P-24 The Comparison of Angiogenic Factor Levels between Mouse Ovarian Tissues with Vitrification and Slow-freezing

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Objectives: Angiogenic factors are essential for neovascularization in frozen-thawed ovarian tissue. This study was conducted to evaluate and compare the vascular endothelial factor (VEGF) and angiopoietin after cryopreservation of mice ovarian tissue using vitrification and slow-freezing method.

Methods: The ovaries recovered from ICR mouse were divided into three groups: 1) ovarian tissue without cryopreservation (control, group I), 2) ovarian tissue vitrified with VFS-40 (vitrification, group II), and 3) ovarian tissue slowly frozen with DMSO (slow-freezing, group III). Thawing was carried out at room temperature. RT-PCR was used to identify the levels of VEGF and angiopoietin in mouse ovarian tissue.

Results: mRNA levels of VEGF-1 and -3 were significantly decreased in group II and III than group I (control) ($p<0.05$). VEGF-1 and -3 mRNA levels was significantly lower in group II than in group III ($p<0.05$). mRNA levels of angiopoietin-1 and -2 were significantly decreased in group II and III than group I (control) ($p<0.05$). Angiopoietin-1 mRNA level was significantly lower in group II than in group III ($p<0.05$), whereas angiopoietin-2 mRNA level was not significantly different between group II and III.

Conclusion: These results show that VEGF and angiopoietin could be damaged by cryopreservation of ovarian tissue. Slow-freezing seem to be better method for preservation of VEGF and angiopoietin than vitrification of the mouse ovarian tissue.

P-25 VEGF Level in Ovarian Tissues after Heterotopic Autotransplantation in ICR Mice

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Objectives: The ovarian tissue banking is a promising technique for the preservation of fecundity in young female cancer patients. Revascularization plays a critical role in successful ovarian tissue transplantation, and vascular endothelial growth factor (VEGF) is a principal factor that promotes neovascularization. This study was designed to access the VEGF levels in cryopreserved ovarian tissue after heterotopic autotransplantation in ICR mosue.

Methods: The ovarian tissues were obtained from 5 or 6 weeks aged ICR mouse. Ovarian tissues were divided into three groups: 1) ovarian tissue without cryopreservation (control, group I), 2) ovarian tissue vitrified with VFS-40 (vitrification, group II), and 3) ovarian tissue slowly frozen with DMSO (slow-freezing, group III). Thawing was carried out at room

temperature. VEGF levels were checked in ovarian tissues of three groups recovered on day 7 after cryopreservation, also checked on day 14 after autotransplantation. Western blot analysis was used to identify the levels of VEGF in mouse ovarian tissues.

Results: In cryopreserved ovarian tissues, VEGF protein levels were significantly decreased in group II and III than group I (control) ($p<0.05$), whereas VEGF protein levels was not significantly different between group II and III. In autotransplanted ovarian tissue, VEGF protein levels showed similar pattern with cryopreserved ovarian tissue. Primary and antral follicular density in autotransplanted ovarian tissue were significantly decrease in group II and III than group I ($p<0.05$), both. No significant differences were found in the density of primary and antral follicles in both groups.

Conclusion: Disruption of VEGF expression induced by cryopreservation seems to affect the angiogenesis and folliculogenesis in the autotransplanted ovarian tissue. Future studies should investigate to improve the preservation of VEGF after cryopreservation.

P-26 설치류 정소에서 Nestin 발현

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Objectives: Nestin은 intermediate filament(IF) protein의 6번째 class에 속하는 protein으로 neural precursor의 marker로 사용된다. Nestin은 주로 신경줄기세포나 근육줄기세포의 초기 발달 단계에서 발현된다. 세포가 분화함에 따라 nestin 발현량은 감소하며, 다른 IF 단백질로 전환된다. 성체조직에서 nestin은 신경줄기세포같은 일부 세포 집단에서만 발현되는데 조직의 손상 후 회복과정에서 발현량이 증가하는 양상을 보이기도 한다. 생쥐나 흰쥐의 정소에서 nestin은 발달과정동안 다양한 세포유형에서 발현하며 출생 후 발현이 감소한다.

Methods: 신생기, 사춘기, 성체기 생쥐 정소에서 total RNA와 protein을 분리하였다. 분리한 total RNA를 이용하여 최적화된 RT-PCR로 nestin mRNA의 발현량을 분석하고, protein을 이용하여 western blot 방법을 이용하여 nestin protein 발현량을 분석하였다. 또한 동시기 흰쥐 정소의 냉동절편을 획득하여 nestin 항체를 이용하여 immunohistochemistry를 수행한 후 confocal microscopy를 이용하여 nestin의 발현위치를 확인하였다.

Results: Immunohistochemistry 결과, 1주령 흰쥐의 정소에서 nestin은 Sertoli cell 세포질에서 강하게 발현하고 Leydig cell에서도 약하게 발현되었다. 2주령과 4주령 흰쥐의 정소에서는 Leydig cell과 Sertoli cell의 세포질에서 강하게 발현되었다. 반면, 8주령 흰쥐의 정소의 경우, Leydig cell과 Sertoli cell의 세포질에서는 약하게 발현하였다. 생쥐 정소의 total RNA에 대한 정량적 RT-PCR 결과 nestin mRNA 발현량은 2주령에서 가장 많이 발현되고, 이 후 급격히 감소하는 것을 확인하였다. 생쥐 정소에서 분리한 interstitium에서 nestin mRNA 발현에 대한 정량적 RT-PCR 결과 1주령과 2주령에서 nestin이 강하게 발현하다가 사춘기가 지나 성체가 되면 발현량이 급격히 감소함을 확인하였다. 반면 steroidogenic enzyme 유전자인 3β-HSD와 17β-HSD는 사춘기 이후 발현이 급격히 증가함을 확인하였다.

Conclusion: Nestin은 흰쥐 정소 내 Sertoli cell과 Leydig cell에서 발현되며 이들 세포의 분화에 따라 발현이 감소한다. 특히 성체정소의 분화된 Leydig cell에서 발현이 감소하므로 미분화 Leydig cell의 표식자로 이용 가능할 것으로 사료된다.