The 5th SRD-KSAR Joint Symposium

“Reproductive Biology in the Next Decade”

September 9 (Wed), 2009, 12:15–16:30
Kinki University, School of Agriculture, Nara, Japan

Organizers:

Prof. **Noboru MANABE** (Chief Director of SRD)

Prof. **Jong Taek YOON** (President of KSAR)

Program

Registration

11:30–

Opening Remarks

12:15–12:25 Prof. **Akira IRITANI** (Kinki University, Japan)

12:25–12:35 Prof. **Kyung-Soon IM** (Seoul National University, Republic of Korea)

Symposium Session

Chair persons: **Noboru Manabe** (The University of Tokyo, Japan)
Hoon-Taek LEE (Konkuk University, Republic of Korea)

12:40-13:05 **"Genetic clues of premature ovarian failure"**
Youngsok CHOI (CHA University, Republic of Korea)

13:05-13:30 **"Regulation of growth hormone pulsatility in female goats: modulation by sex steroids and involvement of neuropeptide Y"**
Tomohiro YONEZAWA (Kitasato University, Japan)

Chair persons: **Yoshihisa UENOYAMA** (Nagoya University, Japan)
Jeong-Mook LIM (Seoul National University, Republic of Korea)

13:30–13:55 **"Factors affecting successful implantation and maintenance of pregnancy at the maternal-fetal interface in pigs"**
Hakhyun KA (Yonsei University, Republic of Korea)

13:55–14:20 **"Neuroendocrine control of the pulsatile GnRH/LH secretion in ruminants"**
Satoshi OHKURA (Nagoya University, Japan)

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Chair persons: **Takashi NAGAI** (National Institute of Livestock and Grassland Science, Japan)
Youngsok CHOI (CHA University, Republic of Korea)

14:50–15:15 **"Developmental potential and reprograming efficiency of porcine embryos cloned with mesenchymal stem cells"**
Gyu-Jin RHO (Gyeongsang National University, Republic of Korea)

15:15–15:40 **"Renewal of techniques related to embryo transfer in cattle – a novel superovulation method using aluminum hydroxide gel"**
Koji KIMURA (National Institute of Livestock and Grassland Science, Japan)

Chair persons: **Kiyoshi OKUDA** (Okayama University)
Gyu-Jin RHO (Gyeongsang National University, Republic of Korea)

15:40–16:05 **"The ovary, as the source of stem cell"**
Jeong-Mook LIM (Seoul National University, Republic of Korea)

16:05–16:30 **"A novel concept for the bovine luteolytic cascade with a focus on luteal blood flow and vasoactive factors"**
Komei SHIRASUNA (Obihiro University, Japan)

Closing Remark

16:30– Prof. **Yukio TSUNODA** (Kinki University, Japan)

Genetic clues of premature ovarian failure

Youngsok CHOI*

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Premature ovarian failure (POF) is defined as a primary ovarian dysfunction characterized by depletion of ovarian follicles before menopause in women. POF has an estimated prevalence of one in 10,000 by age 20, one in 1000 by age 30, and one in 100 by age 40. POF is able to be heritable in up to 30% of patients. POF results from both non-genetic and genetic problem. Non-genetic causes include viral infection, gonadotoxic chemotherapy, radiation treatment, and autoimmune disorder. However, in most case POF is idiopathic and probably genetic. Two chromosome abnormalities such as Turner and fragile X-syndrome are known to be associated with POF. In addition single-gene-mutation related to POF include Fragile X mental retardation 1 (FMR1), forkhead box L2 (FOXL2), POF1B, inhibin alpha (INHA), growth differentiation factor 9 (GDF9), bone morphogenetic protein (BMP15) and follicle stimulating hormone receptor (FSHR). However, the genetic mechanism of POF is poorly understood. Recently Rajcovic laboratory added two oocyte-specific transcriptional factors, newborn homeobox gene (NOBOX) and factor in germline alpha (FIGLA), as candidate gene for premature ovarian failure in human. Mouse Nobox is exclusively expressed in oocytes and encode a homeobox transcriptional regulator. Disruption of mouse Nobox gene causes nonsyndromic ovarian failure in female mice, whereas males are unaffected. Nobox regulates several oocyte and germ cell-specific genes, *Gdf9* and *Pou5f1* (Known as *Oct4*), through binding specific elements (TAATTG) on their promoters. In human, NOBOX is preferentially expressed in the oocyte from primordial follicle through metaphase II oocytes. A missense mutation of NOBOX, p.Arg355His, found in one of POF patient can disrupt NOBOX binding affinity to the element (TAATTG). FIGLA, a basic helix-loop-helix (bHLH) transcriptional factor, is expressed in the embryonic gonad. Figla regulates expression of zona pellucida genes as well as that of other oocyte-specific genes. Like other bHLH transcriptional factors, Figla binds to a specific element, E-box (CANNTG). Figla forms heterodimer with transcription factor 3 (TCF3) to regulate the zona pellucida gene promoters. Figla deficiency loses oocytes rapidly after birth, whereas male gonads are unaffected. A deletion c.419-421 delACA (p.140 delN) in human POF patient was found. The deletion disrupted FIGLA binding to TCF3. These finding shows that mutations in oocyte or germ cell-specific genes in women contribute to pathology of POF through diverse pathways. The addition of two transcriptional regulators as contributors to the etiology of POF might be providing better understanding POF as well as genetic clues of POF.

Regulation of growth hormone pulsatility in female goats: modulation by sex steroids and involvement of neuropeptide Y

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Growth hormone (GH) is secreted in a pulsatile manner, the pattern of which plays an important role in the regulation of growth and metabolism. The Shiba goat, a Japanese miniature goat, is a suitable experimental model for the analysis of GH pulsatility, because they have strictly regular GH pulses. In female Shiba goats, the GH pulse frequency and amplitude in the follicular phase were significantly larger than those in the late luteal phase. Both insulin-like growth factor (IGF)-I and free fatty acid levels in the plasma were higher in the follicular phase than the luteal phase. Estrogen treatment to ovariectomized goats increased the GH pulse amplitude, whereas progesterone treatment decreased it. These observations suggest that the pulsatile pattern of GH secretion in female goats varies with sex steroid levels and thereby affects IGF-I secretion and lipolysis during the estrous cycle. Although GH secretion was classically believed to be modulated by the opposing actions of two hypothalamic peptides, GH-releasing hormone (GHRH) and somatostatin (SRIF), recent studies suggest more complex mechanisms. When GH levels in the peripheral circulation and GHRH, SRIF and neuropeptide Y (NPY) levels in the cerebrospinal fluid (CSF) in ovariectomized goats were simultaneously measured, there was a significant negative cross-correlation and negative synchronicity between GH and NPY profiles, while there was no correlation between GH and GHRH or GH and SRIH profiles. In addition, intracerebroventricular infusion of NPY suppressed GH secretagogue-induced GH release. We further observed that estrogen treatment significantly decreased NPY levels in the CSF. From these observations, we currently hypothesize that the periodic decrease in NPY levels is involved in the generation of GH pulses in goats, and that the modulation of GH pulsatility by sex steroids is also mediated at least partially by NPY.

Factors affecting successful implantation and maintenance of pregnancy at the maternal-fetal interface in pigs

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Implantation process requires a well coordinated interaction between the maternal uterus and the developing embryo in pigs. Embryo implantation in pigs occurs on around Day (D) 12 of pregnancy. During this period, conceptus undergoes a dramatic morphological change and secretes various biological products such as estrogens, proteases, and interferons. Estrogens produced by conceptuses act as the signal for maternal recognition of pregnancy, and the mechanism of estrogen action is explained by the endocrine and exocrine theory. The uterine endometrium becomes receptive to the conceptus by changing cell adhesion molecules, polarizing epithelial cells and increasing secretory activity. Some changes of uterine activity are affected by the ovarian hormone, progesterone, but the presence of conceptus in the uterus also induces changes of endometrial functions, including most importantly maternal recognition of pregnancy. Many factors, such as hormones, cytokines, enzymes, extracellular matrix proteins, and transport proteins are reported to be present at the maternal-fetal interface and function in the establishment of pregnancy in pigs. However, understanding of the cellular and molecular events occurring in the endometrium is not complete. In a recent study that analyzed genes expressed in the endometrium of D12 of pregnancy comparing with those of D12 the estrous cycle to identify the genes that were differentially expressed and some subsequent studies, we made observations that some genes might be critical for the establishment and maintenance of pregnancy. Firstly, we found that calcium regulatory molecules TRPV6 and S100G were dynamically regulated in the uterine endometrium during pregnancy, suggesting that regulation of calcium ion concentration may important for the embryo implantation and the maintenance of pregnancy. Secondly, we observed that salivary lipocalin (SAL1), a lipid-binding protein, was uniquely expressed in the uterine endometrium at the time of embryo implantation, and its expression was regulated by estrogen. Thirdly, we found that lysophosphatidic acid (LPA) was present at the maternal-and fetal interface at the time of implantation and LPA receptor 3 was uniquely expressed in the endometrium during early pregnancy. Further analysis of those molecules will provide insights into the cellular and molecular basis of maternal-and fetal interaction during pregnancy in the pig. More details will be discussed in the presentation. [This work was supported by the BioGreen 21 Program (#20070301034040 and #20080401034003), Rural Development Administration, Republic of Korea]

Neuroendocrine control of the pulsatile GnRH/LH secretion in ruminants

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Pulsatile release of gonadotropin-releasing hormone (GnRH) is indispensable to maintain normal gonadotropin secretion, which in turn regulates the gonadal activity in mammals. The pulsatile secretion of GnRH is associated with synchronized electrical activity in the medial basal hypothalamus— multiple unit activity (MUA)— which is thought to reflect the rhythmic oscillations in the activity of the neuronal network that drives pulsatile GnRH secretion, the GnRH pulse generator. However, the cellular source of this ultradian rhythm in GnRH-releasing activity is totally unknown. The aim of the present paper is to provide an overview of our current understanding of the GnRH pulse generator, the longstanding "black box" mechanism, in goats. Our main focus is to identify where is the location, and what are the components of this hypothalamic mechanism that governs pulsatile release of GnRH.

Kisspeptin (also known as metastin) is the endogenous ligand for the G protein-coupled receptor, Kiss1r (now the term Kiss1r was assigned for the non-human kisspeptin receptor), and there is growing evidence that kisspeptin/Kiss1r system in the brain is involved in the control of reproductive functions. In the hypothalamus of male goats, kisspeptin -immunoreactive cell bodies were exclusively found in the arcuate nucleus (ARC), the neural substrate implicated in the neuroendocrine control of GnRH pulses, suggesting that hypothalamic kisspeptin plays an important role in the regulation of GnRH release in this species. Thus, direct input from the ARC kisspeptin neurons to GnRH cell bodies in the medial preoptic area (MPOA) or their terminals in the median eminence could be the intrinsic source for driving the GnRH pulse generator. To determine whether the kisspeptin signalling could be responsible for producing pulsatile GnRH secretion, we studied male goats, measured plasma levels of luteinizing hormone (LH) and recorded MUA aimed at the posterior ARC, where the majority of kisspeptin neuronal cell bodies are located. Rhythmic volleys of MUA were found to be accompanied by LH pulses with regular intervals in the posterior ARC, where kisspeptin neuronal cell bodies were found. Exogenous intravenous administration of kisspeptin stimulated a sustained increase in LH secretion, without influencing MUA, suggesting that the GnRH pulse generator, as reflected by MUA, originated from outside of the network of GnRH neurons— and could plausibly reflect the pacemaker activity of kisspeptin neurons, whose projections reach the median eminence where GnRH fibers project. These observations suggest that the kisspeptin neurons in the ARC may be the intrinsic source of the GnRH pulse generator.

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Developmental potential and reprograming efficiency of porcine embryos cloned with mesenchymal stem cells

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Errors leading to inappropriate expression could arise at any of the levels at which regulation of gene expression occurs, including the organization of the nucleus, chromatin structure and the availability of regulatory molecules. Studies on cloned embryos during preimplantation development have revealed striking defects to faithfully recapitulate many of the essential early events including transcription/translation, epigenetic modifications such as DNA methylation, histone acetylation and chromatin configuration as well as genetic imprinting. In support of these observations, embryos produced by nuclear transfer (NT) consistently displayed a myriad of developmental abnormalities caused by aberrant expression pattern of genes involved in early development, maintenance of pluripotency, epigenetic reprogramming and apoptosis regulation. Altered levels of expression have also been detected in NT embryos when compared to their in vitro and in vivo counterparts. However, the amount of information gathered on the profile of gene expression in porcine NT preimplantation embryos is still scarce and limited to a handful of genes. One of the factors in determining the efficacy of NT might be the type of donor cells. The oocytes reconstructed from embryonic stem (ES) cells gave rise to an increase in the number of viable offspring compared with somatic cells in mice, and a less differentiated cell type may support greater development of NT embryos compared with terminally differentiated cell types. The nucleus of an undifferentiated cell may require less reprogramming for animal cloning than the genetic material of a differentiated somatic cell. Hence, a comparison of efficacy among donor cells between undifferentiated and differentiated mesenchymal stem cells (MSCs) would be interesting to improve the efficiency.

We found that MSCs as donors in NT offer a greater potential to enhance blastocyst formation, higher total cell number and low incidence of apoptosis compared to somatic cells. Alterations in the expression pattern of genes implicated in transcription and pluripotency (*Oct4*, *Stat3*, *Nanog*), DNA methylation (*Dnmt1*, *Dnmt3a*), histone deacetylation (*Hdac2*), growth factor signaling and imprinting (*Igf2*, *Igf2r*) and apoptosis (*Bax*, *Bcl2*) regulation were observed in NT embryos. The expression of transcripts in MSC-NT embryos more closely followed that of the in vivo derived embryos compared with FF-NT embryos. The in vitro developmental ability of NT embryos derived from undifferentiated MSCs was higher than those from differentiated MSCs or somatic cells.

In conclusion, MSCs with a relatively undifferentiated genome might serve as suitable donors that could be more efficiently reprogrammed to re-activate expression of early embryonic genes in porcine NT.

Renewal of techniques related to embryo transfer in cattle – a novel superovulation method using aluminum hydroxide gel

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In the past two decades, embryo transfer (ET) of cattle has become a commonly used reproductive technique in Japan. However, the pregnancy rate of ET has never been improved, as it has constantly approximated 50%. On the other hand, many new findings and innovations were established during this period not only in reproductive biology but also in machine engineering and materials science. Over the next decade, ET and its related techniques should be reconsidered and improved using these new findings with the aim of increasing the pregnancy rate of ET.

According to the latest investigation, approximately 20,000 calves are produced using ET every year in Japan. Although it is possible to use IVF embryos for ET, more than 80% of ET calves are produced using in vivo-derived embryos. In order to obtain high numbers of in vivo-derived embryos, superovulation (SOV) is usually induced in which FSH is administered to donor cattle in a step-down regime for 3 to 4 days to induce multiple follicular growth and ovulation. This conventional SOV method is both time and labor intensive, and moreover, it can cause great stress in the cattle.

Recently, various types of adsorbants for macromolecules such as proteins or complex medicines have been developed, allowing the chronic release of these macromolecules into the blood stream. Aluminum hydroxide gel (Al-gel) can adsorb macromolecules such as proteins or toxoids and release them gradually. Accordingly, Al-gel is extensively used as an adsorbent or adjuvant for vaccines. Therefore we investigated whether Al-gel could adsorb and release FSH effectively *in vitro* and *in vivo*, and whether a single administration of FSH in Al-gel could successfully induce SOV in cattle.

In the first experiment, it was investigated whether aluminum hydroxide gel could adsorb FSH and release it in the presence of BSA, which is a major protein of interstitial fluid. When the gel was mixed with FSH and centrifuged, the supernatant was recovered. Then the precipitated gel was re-suspended in PBS containing 1% BSA. The solution was centrifuged, and the supernatant was recovered again. The concentrations of FSH in both supernatants were measured by RIA. Almost all of the FSH mixed with the gel was absorbed (99.9%), and more than 70% of the absorbed FSH was released in the presence of BSA, which is a major protein of bovine interstitial fluid.

The aim of the next experiment was to identify whether a single injection of pFSH mixed with Al-gel could induce multiple follicles to grow, ovulate and ultimately produce viable embryos and

to compared this result with that of the conventional 4-day serial injection method. Japanese Black cows were given single injections of 30 mg (AU) pFSH with 5 mL Al-gel (3mg Al/mL) intramuscularly (n=10) or multiple injections of pFSH (control; n=13) at 12 hr intervals for 4 days (6, 6, 4, 4, 3, 3, 2 and 2 mg in saline). Estrus was induced in all cows by using 750 μg of cloprostenol, and the animals were inseminated. The resulting embryos were recovered and classified according to IETS guidelines. At the same time, blood samples were collected from some of the treated cows at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84 and 96 h after the initiation of treatment to measure the concentration of FSH in plasma. The mean numbers of CLs and collected and transferable (grades 1-2) embryos were 12.3 ± 2.7, 10.0 ± 2.5 and 8.6 ± 2.3/cow with the Al-gel single administration versus 11.7 ± 1.8, 9.3 ± 1.7 and 8.0 ± 1.8/cow with the standard multiple administrations, respectively. No significant differences were found between the two methods for any of the measurements. In the case of the single administration of pFSH in Al-gel, pFSH was not detected in the circulation during the first 2 h after administration. The subsequent concentration of pFSH rapidly increased to a peak at 12 h post-administration and gradually decreased thereafter. On the other hand, in the case of multiple administrations, pFSH was detected in the peripheral blood stream immediately after the start of treatment, reached a peak at 6 h and then decreased.

Al-gel is widely used as an adjuvant for vaccinations. Therefore, it was anticipated that repeated administrations of pFSH with the gel would induce immune responses against this hormone and that, consequently, the cattle would not respond to SOV treatment again. Cows were subjected to five successive SOV treatments at 2- to 3-month intervals over a year. However, the successive SOV treatments using Al-gel did not result in a significant decrease in the number of CLs, recovered eggs or recovered transferrable embryos among the SOV periods.

Our results suggested that Al-gel could adsorb and release pFSH effectively *in vitro* and *in vivo* and that a single administration of FSH in Al-gel could successfully induce SOV in cattle.

The ovary, as the source of stem cell

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It has generally been accepted that the utilization of the stem cells that have either multipotent or pluripotent activity is the key factor for developing cutting-edge technologies for cell replacement, organ regeneration, tissue engineering and rejuvenation therapy. Extensive and continuous efforts have been made to establish histocompatible stem cells to date. At the very early stage, a technology to transfer a somatic cell into an oocyte being enucleated, named somatic cell nuclear transfer (SCNT), was suggested for the derivation of patient-specific stem cells (1). However, R & D activity on SCNT definitely accompanies with sacrificing of huge amount of viable oocytes and embryos, which incurs great concern on abusing of human oocytes, as well as the concern on reproductive cloning of the human (2,3). To overcome such limitation, parthenogenetic activation of human oocytes, interspecies nuclear transfer of human somatic cells into animal oocytes and alternative technique of nuclear transfer for generating unimplantable embryos have been subsequently suggested (4-6).

We have additionally sought for other alternatives and our effort has focused to develop clinically feasible technology with minimizing of ethical issue. We have selected the research using ovarian tissue based on our previous experience of research. Considering on general procedure of drug discovery and development, however, the boundary of our research has restricted within animal model experiment at present. We have attempted to derive histocompatible ESC from inactivated germline cells. In mammalian ovaries, there exist numerous immature follicles called preantral (primordial, primary and secondary) follicles in the ovaries. In one's human life, extremely small number of follicles, which is as less as 1% of total number, can develop into the Graafian follicles releasing developmentally competent, mature oocytes into the fertilization site (7). Most immature follicles gradually disappeared after birth and except for mobilized follicles, the rest remaining 'developmentally dormant' in the ovarian cortex or medullary tissues finally became degenerated via apoptosis. There has been no attempt to establish histocompatible embryonic stem cell (ESC) by culturing of preantral follicles, which avoids the use of developmentally competent oocytes for stem cell establishment. We, therefore, have attempted to develop a technique for in vitro-culture of preantral follicles. To retain autologous ESC, parthenogenetic activation of oocytes matured in in vitro-cultured follicles was employed.

As results, we established histocompatible stem cells from the culture of preantral follicles at the secondary stage. Also, we can derive histocompatible ESC and viable blastocysts from adult ovarian tissue and the culture of primary follicles. Follicle bank system was established by

comparison of different freezing protocols. A technique for preantral follicle culture can be employed for reproductive medicine and cancer therapy and considerable number of preantral follicles can be retrieved from the patients suffering from the infertility of uterine factor and from the cancer patients scheduled for chemotherapy. Accordingly, the preantral follicle culture contributes to developing various therapeutic technologies.

References

1. French AJ, Adams CA, Anderson LS, Kitchen JR, Hughes MR, Wood SH. Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts. Stem Cells 2008;26:485-493.
2. Taupin P. Stem cells engineering for cell-based therapy. J Neural Eng 2007;4:59-63.
3. Mertes H, Pennings G. Oocyte donation for stem cell research. Hum Reprod 2007;22:629-634.
4. Kim K, Lerou P, Yabuuchi A et al. Histocompatible embryonic stem cells by parthenogenesis. Science 2007;315:482-486.
5. Chang KH, Lim JM, Kang SK, Lee BC, Moon SY, Hwang WS. An optimized protocol of a human-to-cattle interspecies somatic cell nuclear transfer. Fertil Steril 2004;82:960-962.
6. Meissner A, Jaenisch R. Generation of nuclear transfer-derived pluripotent ES cells from cloned Cdx2-deficient blastocysts. Nature 2006;439:212-215.
7. Moore KL, Persaud V. Before we are born; Essentials of embryology and birth defects, 6th ed. Philadelphia: Saunders, 2003;10-26.

A novel concept for the bovine luteolytic cascade with a focus on luteal blood flow and vasoactive factors

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The corpus luteum (CL) undergoes drastic changes in its function and structure during the estrous cycle. If pregnancy does not occur successfully, luteolysis is caused by prostaglandin F2α (PGF2α) released from the endometrium around days 17-19 of the estrous cycle in the cow. During the past 30 years, it has been proposed that a rapid decrease in luteal blood flow is one of the essential impacts of PGF2α. As the CL matures, the steroidogenic cells establish contact with many capillary vessels, so that the CL is composed of a large number of vascular endothelial cells that can account for up to 50% of all cells in the bovine CL. Also, luteal endothelial cells secrete several vasoactive substances such as PGF2α, nitric oxide (NO), endothelin-1 (EDN1) and angiotensin II (Ang II). Therefore, blood vessels and endothelial cells within the CL should have an essential role in luteal function in the cow, in which the vasoactive molecules above may regulate luteolysis locally.

Luteal blood flow: Recently, we discovered that a PGF2α treatment of cow having the mid CL (Day 10 of the estrous cycle), but not the early CL (Day 4 of the estrous cycle), induced an acute increase (0.5-2 h) in luteal blood flow at the periphery of the CL. During spontaneous luteolysis in the cow, an increase in blood flow in the periphery of the CL was associated to peak of plasma PGFM (a product of the metabolism of PGF2α), just prior to the decline in P4 secretion, strongly suggesting that PGF2α from the uterus induces an acute blood flow increase in the CL.

NO and luteal blood flow: NO, a strong vasorelaxant, could be a good candidate in the blood flow increase. Consistent with the luteolytic ability of PGF2α, PGF2α stimulated the expression of endothelial NO synthase in the periphery of the mid CL after PGF2α administration but not in the early CL. In the peripheral area, resistance vessels (>20 μm) having vasomotor function localized mainly in the mid CL, but they were not observed in the early CL. A direct injection of the NO donor (SNAP) into the mid CL clearly induced an increase in blood flow, and the injection of the NOS inhibitor (L-NAME) completely suppressed the increase in luteal blood flow induced by PGF2α.

Vasoactive factors: Luteal EDN1, Ang II and PGF2α are involved in the process of luteal regression. Using in vivo microdialysis system for the bovine CL, intraluteal EDN1, Ang II and PGF2α secretion increased after onset of spontaneous and PGF2α-induced luteolysis. For the luteal function, EDN1 and Ang II inhibited P4 secretion in luteal cells. Moreover, the intraluteal EDN1 or Ang II injection after 1/4 dose of PGF2α administration (sub-luteolytic dose) resulted in a reduction of

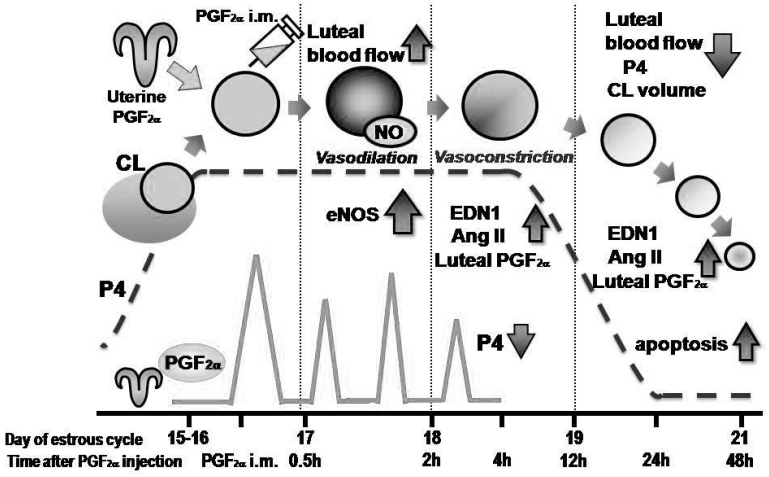
plasma P4.

On the basis of the results of the series of experiments, the following scenario can be drawn for the luteolytic cascade in the cow (Fig. 1). Endogenous (from the uterus) or exogenous PGF2α directly activates eNOS/NO in the peripheral area of the CL, so that strong vasorelaxant NO is drastically produced in periphery of the CL especially large luteal vascular vessels. Therefore, luteal blood flow in the peripheral area are increased acutely by the vasodilative action of NO, suggesting that PGF2α-induced increase in luteal blood flow is one of the earliest physiological events in the luteolytic cascade in the cow. Following an increase in luteal blood flow, CL-derived PGF2α now stimulates production of ET-1, Ang II and luteal PGF2α. These vasoactive substances decrease the P4 secretion. After this stage, these vasoactive molecules are kept at high levels in the CL and induce vasoconstriction to shut-off the blood supply to the CL.

In conclusion, the bovine CL is a large and heterogeneous endocrine organ, and PGF2α has a site-restricted action depending not only on the luteal phase (i.e., early vs. mid) but also on the region of the CL. A three-dimensional vascular structure in the CL and interactions among several vasoactive factors are required for maximal responsiveness to PGF2α to achieve a rapid regression of the CL in the cow.

Fig. 1

Hypothesis for luteolytic cascade with a main focus on vasoactive molecules



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