Effect of Serum IGF-I on Progesterone Concentration during Early Pregnancy in Korean Native Cattle (Hanwoo)

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ABSTRACT : Insulin-like growth factor-I (IGF-I) is a polypeptide that has the function of regulating the expression of steroid hormones through endocrine, paracrine, and autocrine actions in reproductive organs. Moreover, IGF-I is involved in ovulation, implantation, maintenance of pregnancy, and development of fetuses in animals. Therefore, this study was conducted to investigate the effects of serum IGF-I concentration on progesterone (P_4) concentration and pregnancy rates in Korean native cattle (Hanwoo). Blood was collected at estrus (Day 0) and Day 11. Artificial insemination was performed at Day 0. Serum IGF-I and P_4 concentrations were measured by radioimmunoassay (RIA). Overall, P_4 concentration was higher at Day 11 than Day 0, whereas the pattern of IGF-I concentration was reversed. When animals were divided into two groups depending on the pregnancy status, P_4 concentrations of the pregnant group was significantly higher than that of the non-pregnant group at Day 0 ($p<0.05$) and Day 11 ($p<0.05$). But, lower IGF-I concentrations were detected in the pregnant group at Day 0 ($p<0.05$) and Day 11 ($p<0.05$) compared to the non-pregnant group. In conclusion, these results indicated that serum IGF-I is inversely associated with P_4 concentration during early pregnancy in Hanwoo. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 2 : 176-179)***

Key Words : IGF-I, Progesterone, Early Pregnancy, Korean Native Cattle

INTRODUCTION

Insulin-like growth factor-I (IGF-I) is a 7.5 kDa polypeptide composed of 70 amino acids. It has the function to stimulate proliferation and development of the cell by mediating the growth stimulation effect of growth hormone (GH) (Rotwein, 1991). IGF-I has not only endocrine action for animal reproduction, but also autocrine and paracrine actions.

IGF-I has a controlling function of litter size (Yun et al., 2001), follicular development (McWilliam et al., 1995), conceptus and oviduct (Watson et al., 1999). It also acts by autocrine, paracrine action on the uterus in mice (Kapur et al., 1992). Moreover, IGF-I stimulates the synthesis of progesterone (P_4) in corpus luteum (CL) by the mediate action of GH (Lucy et al., 1995). The P_4 level is an important factor for implantation and maintenance of pregnancy (Csapo et al., 1973). Their synthesis and functions are regulated by numerous endocrine factors (Luigi et al., 1995), and it was recently reported that the P_4 synthesis in CL is controlled by growth factors, including locally expressed IGF-I.

Locally expressed and synthesized IGF-I plays an important role in CL where the P_4 is synthesized, during the estrous cycle and pregnancy (Lievermann et al., 1996). IGF-I, secreted by CL itself, has controlling functions to CL by autocrine and paracrine actions (Einspanier et al., 1990). And Lievermann et al. (1996) also suggested that there are IGF-I receptors in CL and locally expressed IGF-I has the CL regulating function. These results indicate that IGF-I plays an important role in P_4 synthesis in CL by autocrine and paracrine actions.

Moreover, Hashizume et al. (2000) reported serum IGF-I concentrations during the estrous cycle in goats, but there have been few observations about the correlation between serum IGF-I and P_4 in bovine. Therefore, this study was conducted to investigate the effects of serum IGF-I concentration on P_4 concentration during early pregnancy in Korean native cattle (Hanwoo).

MATERIALS AND METHODS

Animals

Three multimiparous and 39 primiparous Korean native cattles (Jang-Su Livestock Cooperative, Korea) were used. Artificial insemination (AI) was performed at Day 0, when the behavioral signs of estrus were observed. Pregnancy diagnosis were performed two times using the non-return (NR) method and rectal examinations. Animals were fed diet of total mixed ration (TMR) made by Jang-Su Livestock Cooperative, and the major component of the feed was brewers dried grain. Restricted feeding was performed, and water was available continuously.

Blood collection and preparation of serum

Blood (15 ml) was collected from the jugular vein at Day 0 (estrus) and Day 11 (early luteal phase). Blood samples were centrifuged at 1,000×g for 15 min after being incubated 1-2 h at room temperature, and serum was stored

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at -20°C until being analyzed for hormone and IGF-I concentrations.

Progesterone RIA

Serum progesterone concentration was measured using an RIA kit (Coat-A-Count., DPC, USA). Briefly, 50 μl samples were aliquoted in coated tubes with anti-progesterone and incubated with [^{125}I] progesterone for 3 h at room temperature. Then the pellet was measured in a gamma-counter (Packard, USA).

IGF-I RIA

RIA was used to measure the serum IGF-I concentration. One μg of recombinant human IGF-I (Gropep Pty Ltd., Australia) was iodinated to a specific activity of 270-300 μCi/μg protein using 1 mCi Na[^{125}I] (Amersham Co., U.S.A) by the chloramine-T method (Lee and Henricks, 1990). Iodinated IGF-I was purified on a Sephadex G-50 column and stored at -20°C until used.

Serum IGFBPs were removed using the acid-ethanol method and serum IGF-I concentration was measured by RIA (Daughaday et al., 1980). Anti-human IGF-I (GroPep Pty Ltd., Australia) was used as the primary antibody and radioactivity was tested in a gamma-counter (Packard, U.S.A). Amounts of IGF-I was determined by logit-log plots and the intra-assay coefficient of variation was 11%.

Statistical analysis

Serum concentrations of IGF-I and progesterone in pregnant and non-pregnant cows were analyzed using the Duncan's One-Way ANOVA procedure of Statistical Analysis System (SAS, 1995).

RESULT

Serum P_4 concentration

Serum P_4 concentrations at Day 0 and 11 in the pregnant and non-pregnant groups are shown in Figure 1. As a whole, P_4 concentration at Day 11 was ten fold higher than Day 0. There were no significant differences at Day 0 (P_4 concentrations of the pregnant and the non-pregnant groups at Day 0 were 0.208±0.095 and 0.243±0.115 ng/ml, respectively). But the P_4 concentration at Day 11 was significantly higher ($p<0.05$) in the pregnant group (3.343±0.949 ng/ml) than in the non-pregnant group (2.182±1.009 ng/ml).

Serum IGF-I concentration

Serum IGF-I concentrations at Day 0 and 11 in the pregnant and non-pregnant groups are shown in Figure 2. There was a tendency for serum IGF-I concentrations at Day 11 to be lower than at Day 0. And the serum IGF-I concentration at both Day 0 and 11 was significantly higher in the non-pregnant group (465.813±82.263, 416.568± 159.119 ng/ml) than in the pregnant group (383.938± 119.770, 344.397±87.289 ng/ml).

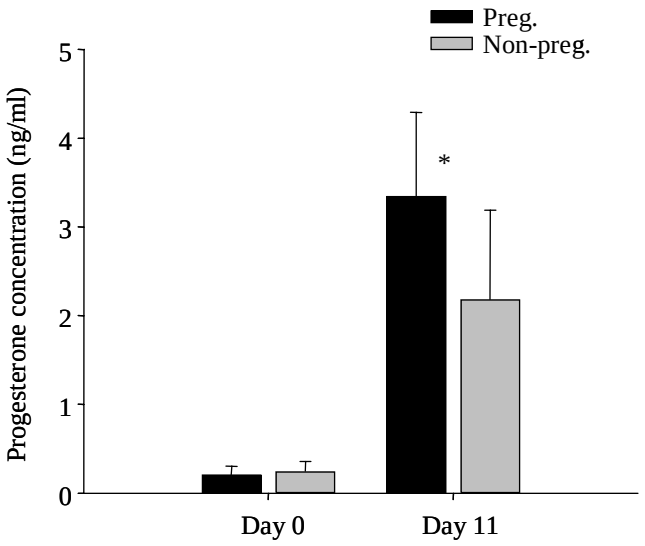

Figure 1. Serum P_4 concentrations at Day 0 and Day 11 in pregnant and non-pregnant groups.
* Indicates the mean differed ($p<0.05$) from non-pregnant group.

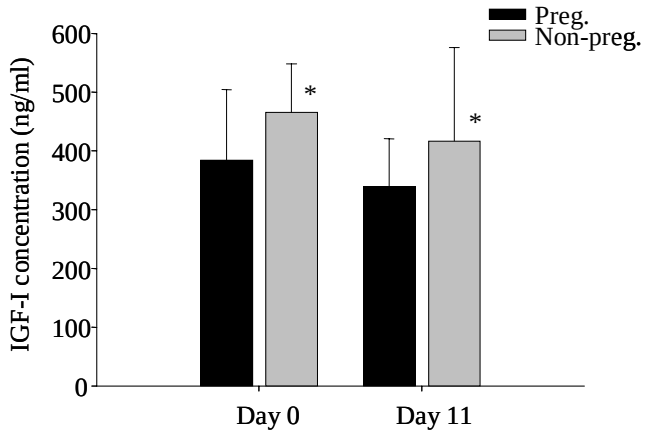

Figure 2. Serum IGF-I concentrations at Day 0 and Day 11 in pregnant and non-pregnant groups.
* Indicates the mean differed ($p<0.05$) from pregnant group.

Pregnancy rate at Day 11

The animals were divided into two groups depending on the IGF-I concentration at Day 0 to investigate the pregnancy rate followed by serum IGF-I concentration. The pregnancy rate of the IGF-I low concentration group (284.710±56.763 ng/ml serum IGF-I) was higher than the IGF-I high concentration group (532.461±34.082 ng/ml serum IGF-I) (Table 1). Moreover, the pregnancy rate of the IGF-I high concentration group was lower than that of all animals, whereas the pattern of the IGF-I low concentration group was reversed.

Table 1. Pregnancy rate in high or low IGF-I concentration group

	IGF-I group[1]		Total [2]
	High	Low	
No. of Cattles	15	15	42
IGF-I conc. (ng/ml)	532.461 ±34.082	284.710 ±56.763	411.23 ±114.535
P_4 conc. (ng/ml)	0.262 ±0.144	0.198 ±0.068	0.219 ±0.102
No. of pregnant cattles	8	14	28
Pregnancy rate (%)	53	93	67

[1] Serum IGF-I concentration was measured at Day 0.
[2] All of the investigated cattles.

DISCUSSION

There was no significant difference in serum IGF-I concentrations at Day 0 between the pregnant and non-pregnant groups. When the animals were divided into two groups depending on the IGF-I concentration, the high IGF-I concentration group showed the lower pregnancy rate than the low concentration IGF-I group. It suggests the possibility that the serum IGF-I concentration affects pregnancy, and therefore, suggests the possibility of the selection of cows with the highest likelihood of pregnancy using the serum IGF-I concentration at Day 0.

Although significant stimulation of P_4 by luteal IGF-I has been detected in rabbits (Chen et al., 1997), sheep (Khan-Dawood et al., 1994), and human luteal cells (Apa et al., 1996), the present study indicates that a low concentration of IGF-I is related to a high level of P_4 in serum. Both growth hormone and prolactin are synergistic with IGF-I for increased P_4 secretion and regulate the CL function of steroidogenesis (Yuan and Lucy, 1996). Rabbit luteal cells also have the capacity to respond to IGF-I, raising the possibility that IGF-I has a role in the regulation of steroidogenesis (Constantino et al., 1991). Increased expression of insulin-like growth factor binding protein–1 (IGFBP-1) during induced regression by treatment with prostaglandin F_2 alpha ($PGF_{2\alpha}$) of bovine CL was also reported (Sayre et al., 2000). These previous studies suggest that luteal IGF-I regulates the CL function of P_4 secretion.

However, serum IGF-I increases approximately two days before behavioral estrus and the IGF-I peak is detected in accordance with the appearance of estrus in goat. The elevated IGF-I levels then decline to basal values 4 to 5 days after estrus (Hashizume et al., 2000). Therefore, these results suggest that plasma IGF-I concentration is reversely associated with P_4 concentration at day 11. The present study in bovine serum was similar to this study. Tissue levels of IGF-I increase from Days 1-5 to Days 12-17 (Day 0 is estrus) of the cycle followed by a rapid decrease at luteolysis (Einspanier et al., 1990).

The significance of P_4 up-regulation by low concentration of serum IGF-I is not readily apparent. One possibility for consideration might be increased expression of $PGF_{2\alpha}$ in a dose-dependent manner by serum IGF-I concentration (Shuichi et al., 2001). Nicholson et al. (1999) reported that IGF-I stimulates the luteolytic actions of $PGF_{2\alpha}$. IGF-I and IGF-II are able to stimulate prostaglandin E_2 (PGE_2) synthesis in a time- and dose-dependent way, whereas they both inhibit $PGF_{2\alpha}$ production (Apa et al., 1999). The functional regression of the corpus luteum is correlated with the increased expression of IGF-I mRNA in luteal tissue in rats (Tamada et al., 1995). Moreover, there is a report about the synergism between IGF-I and LH on increasing steroidogenesis (Stewart et al., 1995), and it is thought that IGF-I is related with LH and of $PGF_{2\alpha}$. The $PGF_{2\alpha}$-induced stimulation of IGFBP-3 production may be one of the mechanisms whereby $PGF_{2\alpha}$ exerts its luteolytic effect via the IGF system, because IGFBP-3 inhibits progesterone production stimulated by IGF-I (Sarvas et al., 1994).

In conclusion, the negative regulation of IGF-I to P_4 may be via up-regulation of $PGF_{2\alpha}$ (Figure 3).

IMPLICATION

Recent studies have investigated P_4 concentration and pregnancy by monitoring serum IGF-I concentration. But because regulation of CL function and maintenance of pregnancy are associated with other hormones, growth factors, and physical environment, a study only about IGF-I concentration may not be an accurate estimation. Therefore, if growth factors and other hormones, for example, IGF-II, $PGF_{2\alpha}$, etc. were also monitored during early pregnancy, early pregnancy diagnosis and selection of cows having high reproductive performance could be more accurately assured.

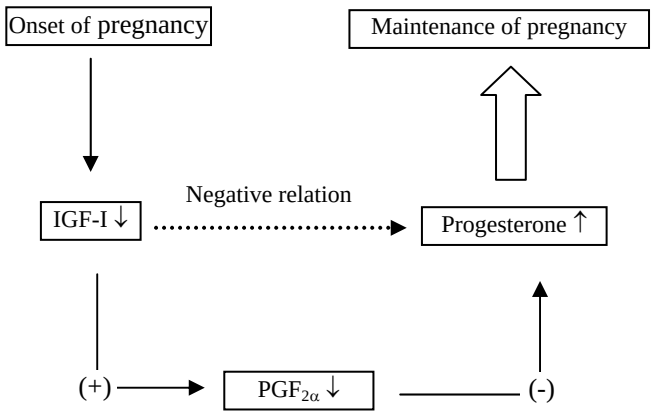

Figure 3. The possible process of serum IGF-I to up-regulate the P_4 expression for maintenance of pregnancy.

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REFERENCES

Apa, R., F. Miceli, E. Pierro, F. Minici, P. Navarra, A. Caruso, M. Napolitano, S. Mancuso and A. Lanzone. 1999. Paracrine regulation of insulin-like growth factor-I (IGF-I) and IGF-II on prostaglandins $F_{2\alpha}$ and E_2 synthesis by human corpus luteum in vitro: a possible balance of luteotropic and luteolytic effects. J. Clin. Endocrinol. Metab. 84:2507-2512.

Apa, R., N. Di Simone, E. Ronsisvalle, F. Miceli, D. de Fed, A. Caruso, A. Lanzone and S. Mancuso. 1996. Insulin-like growth factor (IGF)-I and IGF-II stimulate progesterone production by human luteal cells: role of IGF-I as mediator of growth hormone action. Fertil. Steril. 66:235-239.

Chen, S. H., V. Zanagnolo, S. Preutthipan, K. P. Roberts, S. B. Goodman and A. M. Dharmarajan. 1997. The role of insulin-like growth factor-I (IGF-I) and estradiol in rabbit corpus luteum progesterone production. Endocrine 6:73-77.

Constantino, C. X., P. L. Keyes and J. L. Kostyo. 1991. Insulin-like growth factor-I stimulates steroidogenesis in rabbit luteal cells. Endocrinology 128:1702-1708.

Csapo, A. I., M. O. Pulkkinen and W. G. Wiest. 1973. Effects of lutectomy and progesterone replacement therapy in early pregnant patients. Am. J. Obstet. Gynecol. 115:759-765.

Daughaday, W. H., I. K. Mariz and S. L. Blethen. 1980. Inhibition of access of bound somatomedin to membrane receptor and immunobinding sites: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acidic-ethanol-extracted serum. J. Clin. Endocrinol. Metab. 51:781-788.

Einspanier, R., A. Miyanoto, D. Schams, M. Muller and G. Brem. 1990. Tissue concentration, RNA expression and stimulation of IGF-I in luteal tissue during the oestrus cycle pregnancy of cows. J. Reprod. Fertil. 90:439-445.

Hashizume, T., K. Ohtsuki and N. Matsumoto. 2000. Serum insulin-like growth factor-I concentrations increase during the estrous phase in goats. Domest. Anim. Endocrinol. 18:253-263.

Kapur, S., H. Tamada, S. K. Dey and G. K. Andrews. 1992. Expression of insulin-like growth factor-I (IGF-I) and its receptor in the peri-implantation mouse uterus, and cell-specific regulation of IGF-I gene expression by estradiol and progesterone. Biol. Reprod. 46:208-219.

Khan-Dawood, F. S., R. Gargiulo and M. Dawood. 1994. In vitro microdialysis of the ovine corpus luteum of the ovine corpus luteum of pregnancy: effects of insulin-like growth factor on progesterone secretion. Biol. Reprod. 51:1299-1306.

Lee, C. Y. and D. M. Henricks. 1990. Comparisons of various acidic treatments of bovine serum on insulin-like growth factor-I immunoreactivity and binding activity. J. Endocrinology 127:139-148.

Lievermann, J., D. Schams and A. Miyamoto. 1996. Effects of local growth factors on the secretory function of bovine corpus luteum during the oestrus cycle and pregnancy *in vitro*. Reprod. Fertil. Dev. 8:1003-1011.

Lucy, M. C., W. W. Thatcher, R. J. Collier, F. A. Simmen, Y. Ko, J. D. Savio and L. Badinga. 1995. Effects of somatotropin on the conceptus, uterus, and ovary during maternal recognition of pregnancy in cattle. Domest. Anim. Endocrinol. 12:73-82.

Luigi, D., K. Paulina, C. Olga, V. Margarita, L. T. Jose and C. Eduardo. 1995. Multihormonal regulation of progesterone synthesis in cultured human midluteal cells. J. Clin. Endocrinol. Metab. 80:1566-1570.

McWilliam, R., R. E. Leake and J. R. Coutts. 1995. Growth factors in human ovarian follicle fluid and growth factor receptors in granulosa-luteal cells. Int. J. Biol. Markers. 10:216-220.

Nicholson, W. C., Z. Ge, D. M. Plotner, C. E. Farin and J. E. Gadsby. 1999. Insulin-like growth factor (IGF)-I, IGF-I receptor, and IGF binding protein-3 messenger ribonucleic acids and protein in corpora lutea from prostaglandin $F_{2\alpha}$-treated gilts. Biol. Reprod. 61:1527-1534.

Rotwein, P. 1991. Structure, evolution, expression and regulation of insulin-like growth factors I and II. Growth Factors 5:3-18.

Sarvas, K., M. Angervo, R. Koistinen, A. Tiiinen and M. Seppala. 1994. Prostaglandin $F_{2\alpha}$ stimulate release of insulin-like growth factor binding protein-3, from cultured human granolos-luteal cells. Horm. Reprod. 1643-16496.

SAS. 1995. SAS/STAT Software for PC. Release 6.11, SAS Institute, Cary, NC, USA.

Sayre, B. L., R. Traft, E. K. Inskeep and J. Killefer. 2000. Increased expression of insulin-like growth factor binding protein-1 during induced regression of bovine corpora lutea. Biol. Reprod. 63:21-29.

Shuichi, K., M. Akio, B. Bajaram and S. Dieter. 2001. Growth hormone, but not luteinizing hormone, acts with luteal peptides on prostaglandin F2α and progesterone secretion by bovine copora lutea *in vitro*. Prostaglandins Other Lipid Mediat. 63:79-92.

Stewart, R. E., L. J. Spicer, T. D. Hamilton and B. E. Keefer. 1995. Effects of insulin-like growth factor I and insulin on proliferation and on basal and luteinizing hormone-induced steroidogenesis of bovine thecal cells: involvement of glucose and receptors for insulin-like growth factor I and luteinizing hormone. J. Anim. Sci. 73:3719-3731.

Tamada, H., H. Kitashin, T. Sawada and J. Mori. 1995. Expression of insulin-like growth factor I mRNA in rat luteal tissue increases with functional regression of the corpus luteum. Prostaglandin 50:151-160.

Watson, A. J., M. E. Westhusin and Q. A. Winger. 1999. IGF paracrine and autocrine interactions between conceptus and oviduct. J. Reprod. Fertil. Suppl. 54:303-315.

Yuan, W. and M. C. Lucy. 1996. Effects of growth hormone, prolactin, insulin-like growth factors, and gonadotropins on progesterone secretion by porcine luteal cells. J. Anim. Sci. 74:866-872.

Yun, J. S., W. J. Kang, D. S. Seo, S. S. Park, K. C. Hong, C. Y. Lee and Y. Ko. 2000. Association of endocrine factors (insulin-like growth factor-II and binding protein-3) with litter size in pigs. Asian-Aust. J. Anim. Sci. 14:307-315.

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