

결론: 본 연구의 결과에 따르면 습관성 유산의 기왕력을 가진 환자가 임신한 경우 임신성 고혈압과 조산 등의 산과적 합병증이 의미있게 그 빈도가 증가함을 알 수 있었으며, 산전 기간 동안 보다 면밀한 관찰을 요구하는 고위험 임신으로 관리되어야 한다.

P-5 Culture Condition Affects the Maturation Rate and the Expression of Cyclin B1 Protein during the First Meiotic Maturation on Bovine Immature Oocytes

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Objective: Germinal vesicle (GV) stage mammalian oocytes are arrested at the G2/M border of the first meiotic division. Reinitiation of meiosis and transition to metaphase is correlated with M-phase promoting factor (MPF) activities. The increase of MPF activity is strongly correlated with increased cyclin B1 synthesis. Cyclin B1 is known as a positive cell cycle progression regulator through G2/M phase. In this study, we investigate whether culture condition may affect maturation rate and the expression of cyclin B1 protein during first meiotic maturation on bovine immature oocytes.

Materials and Methods: The cumulus oocytes complexes (COC) were aspirated from 2~6 mm sized bovine follicles and cultured in TCM-199 medium containing 50 M 1-isobutyl-3-methylxanthine (IBMX) to maintain meiotic arrest. Meiotic maturation was initiated by washing the oocytes three times in culture medium without IBMX. The oocytes were matured at 39 °C in 5% CO₂ in air according to the following protocols; Experiment 1: denuded oocytes only, denuded oocytes + granulosa cells (GCs, 1×10⁶) isolated from Graafian follicles, COC only and COC + GCs; Experiment 2: TCM 199 medium + 10% FBS, TCM 199 + 10% synthetic serum substrate (SSS), TCM 199 + 0.4% BSA and TCM 199 + 10% FBS + 10 µg/ml FSH and LH (FBS + hormones); Experiment 3: non-activation (control), 7% ethanol for 5 min and 10 µg/ml ionomycin for 5 min at immediately before maturation. Following 18 hrs of maturation, the maturation rates were scored by first polar body extrusion and then the oocytes were lysed in 20 µl of RIPA buffer for 30 min on ice. The protein levels of cyclin B1 were measured with Western blot analysis. The differences were evaluated using Chi-square test and Kruskal-Wallis test. The correlation between maturation rates and cyclin B1 protein were tested by Pearson correlation coefficient. P value less than 0.05 was considered to be statistically significant.

Results: In experiment 1, the maturation rate and the protein level of cyclin B1 in denuded group were significantly lower than those of co-culture groups ($p < 0.05$). The maturation rate showed positive correlation with protein level ($R = 0.64$, $p < 0.03$). In experiment 2, BSA supplementation group showed significantly lower maturation rate than other groups ($p < 0.05$), however, cyclin B1 levels of 4 groups were not different. Unlike experiment 1, the maturation rate showed no significant correlation with protein level. And in experiment 3, the maturation rate and protein level of activated groups were significantly higher compare to control group ($p < 0.05$). And also the maturation rate showed good positive correlation with protein level ($R = 0.865$, $p < 0.01$).

