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.