을 2번 반복하였다. 이런 과정을 일주일에 3회씩 4주간 시행하고 고환을 적출하였다. 적출된 고환은 hematoxylin-eosin으로 염색하였고, 고환으로부터 추출한 단백은 Western 분석을 시행하였으며 HSP 70 단백 발현을 보기 위해 면역조직화학 염색을 시행하였다.

결 과: 조직학적 소견상 II군에서 정조세포와 정모세포의 수가 감소하고, 성숙한 정자세포는 관찰되지 않았다. III군에서는 I군과 비슷한 정자생성이 관찰되었다. Western 분석 소견에서 HSP 70 단백은 3군 모두에서 상당량 발현되었다. 특히 II군에서 I군에 비해 HSP 70 단백은 약 1.5배 정도 증가되어 발현되었으나 ($p=0.075$), III군에서는 I군과 비교해 HSP 70 단백 발현은 비슷하였다 ($p=0.934$). 면역조직화학적 소견에서 HSP 70 단백에 대한 면역반응성은 Leydig 세포와 섬유모세포에서 관찰되었고, 3군 모두에서 발현부위는 유사하였다. Western 분석 소견과 동일하게 고온욕군에서 면역반응성이 증가하였으나 고온욕 후 저온욕을 시행한 군에서는 대조군과 유사하였다.

결 론: 고온에 노출된 고환은 정자생성능력이 감소될 뿐만 아니라 HSP 70 단백이 현저히 증가하여 고온에 대한 방어기전으로 HSP 70 단백이 과발현되었다. 그러나 고온직 후 저온에 노출했을 때 정자생성능력이 보존되었고 HSP 70 단백의 발현이 대조군과 유사한 소견을 보였는데, 이러한 결과는 온도의 변화가 고환에서 HSP 70 단백 발현에 영향을 미친다는 것을 시사한다. 향후 고환이 고온에 노출시 HSP 70 단백이 증가하였다가 저온시 감소하였는지 혹은 고온직 후 저온에서는 고온에 대한 HSP 70 단백과 발현이 영향을 받지 않았는지에 대한 추가적인 연구가 필요하다.

O-5 Expression of Fra1 and Fra2 mRNA Regulated by 17β-estradiol in the Ovariectomized Mouse Uterus

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Objectives: The early response genes induced by steroid hormones include important regulatory genes, such as transcriptional regulatory factors. The aim of this study was to determine the expression of Fos-related antigen (Fra-1, Fra)-2 mRNA gene and the localization of protein regulated by 17β-estradiol in ovariectomized mouse uterus.

Materials and Methods: Ovariectomized (Ovx) ICR mice were treated with injection of 17β-estradiol (300 ng/mouse) that was dissolved in sesame oil vehicle, and they received a single S.C (0.1 ml/mouse) injection. A pure estrogen receptor (ERs) antagonist, ICI 182780 (500 μg/mouse) was injected 30 min before steroid treatment. Mice were killed 0, 2, 4, 6, and 12 hr after estrogen injection by cervical dislocation. The levels of transcription factors were examined by reverse transcription - polymerase chain reaction (RT-PCR). To determine whether these mRNAs were translated, cellular distribution of these protein was investigated by immunohistochemistry.

Results: The levels of Fra1 and Fra2 were peaked at the time of 4 and 2 hr in the uterus of Ovx mouse

after 17β-estradiol injection, respectively, then these mRNAs were returned to basal levels after 12 hrs. Also ICI 182780 clearly blocked the effect of estrogen. In the pregnancy day, Fra1 level was not increased, but Fra2 level was increased to peak at the time of day 1 and 2. In the preimplantation mouse embryo, these mRNA were detected from germinal vesicle oocyte stage to blastocyst stage *in vivo*.

Conclusions: Taken together, these results suggest that the expression of Fra1, Fra2 is up-regulated by estrogen, and may play an important role in the response of the uterus to estrogen.

O-6 Expression of Apoptosis Gene Bok in the Rat Ovary

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Bok, Bcl-2-related ovarian killer, is a proapoptotic Bcl-2 family protein identified in the ovary based on its dimerization with the antiapoptotic protein Mcl-1. The present study examined the hormonal regulation and localization of Bok messenger RNA levels in the rat ovary during the follicle development by Northern blot analysis and in situ hybridization, respectively. Northern blot analysis of ovaries obtained from immature rats revealed increasing levels of Bok mRNA during postnatal development. The major cell types expressing Bok mRNA were the granulosa cells of preantral and atretic follicles. Treatment of immature rats with diethylstilbestrol (DES) for 24~48 h increased ovarian Bok mRNA levels. Bok mRNA was remained the same levels in rats removed DES for 24~48 h to induce apoptosis. High signals of Bok mRNA after DES treatment were detected in granulosa cells of early antral follicles. Treatment of immature rats with pregnant mares' serum gonadotropin (PMSG) for 12 h increased markedly ovarian Bok mRNA expression which was detected mainly in preantral and atretic follicles. Interestingly, low levels of Bok mRNA were also expressed in granulosa cells of preovulatory follicles. Treatment of PMSG-primed rats with human chorionic gonadotropin (hCG) stimulated strongly ovarian Bok mRNA expression at 6~12 h. At that time, Bok mRNA was expressed in granulosa cells of atretic and small growing follicles. In adult estrus cyclic ovaries, Bok gene expression was higher on proestrus and estrus. Moreover, the highly increased expression of Bok mRNA was found in rat ovaries at 48 h after hypophysectomy. These results demonstrate Bok is one of proapoptotic Bcl-2 members expressed in early growing and atretic follicles during the ovarian follicular development. Gonadotropins induce a transient increase of Bok gene expression in granulosa cells of preantral and preovulatory follicles indicating some role in ovulatory process.