論　文　内　容　の　要　旨

Synopsis of Thesis

氏　　名 Name	伴田 美佳
論文題名 Title	Trends in Plasma Kisspeptin Level and Cellular Experiment Insights During Progestin-Primed Ovarian Stimulation in Assisted Reproductive Technology (生殖補助医療におけるProgestin-Primed Ovarian Stimulation法の調節卵巣刺激周期中のヒト血漿キスペプチン値の変動と細胞実験による洞察)

論文内容の要旨

[目的：Purpose]

The global prevalence of infertility is 8%–12%, with assisted reproductive technology (ART) as an effective treatment method. The appropriate choice of controlled ovarian stimulation (COS) is crucial for high-quality oocyte collection. Recently, progestin-primed ovarian stimulation (PPOS) has become popular, although the exact mechanism by which it suppresses ovulation remains unclear. Kisspeptin, a hypothalamic neuropeptide hormone encoded by the Kiss1 gene, acts through the hypothalamic kisspeptin 1 receptor (Kiss1R) to induce the release of endogenous gonadotropin-releasing hormone (GnRH), which in turn increases follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion from the pituitary gland, thereby inducing ovulation. One of the functions of progesterone (P4) in humans is to suppress GnRH and LH secretion, and thus ovulation, but the detailed mechanism of action remains unclear. This study aimed to investigate the trends in plasma kisspeptin levels during PPOS and to explore changes in kisspeptin expression with progestin administration in mHypoA-50 cells, derived from mouse AVPV kisspeptin-expressing neurons.

[方法：Methods]

Plasma kisspeptin-54 levels were measured using an enzyme-linked immunosorbent assay kit, and serum levels of FSH, LH, E2, and P4 were determined. mHypoA-50 cells were cultured and treated with E2, GnRH, and Progestin, chlormadinone acetate (CMA). Quantitative real-time PCR with Taqman™ Fast Advanced Master Mix was used to measure the mRNA expression of kisspeptin. Gene expression was normalized to histone 3a mRNA levels, which were used as internal controls for the gene expression assay. Each measurement is based on three biological replicates, and the values are presented as the means ± standard error of the mean (SEM).

[結果：Results]

Plasma kisspeptin levels during PPOS

The median age and anti-müllerian hormone level were 32.0 years (interquartile range [IQR]: 30.0–37.0) and 4.88 (ng/mL) (IQR: 4.12–5.42), respectively. While E2 serum levels continuously increased, no significant differences in plasma kisspeptin-54 and serum LH values were found during PPOS.

Effect of CMA on Kiss-1 gene expression in mHypoA-50 cells

We investigated the effect of CMA on *Kiss-1* gene expression, which was increased with E2 and GnRH treatment, in mHypoA-50 cells. E2 stimulation significantly increased *Kiss-1* mRNA expression in mHypoA-50 cells by 1.47 ± 0.13-fold at 100 nM of E2 ([vehicle vs. E2], $P < 0.05$) and 1.06 ± 0.13- fold at 100 nM of E2 + 1.5 ng/mL of CMA ([E2 vs. E2 + CMA], $P < 0.05$). GnRH stimulation significantly increased *Kiss-1* mRNA expression in mHypoA-50 cells by 1.41 ± 0.01-fold at 100 nM of GnRH ([vehicle vs. GnRH], $P < 0.001$) and 1.05 ± 0.07-fold at 100 nM of GnRH + 1.5 ng/mL of CMA ([GnRH vs. GnRH + CMA], $P < 0.001$).

[総括：Conclusion]

Our human plasma analysis during PPOS and the additional experiments with mHypoA-50 cells indicate that PPOS with progestin significantly suppressed the LH surge rate, which may be due to the suppression of *Kiss-1* gene expression in the hypothalamus. PPOS effectively suppressed the LH surge rate, possibly through the inhibition of *Kiss-1* gene expression in the hypothalamus. These findings suggest that PPOS with progestin may offer a promising approach for controlling ovulation in ART.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究では、不妊治療（生殖補助医療）における調節卵巣刺激法（COS）の一つである「プロゲスチンを用いた卵巣刺激法（Progestin-Primed Ovarian Stimulation: PPOS法）」の排卵抑制の機序に関する知見を示した。PPOS法による卵巣刺激中のヒト患者の血漿キスペプチン濃度の推移及びマウス視床下部由来のキスペプチンニューロン細胞株における検討からプロゲスチン製剤がキスペプチンの発現を抑制し、排卵抑制に寄与している可能性を示唆した。

PPOS法の治療有効性は議論の余地があると報告されているが、本研究はPPOS法の生理学的作用機序の解明に着目し、キスペプチンの発現の抑制を介してLHサージ（排卵を引き起こすホルモンの急増）を効果的に抑制しCOSとして有効な治療法であることを示した。

本研究はPPOS法の生理学的評価や作用機序の解明の一端を示し、現在汎用されているPPOS法の更なる治療有効性の検討に繋がる有益な知見であり、学位に値すると考える。