P-28 Expression of matrix metalloproteinases of mouse reproductive organs during estrous cycle

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Protein expression patterns of matrix metalloproteinases (MMPs) were examined in mouse reproductive organs during estrous cycle. Estrous cycles were classified into diestrus, proestrus, estrus or metestrus and MMP expressions were analyzed by zymography using gelatin as a substrate. Total protein content of uterine fluid (UF) was the largest at estrus and the least at diestrous. In contrast, overall MMP activities of UF was the highest at diestrus and the lowest at estrus. UF examined at all stages exhibited 4 MMPs including 106kDa, 64kDa, 62kDa and 59kDa and some minor bands. Of these, the gelatinase activities of 64kDa and 62kDa MMPs were higher in UF collected at diestrous but dramatically decreased at estrous. Ovarian tissue homogenate revealed many MMPs of which 4 MMPs were similar to the MMPs of UF. However, the relative activities of them did not correspond to the UF MMPs. Others were 140kDa, 89kDa, 45kDa and 42kDa MMP. These MMPs did not show distinct changes regardless of the estrous cycle. Similarly, oviductal tissue homogenate also exhibited 4 MMPs as those of UF MMPs and their patterns were also similar to the UF MMPs. An inhibitor of MMP, 1,10-phenanthrolline, or a metal chelator, EDTA, abolished the appearance of MMP activity in gelatin gel whereas a serine protease inhibitor, phenylmethylsulfonyl fluoride failed to inhibit the appearance of MMP activities.

From these results, it is concluded that the protein expression of MMPs of mouse reproductive organs, particularly uterus, is regulated during estrous cycle suggesting to play a role in cyclic reproductive events of these organs.

P-29 Developmental Capacity of Bovine Follicular Oocytes after Ultra-Rapid Freezing by Electron Microscope Grid

I. Cryopreservation of Bovine Immature Oocytes

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Bovine immature oocytes were frozen ultra-rapidly using cryoprotectant solution consisting of 30% ethylene glycol, 0.5M sucrose, 18% Ficoll and 10% FBS added D-PBS (EFS30). For freezing, the electron microscope copper grid was used as a physical support to achieve very high

cooling rates when it was plunged into liquid nitrogen. As criteria of oocyte survival, maturation, fertilization and embryonic development were determined. Also, cytoskeletal alteration of immature bovine oocytes after freezing/thawing was examined. The results obtained in this experiment were summarized as follows; After ultra-rapid freezing and thawing, 42.7% immature oocytes were survived. When maturation was identified at 24 h after thawing, the maturation rate in control and freezing group was 92.0% and 83.5%, respectively. Also, fertilization rate at 18 h after insemination was evaluated as total penetration (92.5, 88.2%), normal 2 pronuclei formation (62.4%, 53.3%) and mean number of sperm/oocyte (1.53, 1.40), respectively. The rate of two-cell formation to freezing group was 65.8%, and was not significant different when compared to control (73.6%). In addition, development to the blastocyst on day 8 after fertilization of freezing group was 32.0% from the cleaved oocytes. Development to the blastocysts of frozen-thawed oocytes was very similar to that of the control (33.3%). These results demonstrate that developmental capacity of frozen-thawed bovine immature oocytes can be successfully obtained by ultra-rapid freezing method using electron microscope grid and EFS30.

P-30 *In situ* detection of apoptosis induced by GnRH antagonist in mouse testis

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In mammals, including human, germ cell death is conspicuous during spermatogenesis and play an important role in sperm output. The survival of male germ cell is probably dependent on gonadotropins as well as intratesticular androgens induced by LH. It is well known that early deprivation of gonadotropin and testosterone by GnRH antagonist treatment is followed by a stage-specific degeneration of germ cell death in the rat testis. In this study, we examined the involvement of apoptosis in the induction of germ cell degeneration in GnRH antagonist treated mouse. Immature (3 week, 4 week) and adult (12 week) male mouse were given a daily injection of GnRH antagonist (1 mg/kg BW) and PMSG (5 IU) for 0 (control), 3 or 5 days. The occurrence of apoptosis was characterized by direct immunoperoxidase detection of digoxigenin-labeled genomic DNA in specific cell types. The weight of GnRH antagonist treated mouse testis had decreased to about 70% that of control group. But co-treatment with PMSG increased the testis weight to control level. In GnRH antagonist treated immature mouse, the number of apoptotic germ cell was significantly higher than that of control group. In contrast, the mouse treated with both GnRH antagonist and PMSG was not affected on germ cell apotosis. But in adult mouse, the number of apoptotic germ cell in GnRH antagonist treated animals was higher than control group, but it was not significantly different. In conclusions, the present study demonstrated that GnRH antagonist induced apoptosis of the male germ cells was found different manner according to the developmental stage. And the gonadotropin were essential for optimal germ cell survival.