Preface

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In the recent decades, our knowledge about the regulating mechanisms of many reproductive phenomena has dramatically increased. The new technologies, such as *in vitro* fertilization, embryo manipulation and so on, have been developed based on the understandings of the endocrine, cellular and molecular physiology controlling central nerve system, ovarian and uterine functions, gamete and embryo.

In the long history of the scientific cooperation between Poland-Japan, the most recent achievements seem to concentrate on the ovarian and uterine physiology around luteolysis or early pregnancy. The research developments in reproduction of both countries are conspicuous in all over the fields, resulting in increasing number of collaborations among the researchers, especially after the first joint seminar of Poland-Japan held in Krakow/Poland, 2005, with the title “**Cutting-edge Reproductive Physiology -Regulation of Ovarian Function-**”. Since both countries have huge potential to develop reproductive research, it has been planed to hold the second joint seminar for exchanging information between both countries. This second seminar “**Cutting-edge Reproductive Physiology –from Gamete to Baby-**” is expected to be a crucial event which contributes in promoting future collaboration among young scientists of the both sides. The participants, especially the young scientists have been expected to exchange the advanced knowledge in the field of reproduction through their research presentations and discussions.

Since Reproductive Physiology covers a variety of fields, the subtitle “From Gamete to Baby” has been added to include the wide research fields such as “central nerve system”, “follicular development”, “regulation of corpus luteum function”, “implantation and maternal recognition”, and “parturition”. Furthermore, the reproductive technology should also be included in the session, because the final goal of our research is to apply to animal reproduction. In the present seminar, only the young top scientists of both countries in the above research fields have been invited as speakers.

I hope and believe that this seminar will encourage future collaborations among researchers of both countries.

JP1-1 Cumulus Cells Play an Important Role to Induce Meiotic Resumption and Progression of Oocyte

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In preovulatory follicles, oocytes are surrounded by numerous layers of cumulus cells in a known as the cumulus cell-oocyte complex (COC). After stimulation of ovulation by the LH surge, the morphology of COC is dramatically changed as a hyaluronan rich matrix accumulates within cumulus cell layer, and the oocyte resumes meiosis by progressing to the metaphase II. Although both changes induced by LH surge are essential for successful fertilization *in vivo*, the expression of LH receptors (*Lhcgr*) is not detected in the oocyte and is minimal (negligible) in cumulus cells compared with granulosa cells. However, cumulus cells express members of the EGF receptor family (ErbB family), prostaglandin receptors (EP2 and EP4) and cytokine family receptors that respond to specific ligands secreted by granulosa cells during the ovulation process. EGF-like factors, especially amphiregulin, that are expressed in a cAMP-PKA-CREB dependent manner in granulosa cells, act on EGFR expressed on cumulus cells. The phosphorylated EGFR induces the RAS-cRAF-MEK-ERK1/2 pathway, which increases the expression of genes involved in cumulus expansion and oocyte maturation. One of the target genes is *Ptgs2* that encodes the rate-limiting enzyme in the synthesis of PGE2. PGE2 stimulates cAMP production, which results in the maintenance of cAMP level at the maximum level in cumulus cells to progress oocyte meiosis to the MII stage. Cytokine family, especially IL-6 secreted from granulosa cells also acts on cumulus cells. When COCs were cultured with IL-6, the developmental competence after *in vitro* fertilization is significantly improved as similar to that in *in vivo* matured oocytes. Thus, by these intermediary steps, the cumulus cells mediate LH signaling from granulosa cells to induce oocyte maturation.

JP1-2 Susceptibility of Particular Sperm Cell Features to Damages of Long Term-storage of Liquid Semen in Normospermic Boars

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The aim of this study was to analyze the dynamic changes of sperm cell characteristics in boar semen extended in long-term extender, in order to detect susceptibility to damage of particular structures of spermatozoa during cooling and storage at 17 C for 240 h post collection. The study included ejaculates collected from seven boars of Polish Large White breed in five replicates (n=35 ejaculates). The sperm characteristics were assessed at 0, 48, 96, 168 and 240 hr post collection with the use of flow cytometer and computer assisted sperm analyzer. The significant decrease of motility parameters was recorded during the whole time of semen storage. There were observed the gradual increase of the percentage of live spermatozoa with inactive mitochondria. The use of combination of SYBR-14/PI stains did not reveal any significant changes in the percentage of live sperm cells up to 168 h of semen storage. Significant ($P<0.01$) deterioration of acrosome integrity was observed beginning from 168 h of incubation. The sperm chromatin structure assay (SCSA) showed the significant increase ($P<0.01$) in DNA fragmentation index (%DFI) after 48 h of semen incubation. Further increase of %DFI was observed during the rest of time of semen storage. In conclusion, storage of boar semen extended in long-term diluent at 17 C for 48 h induced the decrease of the integrity of sperm DNA and rapid decrease of population of motile cells as well as all kinetic parameters of sperm cells such as velocity and linearity. It seems that DNA structure of boar sperm cells in liquid stored semen is more susceptible to damage than the integrity of spermatozoal membranes.

JP1-3 Isolation of Ovarian Components Essential for Oocyte Growth *In Vitro*

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Mammalian ovaries contain a large number of oocytes that degenerate either before or at various stages of growth. Such oocytes can be rescued under suitable culture conditions. Because offspring have been derived from *in vitro* grown murine and bovine oocytes, it is evident that oocytes can grow into "normal" ova *in vitro*. Therefore, some growing oocytes can fulfill their original function during culture. However, how faithfully the follicle, which contains the oocyte, should mimic the original *in vivo* conditions remains a question. Finding an answer to this question is essential for a better understanding of the regulation of oocyte growth. In the culture system for murine and bovine oocytes used in this study, the oocyte–granulosa cell complexes, at approximately the late mid-growth stage, spread on a substratum. However, no theca cells were involved. The structural simplicity of this type of a system gives it an advantage with regard to narrowing down the basic conditions required for the regulation of oocyte growth. Among the factors playing crucial roles in the ovary, FSH stimulated follicular growth, but was not indispensable to oocyte growth *in vitro*. Androstenedione was used to compensate for the absence of theca cells, and it promoted both follicular growth and acquisition of meiotic competence of oocytes. Besides the biological factors, high concentrations of polyvinylpyrrolidone (molecular weight: 360000) improved the growth of bovine oocytes. In addition, some oocyte-mediated bidirectional interactions between oocytes and granulosa cells have been observed. Most of the oocytes cultured in a group were viable after long-term culture, suggesting that unlike the events in the ovary, there was no exhaustive follicle selection. Collectively, the oocytes and the associated granulosa cells can establish independent units capable of supporting oocyte growth in appropriately modified culture media.

JP1-4 Impairments of Reproductive Tract Function in Cows Under Influence of Environmental Pollutants

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The aim of the studies was to investigate the effect of xenobiotics on function of bovine reproductive system. As a treatments we used the chloroorganic compounds - polychlorinated biphenyls (PCBs), pesticide DDT and its metabolite (DDE), which are recognized as an persistent environmental pollutants. Uteri and oviducts were collected from cows on day 1–5 of estrous cycle, while ovaries were obtained on day 8–12 after ovulation. Endometrial, myometrial, epithelial of oviduct, granulosa and luteal cells, as well as myometrial strips were exposed (2–72 h) to environmental pollutants (0.1–100 ng/ml): PCBs mixture (Ar1248), individual PCBs congeners (30, 77, 126 or 153), PCBs hydroxy-metabolites (30 OH, 50 OH), DDT or DDE. Next, (a) viability of cells, (b) expression of mRNA for genes involved in the synthesis of prostaglandin (PG) F2a (cyclooxygenase-2 - COX-2; PGF2a synthase - PGFS) in myometrium, and oxytocin (OT) (neurophysin-I/oxytocin - NP-I/OT; peptidyl-glycine-a-amidating mono-oxygenase - PGA) in ovarian cells, (c) secretion of PGF2a from uterine and oviductal cells and OT from ovarian cells or (d) force of myometrial strips contractions (basal and OT-stimulated), were measured. Some myometrial strips were also pre-incubated with indomethacin, which blocks COX-2 and therefore synthesis of prostaglandins. None of used xenobiotics affected ($P>0.05$) the viability of studied cells. However, they increased ($P<0.05$) both, mRNA expression of studied genes and the secretion of OT and PGF2a secreted from the studied cells. PCBs, DDT and DDE increased ($P<0.05$) the basal and OT-stimulated force of myometrial strips contractions. However, the this effect was reduced ($P>0.05$), when the synthesis of prostaglandins was inhibited. It can be concluded that the synthesis and secretion of OT in ovary and PGF2a in uterus is a part of the mechanism by means of which used xenobiotics may affect the force of myometrial contractions in cattle.

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JP1-5 Regulation Mechanisms of Gene Expression in Bovine Embryos Derived from Somatic Cell Nuclear Transfer

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High rates of early embryonic, neonatal, or postnatal abnormalities have consistently been observed in bovine cloning. The cause of such abnormalities might be correlated with abnormal epigenetic modifications and gene expression in somatic cell nuclear transfer (NT-SC) embryos. We examined expression patterns of several genes involved in segregation of inner cell mass (ICM) and trophectoderm (TE) such as *OCT-4*, *CDX2*, *NANOG*, and *FGF4*, and also DNA methylation level in bovine NT-SC embryos. In NT-SC embryos at the blastocyst (BC) stage, transcript levels of all genes except *CDX2* were lower than that *in vivo* embryos. DNA methylation level in BC embryos derived from NT-SC was higher, and the value remained unchanged in the embryo disc and significantly decreased in the TE. *Dnmt-1* expression in BC embryos derived by NT-SC was significantly lower than that *in vivo* embryos; thus, differences in the DNA methylation status may reflect transcript levels of *Dnmt-1*. In order to clarify reasons of epigenetic errors, we examined the effect of torichostatin A (TSA) on gene expression in bovine NT-SC embryos, and also analyzed Xist mRNA expression in NT-SC embryos. TSA-treated NT-SC embryos developed to BC stage depending on TSA concentrations with 50 nM TSA showing the highest rate compared with NT-SC embryos without TSA. Histone H3 acetylation level in NT-SC embryos treated with TSA was higher than that in NT-SC embryos without TSA. Xist expression level in bovine NT-SC embryos was higher in both sexes than that in IVF embryos. These results raise the possibility that abnormalities in the cloned fetus and placenta are related to the aberrant expression of genes involved in segregation and differentiation process in the early developmental stage. Although aberrant gene expressions may be caused by DNA methylation and Histone modification, these statuses are corrected as a result of demethylation and retention of methylation as the embryo develops and differentiates and TSA treatment of NT-SC embryos.

JP1-6 Searching for Optimal Conditions and Time Point for the Derivation of Bovine Embryonic Stem Cells (bESC): Pluripotency Markers Gene Expression

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The identification of stem cell specific markers is critical to the establishment of stable ESC form various species. Despite many efforts, self-renewing ESC have only been derived from mouse (Evans, 1981) human (Thomson, 1998) and rat embryos (Buehr, 2008). It is now evident that although there are some shared mechanism regulating pluripotency and self-renewal in the ESC, mouse and human ESC differ in morphology, surface marker or gene expression patterns. This question has not been fully addressed in bovine and the existing data is often contradictory. We aim to analyse the canonical mouse and human pluripotency and cell lineage markers in bovine. ICM and TE were mechanically dissected from hatched blastocysts (9dpi) and subjected to quantitative gene expression analysis. The preliminary data indicates, that at least at the mRNA level, the expression of *NANOG, OCT4, KLF4, FN1* is higher in bovine ICM and *CDX2* and *ELF5* in TE, what corresponds to the mouse model. Elf5 was recently indicated as crucial for the induction of differentiation of mouse ESC to the TE lineage *in vitro*. A higher level of *GATA6, REX1, KRT-18* expression in bovine TE probably reflects species specific differences. Mouse implantation begins around 4.5dpi and bovine around 18–20dpi. Although, Rex1 is one of the main factors distinguishing mouse ESC from the epiblast stem cells, its transcript was discovered at a very low level in TE (Rogers,1991). *GATA6* is mouse primitive endoderm marker. Its higher expression in bovine TE may reflect the fact that unlike in the mouse, bovine TE is formed from the cells surrounding the blastocyst cavity. Immunofluorescent analysis localises Cdx2 predominantly in the trophoblast and Oct4 in the ICM of bovine blastocysts. We are at a very early stage of deriving bESC, but we are able to obtain uniform colonies from ICMs and maintain them in culture without morphological signs of differentiation. The project is financed by the Foundation for Polish Science (Programme Powroty/Homing; HOM/2009/6B) as a part of Fellowship awarded to Dr Z. Madeja.

JP2-1 Kisspeptin: A Key Molecule Controlling the Activity of the Hypothalamo-pituitary-gonadal Axis

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Gonadotropin-releasing hormone (GnRH) neurons located in the preoptic area (POA) are final common pathway to control gonadotropin release, which is critical for steroidogenesis and gametogenesis by the gonads. GnRH neurons are modulated by feedback actions of sex steroids and several environmental factors. The afferent inputs to the GnRH neurons in the hypothalamus remain elusive. Kisspeptin neurons, a potent candidate for afferent inputs to the GnRH neurons, emerged from genetic linkage analyses of the patients of hypogonadotropic hypogonadism. These studies showed that loss-of-function mutations in *GPR54* gene, a kisspeptin receptor, result in the hypogonadotropic hypogonadism. Ever since then, researchers found potent stimulatory effects of full-length kisspeptin or its C-terminal decapeptide on gonadotropin release through GnRH release. The present paper focuses on the role of the two populations of brain kisspeptin neurons in controlling two modes of GnRH/luteinizing hormone (LH) release in mammalian species. Kisspeptin neurons were limitedly localized in the anteroventral periventricular nucleus (AVPV) and hypothalamic arcuate nucleus (ARC) of female rats, which are prospected regions controlling GnRH/LH surges and pulses, respectively. In male rodents, kisspeptin neurons are not found in the AVPV. The sexual dimorphism of the AVPV kisspeptin neurons is well consistent with the sexual difference in LH surges in rodents. In addition, estrogen enhances AVPV kisspeptin expression in females. Thus, AVPV kisspeptin neurons would be responsible for surge mode of GnRH/LH release. On the other hand, ARC kisspeptin neurons are still unknown but are considered to control pulse generation. Neuronal activities synchronized with LH pulses are recorded in goat hypothalamus, when the electrode is placed at the kisspeptin neuronal cluster in the ARC. Kisspeptin biology provides a clue as to the mechanism controlling two modes of GnRH release in mammalian species.

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JP2-2 Neuroendocrine Regulation of Seasonality in Sheep

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In temperate latitudes, sheep are seasonal breeders whose reproductive activity is controlled mainly by photoperiod through nocturnal secretion of melatonin. However, neither the target sites for its action in the brain nor the neuropeptide circuits engaged by the melatonin signal are well defined. Intense research has been carried out to provide explanations for the relationships between hormones related to the regulation of energy homeostasis, metabolism and reproduction. Leptin, ghrelin and orexin form a close web of interrelationships in the regulation of food intake and energy balance. The aim of this study was to determine whether day-length modulates the interactions between leptin, ghrelin, growth hormone (GH) and orexin in seasonally breeding sheep. Ovariectomised, E2-implanted ewes (n=24) were utilised. Ewes were assigned randomly to one of six treatments and infused into III-ventricle three times at 0, 1 and 2 hr beginning at sunset. Treatment consisted of: 1) control; 2) leptin; 3) ghrelin; 4) orexin B; 5) leptin antagonist then ghrelin; and 6) leptin antagonist then orexin. Blood samples were collected at 15-min intervals for 6 h. Leptin increased ($P<0.001$) plasma concentrations of melatonin during short-day (SD) and decreased them during long-day (LD) photoperiods. Ghrelin decreased ($P<0.01$) melatonin during SD and LD, and orexin increased melatonin ($P<0.001$) only during LD. Leptin attenuated ($P<0.05$) ghrelin concentrations compared to controls during SD. Plasma concentrations of GH and orexin were lower ($P<0.05$) after leptin infusions during LD and SD; however, ghrelin had the opposite effects ($P<0.01$) on orexin concentrations. Orexin increased ghrelin concentrations during LD. Ghrelin and orexin concentrations were increased ($P<0.001$) after leptin antagonist infusions. Our data provide evidence that secretion of leptin, ghrelin and orexin are not only seasonally dependent, with relationships that are under photoperiodic regulation, but that leptin is the predominant factor regulating ghrelin and orexin release in sheep.

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JP2-3 The Corpus Luteum Development Regulated by Angiogenic Factors and Polymorphonuclear Neutrophils in the Cow

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Development of the corpus luteum (CL) in ruminants occurs rapidly and time-dependently within 1 week after ovulation. These changes involve luteinization of steroidogenic cells and angiogenesis to establish luteal function (progesterone (P) secretion). The CL produces angiogenic factors including basic fibroblast growth factor (FGF2) and vascular endothelial growth factor A (VEGFA). FGF2 and VEGFA systems exists higher levels in the early CL during the estrous cycle. Both FGF2 and VEGFA stimulate P secretion from the early CL and progresses proliferation of luteal and endothelial cells. To investigate the impact of FGF2 and VEGFA, we injected the specific FGF2 and VEGFA antibodies directly into the CL in the cow. These treatments markedly suppressed the increase in CL volume and P synthesis (plasma P concentrations and mRNA expression of StAR) together with changes in mRNA expression of FGF2 and VEGFA. Therefore, FGF2 and VEGFA promote the establishment of a new vascular network and luteal function during CL formation in the cow. CL formation resembles an inflammatory response due to the influx of immune cells. Polymorphonuclear neutrophils (PMN) play a role in inflammation, and secrete chemoattractants to stimulate angiogenesis. We hypothesized that PMNs infiltrate in the developing CL and may play a role in angiogenesis of the CL. Considerable amounts of PMNs and the high level of interleukin-8 (IL-8, a neutrophil-chemoattractant) were observed during the early luteal phase. The PMN migration *in vitro* was stimulated by the supernatant from the early CL, and this activity was inhibited by neutralizing with an anti-IL-8 antibody. Moreover, the supernatant of activated PMNs and IL-8 stimulated formation of capillary-like structures of luteal endothelial cells. In conclusion, angiogenic factors (FGF2 and VEGFA) are essential for the development of the CL, and PMNs is a potential regulator of angiogenesis together with IL-8 in developing CL in the cow.

JP2-4 Regulation of Corpus Luteum Function by Factors Associated with Control of Energy Homeostasis

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Reproduction is connected with metabolic status in mammals. Recent studies have indicated that the specific hormones – leptin, orexin A, orexin B and adiponectin – create link between feed intake regulation and control of reproductive system. The expression of leptin, orexins, adiponectin and their receptors mRNAs and proteins has been found in the human, rat and porcine (our studies) corpus luteum (CL). The expression of these hormones and their receptors depends on phase of the oestrous cycle. These cycle-related changes in expression level seem to be associated with hormonal milieu of animals and imply and important role for ovarian steroids as key regulators of these hormonal systems. The identification of leptin, orexins, adiponectin and their receptors mRNAs and proteins expression in CL supports the conclusion that these hormones may act as the autocrine or/and paracrine factors within the ovary, and that CL are target tissues for leptin, orexins and adiponectin, which might have direct effects on their functions. Direct effects of leptin, orexins and adiponectin on regulation of CL function have been investigated in numerous studies. These reports indicate that the hormones regulate steroidogenesis and may play a role in the process of luteinisation. In addition, leptin, orexins, adiponectin and their receptors mRNAs and proteins has been found in human, rat and porcine (our studies) hypothalamus and pituitary. Studies on the hypothalamic-pituitary axis have identified that these hormones regulate the secretion of GnRH, LH and FSH, and may indirectly participate in the control of CL function. Taken together, the evidences were presented that supporting the idea that leptin, orexins and adiponectin are more than factors associated with energy homeostasis only. Their also serve as the metabolic signals controlling directly and indirectly (through hypothalamo-pituitary hormones) functions of corpora lutea.

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JP3-1 Establishment of *In Vitro* Assessment System for Conceptus Attachment to the Uterine Epithelial Cells

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The establishment of pregnancy requires appropriate communication between the developing conceptus and the uterine endometrium. If implantation failure is in fact the major cause of pre-implantation embryo loss, studies should be directed toward an understanding of biochemical communication between the conceptus and mother. The implantation proceeds when the conceptus and uterine developments are synchronized through secretory factors from each tissue. IFNT plays a central role in the crosstalk between the conceptus and mother toward its attachment to the uterine endometrium. Because transcription of *IFNT* gene starts to decline when the conceptus attaches to uterine epithelial cells, *IFNT* would be an ideal maker for evaluation on whether or not the attachment of trophoblast cells to uterine epithelial cell occurs. If the assessment system of attachment *in vitro* were established, each of attachment processes could be dissected for thorough evaluation and more importantly for the study of factors identified and/or yet unidentified required for conceptus attachment to proceed. We, therefore, attempted to establish the *in vitro* coculture system with bovine trophoblast CT-1 cells and uterine epithelial cells, which possibly mimic the attachment process *in vivo*. Results from the coculture experiments with CT-1 and endometrial cells revealed that both the uterine flush from pregnant animals and physical contact with endometrial cells were essential for the down-regulation of *IFNT* in CT-1 cells. Although further study is required for understanding the implantation process, the coculture system developed may become useful for the assessment of biologically active factors during the implantation process.

JP3-2 The Role of HOXA10 as a Marker of Endometrial Receptivity

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The establishment of a uterine environment suitable for successful implantation and further pregnancy establishment is crucial and remains under strict regulation by ovarian steroids and conceptus signals. Search for markers of uterine receptivity revealed that homeobox A10 *(HOXA10)* gene expression is essential for uterus development and embryo implantation in humans, non-human primates and mice. Recently we examined the expression of *HOXA10* in the pig uterus. The endometrial *HOXA10* gene expression was increased on day 15 of pregnancy when compared to day 15 of the estrous cycle. Moreover, higher *HOXA10* transcript level was detected during implantation than at the period of "maternal recognition" of pregnancy. Immunoreactive HOXA10 protein was detected in luminal and glandular epithelium, but also in stromal cells and blood vessels of the endometrium. Estradiol (E2) alone or in the presence of progesterone (P4) increased *HOXA10* transcript level in cultured stromal cells, as well as incubated endometrial explants. Moreover, HOXA10 protein was stimulated in endometrial explants by E2, P4 and both steroids added simultaneously. Administration of PMSG/hCG to induce estrus resulted in decreased expression of *HOXA10* in the endometrium on day 12 of gestation, but had no effect on *HOXA10* transcript levels in conceptuses. The observed down-regulation of *HOXA10* was accompanied by reduced serum P4 content in gonadotropin-treated gilts. Additionally, conceptus products stimulated *HOXA10* and *PTGS2* transcript levels in luminal epithelium *in vitro*. Summarizing, these results indicate that HOXA10 expression in the pig uterus is closely related to implantation process and stimulated by both maternal and conceptus-derived factors.

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JP3-3 Plasma Estrogen Concentration and Placental Regulation of Estrogen Activity at Parturition in Cows Carrying a Somatic Clone Fetus

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The production of viable offspring from nuclear transfer with somatic cells has been reported in a range of domestic species, although the production efficiency is still low. Pre-partum abnormality such as prolonged gestation and a lack of readiness for birth in the recipient cows is one of the factors in decreasing the production efficiency of clone cattle. We analyzed hormonal concentrations during the pre-partum period and placental function regarding regulation of estrogen activity and steroidogenesis in placentome at parturition. Placental estrogens have been suggested to play important roles in the parturition process. In clone pregnancies, pre-partum rises in concentrations of plasma estrone (E1) and estradiol-17b (E2) in the recipient cows were subtle. Estrone sulfate (E1S) is an inactive form of E1 catalyzed by estrogen sulfotransferase (SULT1E1). The plasma concentration of E1S in clone pregnancies increased gradually from pre-initiation of parturition induction whereas control cows that received *in vivo*-derived embryos showed a significant increase at parturition. In clone pregnancies, the ratio of E1/E1S was low during the pre-partum period. Messenger RNA expression of SULT1E1 in the placentome at parturition was significantly higher in clone pregnancies and was localized in binucleate cells. SULT1E1 mRNA abundance was negatively and positively correlated with concentrations of maternal E1 and E1S at parturition respectively. Estrogens are synthesized by aromatase (CYP19) from androgens. Sulfoconjugated estrogens may also be precursors of the production of free estrogens, depending on placental estrogen sulfatase (STS) activity. STS and CYP19 mRNA abundances were similar between clone and control pregnancies and showed positive correlations with maternal E2 concentration. These results suggest that excess estrogen sulfoconjugation is the reason for the perturbed low ratio of active to inactive estrogens and the resulting hormonal imbalance contributes to the lack of overt signs of readiness for parturition in cows pregnant with clones.

JP3-4 The Role of Immunological Disturbances in the Pathogenesis of Pre-eclampsia

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It seems that abnormal activation of the immune system may play a role in the etiology of pre-eclampsia. Many authors have found a number of changes in the innate and adaptive immune system which may contribute to the development of pre-eclampsia. The aim of our study was to estimate the prevalence of $CD3^+CD4^+$ T lymphocytes producing IL-17, IL-2, IFN-g and IL-4, as well as $CD4^+CD25^+FoxP3^+$ T regulatory cells (Tregs) in peripheral blood of patients with pre-eclampsia and healthy women in the third trimester of normal pregnancy. Furthermore, the purpose of our study was to assess the immunosuppressive activity of Treg cells of pre-eclamptic patients in comparison with the controls. *:* The percentages of T $CD3^+CD4^+$ lymphocytes producing IL-17A were significantly higher in pre-eclampsia when compared to healthy normotensive pregnant women in the third trimester of normal pregnancy ($p<0.001$). The population of $CD4^+CD25^+FoxP3^+$ Treg cells was significantly lower in the study when compared to the control group ($p<0.05$). There were no changes in the proliferative response of $CD3^+CD4^+CD25^-$ T lymphocytes of pre-eclamptic patients during *in vitro* assay without Treg cells and after the addition of autologous Tregs. In normal pregnancy the proliferative response of $CD3^+CD4^+CD25^-$ T lymphocytes was significantly higher without Treg cells when compared to this response after addition of autologous Tregs ($p<0.05$). The results obtained suggest the up-regulation of Th17 immune response in pre-eclampsia. It seems that the decreased number and function of Treg cells may be responsible for the activation of inflammatory response in this disorder. The predominance of Th17 immunity can act through the modulation of Th1/Th2 immune response in pre-eclampsia.

Closing Remarks

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Studying the regulatory mechanisms of biology of gametes, ovarian function, early embryo development and implantation is important as a basic data for the establishment of new methods of the diagnostic and treatment for sterility in medical and veterinary sciences. In addition, it is essential for the fields of experimental zoology and animal husbandry to develop new techniques of reproductive control, enable efficient animal production and biodiversity.

Japanese and Polish researchers who are young pioneers in reproductive physiology, biotechnology and pathology have met for four days on our joint Seminar and have discussed the present situation of reproductive biology in both countries. The seminar covered seven main topics of mutual interest and discussion: (1) Gametes biology and biotechnology of reproduction, (2) Embryo, (3) Central mechanisms of reproduction, (4) Ovarian function – including follicular development, (5) Corpus luteum function, (6) Pregnancy recognition and implantation, (7) Pregnancy, placenta, parturition. The main objective of the Seminar was to encourage the young scientists of both countries to establish further long-lasting collaboration which should result in joint research projects. The new directions of the future research were analyzed and defined and we can expect new development in field of reproductive biology and pathology. Finally we hope that the Seminar should enable us to make at least the research in our filed more public and practical.

Here I want to particularly thank Dr. Ken SAWAI for their hard work in organizing the current meeting. Organizing an international meeting is not an easy job. I want to also thank all our speakers and all of you for participating in the meeting. I believe that in the soon future we can further increase our collaboration. We hope that every participants will return home stimulated and with new research ideas and new friendships.