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Successful pregnancy in IVF-ET program by immature oocyte retrieval without ovarian stimulation

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Introduction : In the conventional controlled ovarian hyperstimulation (COH) of IVF-ET program, almost all of the follicles in monthly cohort were matured and could be obtained. However, besides the advantages of COH in ART, the disadvantages also included the economic impact, time loss for follicle monitoring, side effects of gonadotropin, risks of ovarian hyperstimulation syndrome (OHSS), and unknown long term cancer risks. Delivery of healthy babies was succeeded using immature oocytes excised from ovaries of regular or irregular cycle as well as natural cycle. The present study were carried out to evaluate the clinical feasibilities of in vitro maturation (IVM) and IVF, and increase of pregnancy rate in the non-stimulated cycles. And this study was also conducted to evaluate the number and quality of retrieved oocytes, and the assessment of in vitro maturation, fertilization rates, developmental capabilities, and implantation rates of the IVM derived human embryos according to the menstrual cycle of patients, and the endometrial preparation for implantation by hormonal administration.

Materials and Methods : Forty-three IVF candidates (age : 31.6 ± 4.2 yrs, 41 regular menstrual cycles and 28 irregular menstrual cycles) were underwent sixty-nine immature oocyte retrieval cycles. Immature oocytes were aspirated at mid-follicular phase. Intracytoplasmic sperm injection (ICSI) was performed on matured oocytes after in vitro maturation. For endometrium preparation, estradiol valerate (6mg/day) was treated before/after oocyte retrieval day. After embryo transfer, progesterone (50mg/day) was concomitantly treated with or without hCG (5000 IU) treatment. Embryos were transferred into uterine endometrium at 2 to 8 cell stage. The number of oocytes retrieved, fertilization rate, development of embryos, and pregnancy outcomes were measured in prospective randomized studies according to patients characteristics and hCG, E2 administration protocol.

Results : The number of the retrieved immature oocytes in irregular menstrual cycle were more than those of regular menstrual cycle. The rates of maturation, fertilization, and cleavage of healthy immature oocytes were 76.4% (278/425), 79.9% (222/278), and 91.7% (188/222), respectively. The number of the retrieved healthy oocytes in E2 administration after oocyte retrieval were more than that of in E2 administration before oocyte retrieval. Seven pregnancies were obtained without E2 administration before oocyte retrieval, and hCG administration before or after oocyte retrieval. Endometrial thickness was not different between pregnant and non-pregnant group.

Conclusions : From above results, we can conclude that immature oocytes were able to mature and fertilize with reproductive consistency in non-stimulated ART cycle by in vitro culture with supplementation of high dosage gonadotropin. And this protocol is effective on infertile women, especially PCO, OHSS patients and oocytes donation cycle. Also E2 administration before oocyte retrieval were shown the detrimental effect on oocyte number and its quality as well as final pregnancy outcome, however hCG administration would be necessary for pregnancy outcome in non-stimulated IVF-ET cycle.

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Biochemical assisted hatching(BAH) by pronase increased the pregnancy rate in human ART program.

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Objectives : In human IVF-ET programs, women aged >38 years or with elevated basal FSH level were shown to have oocytes with abnormally thick zona pellucida (ZP) and low hatching ability, resulting in low pregnancy rates. To improve hatching and pregnancy rates, assisted hatching using methods such as partial zona dissection and zona drilling has been used and can enhance success. Recently, it was reported that supplementation of pronase and/or proteinase K in culture media did not affect the development of embryos but increased hatching rates by the alteration of ZP structure in mice, and that good pregnancy rate and normal deliveries were achieved by biochemically assisted hatching (BAH); the addition of a low dosage of protease to culture media. In this study, we analyzed the efficiency and stability of BAH by the addition of pronase into the culture medium according to the clinical profiles of patients and fertilization method.

Materials and Methods : From July 1994 to December 1996, 1095 cycles undergoing IVF-ET program in Jeil Women's Hospital were randomly selected for BAH using pronase. The concentration of pronase E(Sigma Chemical Co.) used in this study was $1\mu\text{g/ml}$ in human tubal fluid medium (HTF) with 0.5% human serum albumin. *In vitro* fertilized embryos were cultured for 24 hours in HTF with 10% human serum or synthetic serum substitute (Irvine Scientific). In BAH group, embryos were transferred to HTF with pronase E and cultured for the last 24hours. The development of embryos were observed and transferred to the uterus of patients. The clinical pregnancy rate according to patient age, the number of stimulation cycles, the number of retrieved oocytes and fertilization method were analyzed compared between the BAH and control groups.

RESULTS : The clinical pregnancy rate was higher in the BAH group than in the control group (34.6% vs 29.3%; $P=0.063$). In patients over 35 years old (31.4% (58/185) vs 22.2% (51/230); $P=0.035$), with few oocytes ($4 \leq n \leq 12$) or less than 3 cycles of IVF-ET (36.8% (86/234) vs 27.2% (93/342), $P=0.015$; 36.8% (148/402) vs 29.9% (168/562), $P=0.024$), the clinical pregnancy rate of the BAH group was significantly higher than that of the control group. But, in the case of many oocytes (≥ 13) or nevertheless 4 cycles of IVF-ET, clinical pregnancy rate was not different in both groups. In both the BAH and the control group, clinical pregnancy rates were similar between conventional IVF and ICSI treatments.

CONCLUSIONS : The clinical pregnancy rate was increased by BAH using pronase and, specifically, BAH increased clinical pregnancy rates significantly in patients over 35 years old or with low oocyte retrieval rates. Therefore, BAH using pronase may be a simple, safe and valuable technique compared to the other known assisted hatching techniques in human ART program.