Abstracts for the KSAR and JSAR Joint Symposium

Fertility control in female domestic animals: From basic understanding to application

Current Research Orientation in Livestock Reproduction in Korea

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The genetic improvement of farm animals has been increased greatly by artificial insemination and multiple ovulation-embryo transfer. The nuclear transfer (NT) technology has been developed to increase the frequency of genes of genetically outstanding animals in a population. The introduction of new genetic materials into a population and the production of knock-in and knock-out animals for xenotransplantation have also been attempted by transgenic and somatic cell nuclear transfer (SCNT) technologies. Furthermore the human somatic cell nuclear transfer-embryonic stem (SCNT-ES) cell lines will be contribute to develop new technologies in cell therapy. During the period of 15 years since 1988, the research works in the field of embryo cloning and SCNT have also been performed with successful results in Korea. Some of the interesting results related to embryo cloning, SCNT, transgenic SCNT, and human SCNT-ES cell lines from Korean laboratories are discussed by animal species in my talk.

The Basis of the Oocyte Production

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The ovary is a multi-functional organ and is in particular responsible for producing fertile ova. Each one of ova is derived from a follicle, a specialized structure that encapsulates the oocyte and supports its development up to ovulation. In the mean time, however, many more of other oocytes are to be lost by atresia as a result of ovarian regulatory actions. Therefore, the overall mechanisms for the oocyte (ovum) production in the ovary involve complicated processes, many of which are more likely to permit a limited number of follicles to survive than simply nurture the oocyte. In this review, the focus is on a simple model of an intrafollicular environment that supports oocyte development. In vitro culture of growing oocytes with companion ovarian cells provides us with a way to know the necessary and sufficient conditions for continued oocyte growth. Such culture systems have been developed to understand the regulatory mechanisms of oocyte development in mice, but are also potentially powerful tools for studies of oogenesis in larger animals. From application point of view, establishment of the oocyte culture technology would lead to expand the population of livestock and other valuable animals. In a typical mouse system, oocyte-granulosa cell complexes can grow on the surface of the matrix. Only recently, such a substratum-adherent system has been developed for bovine oocytes, in which the oocytes, starting from about 75% of the maximum diameter, approached the full-size during a two-week culture period. In this case, no cell types other than granulosa cells were necessary to support oocyte growth. A proportion of the oocytes were capable of undergoing in vitro fertilization and embryo development. It should be emphasized that a high concentration of polyvinylpyrrolidone in culture medium, a novel modification, influences the organization of complexes, resulting in a firm association between the oocyte and surrounding granulosa cells. Studies with mice have revealed that the oocyte has key roles in regulation of its own microenvironment through establishing the bidirectional communication with granulosa cells. These findings taken together would imply that a continuous and evolving regulatory loop between the oocyte and surrounding granulosa cells is essential as a basis for the growth and development of animal oocytes.

In Vitro Production of Cloned Porcine Embryos

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Although cloned piglets have been successfully generated from embryos produced in vitro after somatic cell nuclear transfer (SCNT), many problems such as a low developmental rate and a poor quality of SCNT-derived embryos remain to be solved. The acquisition of developmental competence of SCNT-derived embryos during in vitro culture is a prerequisite step to produce cloned piglets. Despite recent improvements in the in vitro culture system for porcine embryos, the efficiency of the production of piglets after transfer of SCNT- or IVF-derived embryos is still inferior to other species such as sheep and cattle. Therefore, our researches are focused on improving in vitro developmental ability and structural integrity of porcine embryos. Firstly, we found that electrical stimulation was more effective for parthenogenetic activation of in vitro matured porcine oocytes than chemical reagents. Then, effects of different activation methods on developmental ability of SCNT embryos were also assessed. Electrical pulse made significant increases in both cleavage rate and development rate to the blastocyst stage ($P<0.05$) of SCNT embryos compared to A23187 only or combined A23187/6-DMAP treatment. And then to investigate the optimal conditions for electrical method, the field strength and activation timing after fusion on the development of porcine SCNT oocytes were examined. The results demonstrate that field strength and activation timing after electrofusion could affect developmental ability and quality of porcine SCNT embryos. SCNT-derived embryos had a lower developmental competence to the blastocyst stage than IVF-derived embryos ($P<0.05$). Total cell number of SCNT-derived blastocysts was also inferior to that of IVF-derived embryos ($P<0.05$), however no difference was detected between the two groups in the ratio of ICM to total cells. To investigate what proportions of in vitro-produced porcine embryos represent normal structural integrity, differentially-stained blastocysts were individually classified into three presumptive groups according to the ratio of ICM to total cells (I; <20%, II; 20 to 40%, and III; >40%). Low proportions of NT- and IVF-derived blastocysts were assigned to Group II, whereas almost all of in vivo-derived embryos were allocated to Group II. From our observations, it is concluded that limited structural integrity may lead to poor survival to the term of SCNT- or IVF-derived porcine embryos produced in vitro.

Hormonal Control of Follicular Maturation, and Luteal Development and Maintenance in Domestic Ruminants

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Ovarian activity in domestic animals is regulated intrinsically and can be, at least partially, controlled extrinsically by hormones. In domestic ruminants, luteinizing hormone (LH) has a pivotal role in the regulation of ovarian functions, such as follicular maturation, ovulation, and luteal development and maintenance. In this joint symposium, I will describe our observations including artificial controls using hormones of ovarian follicular maturation for fertility improvement, and gene expressions of a few luteotropic factors during luteal development and their regulation by LH in ruminants.

In Shiba goats, which are originated from a Japanese native dwarf breed and are non-seasonal breeder, estrus is not observed usually during the suckled early postpartum period. Effects of a low dose of human chorionic gonadotropin (hCG) on the induction of estrus were examined in suckled early postpartum goats pretreated intravaginally with progesterone (Controlled Internal Drug Release dispenser; CIDR). Treatment with three times of a low dose of hCG at 12h intervals after the inclusion of CIDR for 7 days promoted follicular maturation and fertile estrus induction in the goats (Kawate et al, J Reprod Dev, 2002).

In suckled postpartum Japanese Black beef cows, inclusion of a CIDR for 7 days from the start of Ovsynch protocol (GnRH on Day 0, PGF2□ on Day 7, GnRH on Day 9) improved conception rates to timed-AI (Kawate et al, Theriogenology, 2004). To clarify one of mechanisms of the improved conception by the combination of Ovsynch and CIDR, timings of follicular maturation and ovulation of the beef cows were examined when hormonal treatments were started a few days before initiation of corpus luteum regression. These studies showed that dominant follicles matured before the second GnRH treatment and ovulated before AI in the Ovsynch protocol alone. The addition of a CIDR to the Ovsynch prevented such earlier occurrences of follicular maturation and ovulation, and made those phenomena occur at an appropriate time.

To examine a part of mechanisms of corpus luteum (CL) development and maintenance in domestic ruminants, gene expressions of LH receptors, vascular endothelial growth factor (VEGF) and its receptors and prostacyclin (PGI2) synthase, which catalyzes the conversion of PGH2 into PGI2, in the CL, and their regulations by LH were examined using the Shiba goats. The PGI2 synthesis was enhanced at the beginning of CL formation, followed by increases of LH receptors and its mRNA, VEGF165 and KDR/Flk-1 (one of VEGF receptors) mRNAs during the CL development, and then VEGF165 and Flt-1 (another VEGF receptor) mRNAs increased during the CL maintenance (Kawate et al, Mol Reprod Dev, 2002, 2003, 2004). The increase of LH receptor during the CL development was inhibited by

gonadotropin-releasing hormone antagonist, but other factors were not affected. These results suggest that the PGI2 may play a role in initiating caprine CL development, and that the LH receptor, VEGF and KDR/Flk-1 may participate in the CL development. Besides, the LH receptor may be, at least partly, up-regulated by LH itself during the CL development (Kawate, J Reprod Dev, 2004).

Synchronization of estrus and ovulation in dairy cattle

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The failure to accurately detect heat is one of main reasons for increasing the calving interval in dairy herds. However, the estrus periods in nearly half of normal cycling dairy cows may not be detected. In addition to poor heat detection, delayed ovulation and failure to ovulate is one of the most frequent causes of infertility in high yielding dairy herds. Typical protocols for estrus synchronization have focused on extending or restricting the luteal phase by the use of exogenous progestins and/ or $PGF_{2\alpha}$. These protocols allow dairy producers to easily detect cows in estrus within limited periods (2 to 7 days). Previously developed protocols for estrus synchronization using $PGF_{2\alpha}$ show large variations in the time to estrus due to the stage of development of follicular waves at the time of treatment. In fact, the synchrony of estrus and ovulation not only depends on the control of luteal life span but also on the phase of follicular development. Thus, protocols for manipulation of follicular development to induce precise synchronization of ovulation have been developed in cattle. The Ovsynch protocol, which combines GnRH (Day 0), $PGF_{2}\alpha$ (Day 7) and GnRH (Day 9) treatments, may be used to regulate follicular development and subsequent ovulation. This protocol induces synchronized ovulation within 24 to 32 h after a second injection of GnRH, thereby enabling AI without the need to detect estrus. It has been widely used in many beef and dairy herds. However, ovulation failure in response to the initial GnRH injection in the Ovsynch protocol may result in reduced pregnancy rates following timed AI due to asynchronous ovulation. For example, premature estrus between the first GnRH and the injection of $PGF_{2\alpha}$ has been reported in 5 to 11.8% of cows. An alternative is to utilize a progestin-based regimen. However, in order to prevent the development of a persistent follicle that can result in reduced fertility following AI, the induction of a new follicular wave is important in progestin-based synchronization regimens. Recently, researches showed that treatment with GnRH in a CIDR-based protocol improved pregnancy rates to timed AI in beef and dairy cattle. The protocol resulted in the emergence of synchronous follicular waves and synchronous ovulation, which gave acceptable pregnancy rates to timed AI. At the same time, the interval from treatment to the emergence of a new follicular wave appears to influence pregnancy rates, possibly because of the growth and size of the new preovulatory follicle. Further studies on the delicate regulation of follicular dynamics and luteal function may lead to enhanced fertility in dairy cattle.

Improvement of Pregnancy Rates by Efficient Selection of Recipients in Bovine Embryo Transfer

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To improve pregnancy rates in bovine embryo transfer, the correlation of the pregnancy rate with the luteal function in recipients was investigated. Blood concentrations of progesterone (P) and estradiol-17β (E_2), which closely related the luteal function were determined in parous Japanese Black beef cattle on the previous day of frozen embryo transfer i.e., on day 6 (the day of appearance of estrus designated day 0) and the day of the transfer i.e., on day 7. Both on the previous day and on the day of embryo transfer, the pregnancy rate tended to be increased with the increase in blood P concentration of recipients; the pregnancy rate (60.7%) for recipients, which showed a blood P concentration of ≧2.5 ng/ml on the previous day of embryo transfer, was significantly ($P<0.05$) higher than that (38.6%) of recipients showing a blood P concentration of ＜2.5 ng/ml on the previous day of embryo transfer. The pregnancy rate tended to be increased according to the decrease of blood E_2 concentration on the day of embryo transfer. To investigate the mechanism involved in the improvement of pregnancy rate being positively correlated with the high luteal function in recipients, we injected 1,500 IU of human chorionic gonadotropin (hCG) in Holstein dairy cattle on the day of ovulation or 5 days after ovulation, and then reaction of the ovaries to the administration was investigated. The cattle administered with hCG 5 days after ovulation showed the formation of induced corpus luteum resulting in a significantly ($p <0.05$) higher blood P concentration than that of control cattle. They showed a rapid decrease of blood E_2 concentration after hCG administration and maintained it at a low level. Based on these results, the following treatments were conducted prior to embryo transfer and the pregnancy rate was compared; 1,500 IU of hCG was injected in parous Japanese Black beef cattle recipients on days 1 (Day 1-hCG group) and 6 (Day 6-hCG group), and 5 ml of saline was injected in the control cattle (control group) on day 6. The pregnancy rate (67.5%) in the Day 6-hCG group was significantly ($p <0.05$) higher than that (45.0%) in the control group and that (42.5%) in the Day 1-hCG group.

These results revealed that the administration of hCG 1,500 IU to recipients on day 6 improve the pregnancy rate in the non-surgical frozen embryo transfer carried out on day 7 by enhancing luteal function and depressing E_2 secretion in recipients.