P-22 생쥐원시난포의 체외성장, 성숙 및 발생능력에 관한 연구

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대부분의 포유동물 원시난포는 태아 또는 분만직후, 가장 높은 수로 증가되나 이후 매우 급격하게 그 수가 감소되는 것으로 알려져 있다. 비록 이들 태아 또는 태생 후의 성숙개체에서 난포 또는 난자를 회복시키려는 노력으로 난소조직 배양 혹은 체외배양체계를 통한 난자회수에 대한 연구가 되었으나 아직 많은 실험 연구를 요구하고 있다.

본 실험은 생쥐를 모델로하여 생후발달시기별로 난소로부터 회수되는 난자수를 극대화하고 발생능력의 적정시기를 선정하기위하여 실시하였다. 생쥐난포내의 난자를 체외성장(in vitro growth; IVG), 체외성숙 (in vitro maturation; IVM), 및 체외수정 (in vitro fertilization; IVF)의 체계화와 난소내의 원시난포의 회수의 가능성을 조사하기 위하여 첫단계로 생후 0, 5, 10 및 15일령 ICR 생쥐의 난소를 각각 20, 15, 10 및 5일 배양하였다. 또한 현재까지의 예비실험에서 Waymouth medium을 기초배양액에 성장인자를 Fetuin, SIT, Fetuin+SIT를 첨가하여 배양체계를 확립하였으며, 각 배양된 난소로부터 분리된 난포란을 분석한 결과 IVG, IVM의 가능성을 보여줌으로써 이들 난자의 체외수정에 의해 성숙 이후의 수정능력, 발생능력을 검토중이다.

P-23 Expression of Luteinizing Hormone (LH) gene in the rat ovary

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Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are synthesized and secreted by gonadotrophs in anterior pituitary, and they play a pivotal role in both male and female reproduction. The gonadotropins are composed of two subunit, a common α (Cα) and the unique β-subunit, which determine the biological specificity of the hormones. Although the pituitary is thought to be the only source of LH for a long time, recent studies demonstrated the novel expression of LH β-subunit genes in the rat testis. So far, the function of testicular LH is not clearly understood, one possible role of the hormone is modulation of spermiogenesis since the transcripts for LH β-subunit are predominantly detected in differentiating spermatids. The present study was performed to analyze the expression of LH gene in the rat ovary, a female counterpart of testis.

Expression of LH β-subunit gene in the rat ovary was demonstrated by amplification of ovarian RNA by reverse transcription-polymerase chain reaction (RT-PCR). Similarly, the transcripts for the common α-subunit in the ovary were detected by RT-PCR. To assess the LH production in the rat ovary, radioimmunoassay (RIA) was performed using antibody which can recognize rat LH β-subunit polypeptide. Significant amount of LH-like molecules were detected in crude ovarian extracts, and the competition curves with increasing amount of tissue extracts were

parallel with those of standard peptide, indicating that the ovarian immunoreactive LH-like material is similar to authentic pituitary LH molecule. In conclusion, these findings demonstrate that genes for LH subunit are expressed in the rat ovary, and suggest that LH can play an central role in regulation of female reproduction with both endocrine (i.e. pituitary LH) and auto-/paracrine (i. e. ovarian LH) manner. Studies are currnetly carried out to analyze the exact structure of the transcript and the regulation mechanism of the gene expresison.

P-24 Detection of Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and its receptor gene in the rat uterus and oviduct

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a hypothalamic neuropeptide, and with vasoactive intestinal peptide and growth hormone releasing hormone, belongs to the secretin / glucagon hormone family. PACAP exerts a potent stimulatory action on cyclic AMP production in anterior pituitary cells and promotes the release of several hormones from pituitary. Recent evidence clearly shown that PACAP transcript with novel exon 1 and/or transcripts with higher molecular weight than that of hypothalamic form are detected in the rat testis and ovary, indicating the existence of local production and function of PACAP. In fact, PACAP might serve as a autocrine and/or paracrine regulator for gonadal steroidogenesis. The preasent study was performed to analyze the expression of PACAP and its receptors in the rat uterus, a candidate for novel extrahypothalamic source and target.

In the adult rat uterus and oviduct, expression of the PACAP gene was demonstrated by amplification of uterine and oviductal RNA by reverse transcription-polymerase chain reaction (RT-PCR). The 3' rapid amplification of cDNA end (RACE) technique was applied to analyze the PACAP coding region and 3' untranslated region; PCR products with identical size were detected from uterus/oviduct samples and from all known PACAP sources. In addition, RT-PCR using specific primers for the PACAP type I receptor yielded products of expected sizes with RNAs from rat uterus and oviduct. Our findings demonstrate that both PACAP and PACAP receptor genes are expressed in the rat uterus and oviduct, where they could play an autocrine and/or paracrine roles on uterine/oviductal function such as secretion and muscle contraction for the transport of fertilized eggs.