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研究成果の概要(和文)：本研究では、牛の初期胚 - 子宮相互作用を調節するメカニズムについて検討した。以下の項目を明らかにした。1) 上皮成長因子(EGF)は主に子宮内腔上皮細胞によって産生され、ウシ胚では産生されなかった。2) ウシ子宮内膜のEGFタンパク発現は、着床時期(中期黄体期)のほうが初期及び退行期黄体期と比較して高かった。3) 子宮内膜上皮細胞は間質細胞より多くのEGFを産生する。4) EGFは、子宮上皮細胞および間質細胞において、黄体維持因子であるプロスタグランジンE2の分泌を刺激する能力を持つ。5) EGFにより、ウシの妊娠認識因子インターフェロン- τ の産生が刺激されることで妊娠成立する。

研究成果の概要(英文)：The understanding of the interaction between the early embryos and uterine environment is crucial to reduce the embryonic loss in domestic animals. The present research revealed the mechanisms of action of embryonic and uterine products on bovine uterus during implantations and their interactions.

1) We found that epidermal growth factor (EGF) is mainly produced by uterine luminal cells but not by bovine embryos. 2) EGF protein in bovine endometrium was higher around the time of implantation compared with other luteal stages. 3) Cultured endometrial epithelial cells produce more EGF than endometrial stromal cells. 4) EGF has the ability to increase luteolytic (prostaglandin F₂ α) and luteotropic (prostaglandin E₂) in both epithelial and stromal cells. 5) Endometrium contains more EGF around the time of implantation. This growth factor stimulates the production of interferon- τ , the pregnancy recognition factor in cattle, to ensure the establishment of pregnancy.

研究分野：家畜生産科学

キーワード：Bovine Embryo Endometrium Epidermal Growth Factor Prostaglandin Implantation

1. 研究開始当初の背景

Early embryonic mortality is one of the major causes of reproductive failure and causes considerable economic loss to the livestock industry. Reduced pregnancy rate results in slower genetic improvement and substantial economic loss to dairy and beef production. Japanese livestock economy is seriously influenced by embryonic wastages and hence its control is the greatest concern for scientists and farmers. The survival of embryo during early embryonic development depends on the establishment of the maternal recognition of pregnancy (MRP). Impaired communication between the conceptus and the maternal uterine environment leads to early embryonic mortality. In ruminants, the principal signal for MRP is identified to be interferon τ (IFN τ) secreted by the trophoectoderm between days 8 and 20 of pregnancy (Mol Reprod Dev. 2009; 76: 1143-1152). IFN τ exerts antiluteolytic action by suppressing the normal pattern of pulsatile release of prostaglandin F 2α (PGF) at the late luteal stage. PGF is well recognized as the main luteolytic agent in ruminants, whereas PGE 2 has luteotropic and luteoprotective properties (Endocrinology 2004; 145: 5280-5293). The understanding of the complex interaction between the conceptus and its maternal environment including the control of luteotropic prostaglandin E 2 (PGE 2) and luteolytic PGF 2α will facilitate the development of optimal reproductive management and strategies to ameliorate the early embryonic losses.

To investigate the local production of prostaglandins (PGs) by the uterus and the ovary, catheters for frequent blood collection were implanted into the uterine vein, ovarian vein and jugular vein to collect simultaneous,

real-time information on the ovarian uterine and systemic changes of factors regulating ovarian and uterine function *in vivo*. Furthermore, tissue and cell culture model for evaluating the effects of cytokines, prostaglandins and ROS in bovine corpus luteum *in vitro* has been established. Using the above experimental models the principal investigator have demonstrated the following major points:

- 1) Glucocorticoids (GCs) regulate bovine reproductive functions in cows (Research achievement, 2) Cortisol may play roles in preventing excessive uterine PGF production and protecting the corpus luteum against luteolysis, 3) Daily intravaginal application of cortisol between from Day 15 to 18 after artificial insemination increased pregnancy rates compared with that of untreated controls, 4) Treatment of cycling cows with metyrapone, an inhibitor of HSD11B1, reduce the local conversion of cortisol from cortisone at the late luteal stage prolonged the luteal phase. 5) Bovine reproductive tract (uterus and/or vagina) has the capacity to convert biologically inactive cortisone to active cortisol, and that intravaginal application of cortisone increased plasma concentrations of cortisol between 0.5 and 1 h and reduce PGF concentrations. These findings suggest that GCs play crucial roles in regulating uterine PGF secretion. However, it is unknown how cortisol affects uterine PGF production throughout the estrous cycle in cattle, especially at the late luteal stage and during the maternal recognition of pregnancy. The results of the present research will provide novel insights into the mechanism of pregnancy establishment and the regulation

of prostaglandins (PGs) synthesis. Epidermal growth factor (EGF) acting through EGF receptor may have critical function in the establishment of pregnancy and cortisol have crucial roles in modulating bovine conceptus elongation. In the present study, we attempt to characterize the molecular, paracrine and endocrine interaction among the conceptus the endometrium and the corpus luteum by determining the genes and protein expressions for EGF, glucocorticoids (GCs) and prostaglandin synthesis. The actions of EGF and cortisol on conceptus elongation as well as on bovine uterine and CL function during the estrous cycle and early pregnancy will be clarified.

2 . 研究の目的

Early embryonic mortality is one of the major causes of reproductive failure and causes considerable economic loss to Japanese livestock. Impaired communication between the conceptus and the maternal uterine environment leads to early embryonic mortality. The understanding of the interaction between the conceptus and embryonic environment will facilitate the development of new strategies to reduce the early embryonic loss. The present research aimed to reveal the mechanisms of actions of embryonic products of bovine uterus during implantation and their interactions.

3 . 研究の方法

To investigate the cellular localization and physiological roles of epithelial growth factor (EGF) on prostaglandin $F2\alpha$ ($PGF2\alpha$) and prostaglandin $E2$ ($PGE2$) production in bovine endometrial cells, the expression of EGF protein and EGF receptors in bovine endometrium

throughout the estrous cycle was analyzed. The effects of EGF on PGF and $PGE2$ production were examined in luminal epithelial and stromal cells after 24 h of culture. We found that both types of cells express EGF receptors and respond to EGF treatment by increasing $PGE2$ rather than PGF production.

a) Semi-quantitative PCR was performed to identify the exact source of EGF at different stages of the estrous cycle.

b) Gene expressions of EGF and their receptors were analyzed by Real-time PCR and protein expression was determined by Western Blot. The effects of EGF on PGs production was evaluated in cultured endometrial cells and blastocysts.

We validated an *in vitro* co-culture system to study embryo-uterine interactions and secretion of luteotropic and luteolytic factors. The quality of embryos produced *in vivo* was evaluated morphologically and then cultured in the presence of EGF (1, 10 and 100 nM) for 24 hours. The capacity of embryos to produce $IFN\tau$ was also evaluated *in vitro*. The capacity of embryos to produce $IFN\alpha$ *in vitro* may be a useful indicator to predict pregnancy in cattle.

4 . 研究成果

Experimental evidences suggest that HB- EGF, prostaglandins (PGs) and interferon- τ ($IFN\tau$) regulate uterine function for pregnancy establishment in ruminants. In this study, the mRNA expressions of HB-EGF, ErbB1 and ErbB4 in bovine endometrium and the effects of HB-EGF and $IFN\tau$ on $PGE2$ and $PGF2\alpha$ production by endometrial cells were investigated. RT-PCR analysis revealed that HB-EGF mRNA was greater at the mid-luteal stage than at the early and regressed luteal

stages ($p < 0.05$). ErbB1 mRNA expression was greater at the mid- and late luteal stages than at the other luteal stages ($p < 0.05$). IFN- τ increased the expression of HB-EGF, ErbB1 and ErbB4 mRNA in epithelial cells ($p < 0.05$). HB-EGF did not affect PGF2 α or PGE2 production by bovine endometrial epithelial cells, but increased PGF2 α and PGE2 production by bovine endometrial stromal cells ($p < 0.05$). IFN τ significantly decreased HB-EGF-stimulated PGF2 α ($p < 0.05$), but not PGE2 ($p > 0.05$) production by stromal cells. These results indicate that HB-EGF and its receptors expression changed in bovine endometrium throughout the oestrous cycle. IFN τ increased their expression in cultured endometrial cells. HB-EGF and IFN τ have the ability to regulate PGs production by stromal cells and therefore may play a role in the local regulation of uterine function at the time of implantation in cattle.

Epidermal growth factor (EGF) is produced in bovine endometrium throughout the estrous cycle. However, little is known about the expression of EGF receptor (EGFR) and the roles of EGF in bovine endometrium. To clarify whether EGF is involved in local regulation of bovine endometrial function, first, we determined the EGF protein and the expression of *EGFR* mRNA in endometrial tissues throughout the luteal stages. EGF protein concentration was higher ($P < 0.05$) at the mid (Days 8-12) luteal stage than at the other luteal stages. *EGFR* mRNA expression was higher ($P < 0.05$) at the mid and late (Days 15-17) luteal stages than at the other luteal

stages. To investigate the protein concentrations of EGF and *EGFR* mRNA expression in cultured bovine endometrial cells, epithelial and stromal cells, were isolated between Day 0 and Day 4 post-ovulation from 22 uteri. Both EGF protein concentration and *EGFR* mRNA expression were higher ($P < 0.05$) in epithelial cells than in stromal cells. Then, to examine the possible role of EGF in the regulation of prostaglandin F2 α (PGF2 α) and prostaglandin E2 (PGE2), cultured endometrial epithelial and stromal cells were exposed to EGF (0, 1, 10 and 100 nM) for 24 h. In epithelial cells, EGF (10 and/or 100 nM) increased ($P < 0.05$) PGF2 α and PGE2 secretion, but in stromal cells EGF (100 nM) increased ($P < 0.05$) PGF2 α , but not PGE2 secretion. These results indicate, that 1) the highest amount of EGF is produced by bovine endometrium at the mid-luteal stage, 2) endometrial *EGFR* mRNA expressions are higher at mid and late-luteal stages than other stages, 3) EGF is expressed mainly by uterine epithelial cell and 4) EGF has the ability to increase PGE2 and PGF2 α production in both epithelial and stromal cells and therefore may play a role in local regulation of uterine function.

本研究で得られた成果は既に国内外の専門学雑誌に発表済みとなっている。受精卵及び子宮内環境を解明することによって、家畜の効率的生産のための新たな生殖制御方法開発に重要な基礎飼料を提供するばかりでなく、不妊の診断やその治療法確立のための基礎研究としても意義がある。

5 . 主な発表論文等

(研究代表者、研究分担者及び連携研究者には下線)

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6. 研究組織

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