulin, IGF-I and GH) in detail, 9 out of the 22 cows showing ovulation or anovulation of DFs during the first follicular wave post-partum were studied. The number of ovulatory cows was 5 (55.6%), E2 peak could be confirmed on 16.0 ± 1.1 days post-partum, the interval between E2 peak and LH surge being approximately 21 ± 7 h, and the interval between calving and ovulation being 17.0 ± 1.5 days. On the other hand, E2 peak and LH surge could not be confirmed in the anovulatory cows.

In the ovulated cows, E2 peak could be confirmed on 16.0 ± 1.1 days post-partum, and the interval between E2 peak and LH surge was approximately 21 ± 7 h (Fig. 4). On the other hand, E2 peak and LH surge could not be confirmed in the anovulatory cows (Fig. 4). In ovulated cows, insulin concentrations during and after the E2 peak period showed a strong tendency to be higher than those before the E2 peak (P = 0.06; Table 1). The GH concentrations from before until after the E2 peak did not change. However, plasma IGF-I remained high until 7–10 days post-partum, followed by a gradual decrease (Fig. 4). Therefore, IGF-I concentration during the after E2 peak was lower than that during the before E2 peak (P < 0.05, Table 1).

Discussion

The present study focused on the changes in metabolic hormones, such as IGF-I, GH and insulin that occurs during the peri-partum period in dairy cows and their relation to the occurrence of ovulation. The results suggest that the plasma concentration of IGF-I relates to the early growth of the ovulatory DF and insulin relates to the maturation and ovulation of the ovulatory DF at the first follicular wave post-partum.

Our results showed that higher plasma GH concentrations and lower glucose and IGF-I concentrations were the clear features in anovulatory cows during the pre-partum period compared with those of the ovulatory cows. Previous studies have shown that the expression of GH receptor 1A and IGF-I mRNA in the liver declines at parturition, and GH concentrations are elevated during the post-partum period, because IGF-I concentrations decrease to low levels, reducing the negative feedback on GH (Kobayashi et al. 1999, Pushpakumara et al. 2003, Radcliff et al. 2003). The plasma IGF-I levels in the ovulatory cows at the first follicular wave post-partum were higher than those in the anovulatory cows (Beam & Butler 1998). The high GH concentrations in the anovulatory cows in the present study with lower IGF-I concentrations during the peri-partum period may have caused GH insensitivity in the liver. These findings suggest that energy status, which reflects on GH, IGF-I and glucose levels, and ovulation are closely related to each other during the early post-partum period. However, in the present study, the metabolites such as NEFA, AST and T-cho did not differ between ovulatory and anovulatory cows during the peri-partum period, indicating that the lipid metabolism between these two groups was at similar levels despite the different GH levels between two groups. In the anovulatory cows, body fat stores might have already been reduced during the peri-partum period, resulting in lower BCS than that in ovulatory cows. Therefore, ovulatory cows may be able to handle better the negative energy balance after parturition when compared with anovulatory cows. The present study could not examine the exact feed intake and nutritional status, because all cows were provided with feed and water available ad libitum. Thus, further studies are necessary to investigate the effect of feed intake on metabolic hormones and on the maturation and ovulation of the first post-partum DF.

It was observed that the rate of follicular growth did not differ between the ovulatory and anovulatory cows during the post-partum period. The local blood flow was detected in DF of all cows despite ovulation or anovulation. Angiogenesis, the formation of new network of blood vessels, is essential for follicular

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Figure 2 Representative images of dominant follicles during the first follicular wave post-partum of ovulatory and anovulatory cows. Red colour represents the blood flow towards the transducer, and blue indicates blood flow away from the transducer. The colour gain of the flow mode was set to detect the movement of at least 2 mm/s. Scale bar represents 5 mm. The white dotted lines that delineate circles indicate the frame of the follicle and the green dotted lines that delineate circles indicate the frame of the corpus luteum.
development and ovulation (Yamada et al. 1994, Jiang et al. 2003). In addition, a recent report suggested that the angioprotein-tie system that acts on angiogenesis in concert with vascular endothelial growth factor controls the blood vessels to maintain active angiogenesis in developing bovine follicles (Hayashi et al. 2003). The maintenance of follicle vasculature and appropriate blood supply to the large follicles is essential for follicle dominance (Acosta et al. 2005). However, our results showed that an insufficient blood flow supply is not the cause of anovulation. The plasma E2 concentrations during this period increased only in the ovulatory cows, while those in the anovulatory cows did not, which are well consistent with previous reports (Beam & Butler 1997, 1998). Thus, it is likely that an insufficient ability of the granulosa cells to secrete E2 is a determinant for anovulation rather than insufficient angiogenesis.

The plasma concentration of FSH did not differ between the ovulatory and anovulatory cows until 14 days post-partum. Our data confirmed the previous studies that FSH appears to be insensitive to metabolic status (Lamming et al. 1981, Beam & Butler 1998). On the other hand, serum concentrations of IGF-I during the first follicular wave post-partum were reported to be at low levels in anovulatory dairy cows (Beam & Butler 1998). In the present study, the plasma IGF-I remained elevated until 7–10 days post-partum, followed by a gradual decrease, whereas IGF-I in the anovulatory cows was kept at a low level throughout the post-partum period. The IGF-I stimulates steroidogenesis and the proliferation in follicular cells (Spicer et al. 1993, Spicer & Stewart 1996), and the ratio of oestrogen to progesterone in follicular fluid was correlated positively with plasma IGF-I in lactating cows (Lucy et al. 1992a). In addition, IGF-I works with FSH synergically in promoting follicular development and E2 synthesis (Fortune et al. 2004). Thus, the data suggests that IGF-I is an important factor for the development of the DF to the maturation stage by amplifying the endocrine signal of FSH to upregulate E2 secretion from the future DF post-partum.

The present data suggested that plasma insulin level in the ovulatory cows increased together with E2 peak. In dairy cows, the serum insulin levels increase together with E2 during the development of the DF (Armstrong et al. 2003). Insulin has been shown to stimulate E2 production in the granulosa cells (Gutierrez et al. 1997, Glister et al. 2001). In addition, E2 secreted from a follicle also enhances insulin secretion from the

![Figure 3](image-url)
pancreas (Morimoto et al. 2001). These findings clearly indicate that plasma insulin and E2 from follicles positively interact with each other during the development of the DF. The increase in circulating insulin level by the feeding modification results in advanced first ovulation post-partum in the cow (Gong et al. 2002) and the increase in plasma LH concentration in heifer (McCann & Hansel 1986). The LH pulse frequency was reported to be greater in ovulatory cows than in anovulatory cows (Lamming et al. 1981, Canfield & Butler 1990, Jolly et al. 1995). Therefore, plasma insulin levels closely relate to the follicle growth and ovulation by regulating LH secretion. On the other hand, LH was not controlled by insulin infusion in early lactation cows (Butler et al. 2004). The energy status resulting in high insulin levels, but not directly insulin infusion, may induce the change of pulsatile LH release, follicle growth and ovulation. Consequently, the nutritional status which leads to the increase of insulin level together with E2 peak, is one of the key features of ovulatory cows. Undoubtedly, further studies are needed to characterize the factors responsible for the suboptimal GnRH and LH release after parturition in dairy cows.

As shown in Fig. 5, our results showed that the changes in the concentration of two metabolic hormones (IGF-I and insulin) may regulate the development of ovulatory follicle in the first follicular wave post-partum. In ovulatory cows, high IGF-I levels were maintained during the growth of DF, and then insulin levels increased together with the increase in E2 secretion from DF. These findings indicate that IGF-I is an essential factor for the growth of the DF, and insulin may stimulate the DF to mature and reach ovulation. Collectively, it is

**Table 1** The metabolic-endocrine factors between before and after E2 peak period in ovulatory cows.

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<th>Relative to E2 peak</th>
<th>Significance of differences</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>0.29±0.03</td>
<td>0.47±0.08</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>11.7±1.5</td>
<td>9.6±0.8</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>98.2±21.5</td>
<td>61.1±13.7</td>
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NS, not significant. Values are means±S.E.M. Data were divided into two phases which were before E2 peak (−10 to −1 days relative to E2 peak) and after E2 peak (0–10 days relative to E2 peak).
suggested that the DF which is stimulated by high IGF-I levels during the first follicular wave \textit{post-partum}, become ovulatory with the increase in E2 secretion, resulting in the occurrence of LH surge and ovulation.

In conclusion, the present study provides convincing evidence that IGF-I is an important factor for the development of the DF during the first follicular development \textit{post-partum}. After that, the increase in insulin level together with E2 peak may ensure the maturation and ovulation. Our findings strongly support the concept that IGF-I and insulin represent ‘metabolic signals’ for the resumption of ovarian function \textit{post-partum} in high-producing dairy cows. Moreover, we provide the first visual evidence that both ovulatory and anovulatory DFs of the first follicular wave \textit{post-partum} are similarly supplied with the active blood flow, suggesting that an insufficient angiogenesis is not a determinant for anovulation.

Acknowledgements
The authors thank Dr K Okuda, Okayama University, Japan, for P4 antiserum, and Dr Parlow, NIDDK, for the FSH, LH and GH standard, and IGF-I antiserum. This study was supported by the Grant-in-Aid for Scientific Research of the Japan Society for the Promotion of Science (JSPS); the 21st Century COE Programme (A-1) of the Ministry of Education, Culture, Sports, Science and Technology, Japan; Secure and Healthy Livestock Farming Project of the Ministry of Agriculture, Forestry and Fisheries; and the Japan Livestock Technology Association. C Kawashima and E Kaneko are supported by the COE programme. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References


Butler ST, Pelton SH & 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.
insulin-like growth factor I (IGF-I) in lactating cows with positive or negative energy balance during the preovulatory period. Reproduction, Nutrition and Development 32 331–341.


Received 29 May 2006
First decision 25 July 2006
Revised manuscript received 28 August 2006
Accepted 30 October 2006