Objectives: (1) To evaluate sub-lethal plasma membrane damage of sperm during cryopreservation-thawing by the assessment of phosphatidylserine (PS) translocation, (2) to examine the relationship between generation of reactive oxygen species (ROS) and cryopreservation-related membrane alterations, and (3) to study the value of membrane PS translocation for prediction of the ability to survive cryopreservation.

Design: Prospective cohort study.

Materials and Methods: Sub-fertile semen samples (n=10) were studied. Membrane PS translocation was evaluated with annexin V binding and generation of ROS was measured by chemiluminescence using luminol. Motility parameters were assessed by computer analysis. All measurements were performed in purified semen fractions with high and low sperm motility (Percoll density gradient separation) independently, before and after cryopreservation-thawing using TEST-Yolk buffer and glycerol.

Results: Freezing-thawing induced significant levels of PS externalization in both sperm fractions. However, the induction of PS translocation in the fractions with high sperm motility was significantly higher than that of the fractions with low sperm motility. Significantly higher ROS levels were detected in pre-freeze samples of the fractions with low sperm motility compared to the fractions with superior motility. After freezing-thawing, ROS levels were significantly reduced in both fractions. There was a significant and negative correlation between annexin V staining before freezing and the ability to survive cryopreservation in the fractions with high sperm motility ($r=-0.45$, $p=0.006$). Furthermore, levels of annexin V staining $<10\%$ in a given fraction with high sperm motility predicted a $>45\%$ cryosurvival rate, with a sensitivity of 83% and a specificity of 80%.

Conclusions: (1) Cryopreservation-thawing induced membrane PS translocation; (2) the effects of cryopreservation on sperm plasma membrane integrity were not associated with an increase in ROS generation as detected by chemiluminescence; and (3) the degree of annexin V binding before freezing may be used to predict the ability to survive cryopreservation-thawing.

P-380

Effects of Cryopreservation in Hamster Oocytes. [1]M. S. Parisi, [2]J. Notrica, [1]M. N. Parisi, [2]E. Polak de Fried. [1]University of Buenos Aires, School of Medicine, Department of Physiology and [2]CER Medical Institute, Department of Reproductive Medicine, Buenos Aires, Argentina.

Objectives: Osmotic water permeability (Posm) of oocyte membrane would play a fundamental role in cryopreservation and thawing procedures. It has recently reported that rat oocytes lost a mercury sensitive water channel during their arrival to maturity (aquaporin 9). During cryopreservation oocytes suffer dramatic osmotic changes which are reflected in the very low survival rates after thawing. The aim of this study was to evaluate, in hamster oocytes, the eventual role of water channels during the cryopreservation procedure.

Design: Prospective analysis of possible relationship between cryopreservation and biophysical properties of the oocyte membrane.

Materials and Methods: 33 metaphase II Golden Hamster oocytes previously stimulated with gonadotropins were recovered. 17 out of 33 oocytes were cryopreserved by slow freeze-rapid thaw 1.2 propanediol and sucrose planner method (GROUP A). The remaining 17 were not cryopreserved (GROUP B). GROUP A and GROUP B were divided in two groups: control and mercury chloride ($HgCl_2$) treated, in which oocytes were exposed to an isotonic solution 0.3 mM of $HgCl_2$ before Posm measurement. Posm, expressed as cm. $10^{-4}.s^{-1}$, was calculated in all oocytes by creating an osmotic gradient and assessing volume changes in relation to time by means of an original video microscopy technique.

Results:

	Posm (X ± SEM) (cm. $10^{-4}.s^{-1}$)		
	Group A	Group B	
Control	18.07 ± 1.13	12.05 ± 1.03	P<0.01
$HgCl_2$ treated	13.88 ± 1.74	11.56 ± 1.07	
	NS	NS	

Conclusions: Our results are in concordance with the following hypothesis: 1) Mature hamster oocytes, as previously reported in rat, does not express mercury sensitive water channels. 2) The observed increase in Posm, in cryopreserved oocytes, may reflect membrane damage inducing this non specific permeability increase.

P-381

Resumption of the Human Primordial Follicle Growth in Xenografts After Vitrification of the Ovarian Tissues.* [1,2]K.-A. Lee, [2]S. H. Lee, [2]S. J. Yoon, [1,2]D. H. Choi, [1,2]T. K. Yoon, [1,2]K. Y. Cha. [1]College of Medicine, Pochon CHA University, [2]Infertility Medical Center, CHA General Hospital, Seoul, Korea.

Objective: The present study was conducted to evaluate the viability of germ cells from the fetal ovarian tissues after vitrification followed by xenografting.

Design: Case Report.

Materials and Methods: Human fetal ovarian tissues aged 22 weeks in gestation was obtained and sliced (<1 mm^3). Fetal ovarian tissues were cryopreserved by vitrification with 5.5 M ethylene glycol and 1.0M sucrose as cryoprotectants (EG5.5). Tissues were pre-equilibrated with EG5.5 at room temperature for 5 minutes and plunged into liquid nitrogen immediately. Frozen-thawed tissues were xenografted into NOD-SCID mice to evaluate the viability and capacity for further growth of the primordial follicles. Female and male recipients were used to compare the differences. Through a small dorso-lateral transverse incision of the skin, pieces of ovarian tissues were inserted into the subcutaneous space above the flank. Transplantation was performed bilaterally and animals were allowed to recover. Grafts were recovered from the recipients 4 weeks after transplantation and histological analysis was evaluated.

Results: Grafts recovered 4 weeks after transplantation contained less number of oocytes and primordial follicles compared to that of the fresh tissues. Survived follicles were mainly primordial and intermediary with larger diameter and more granulosa cells. The mean diameter of follicles from the male recipient mice was 40.28 ± 1.10 μm (n=76), while that from the female recipient mice was 40.45 ± 0.67 μm (n=36). There was no statistical difference in the follicular diameter between female and male recipients ($p>0.5$). The follicular diameter increased significantly ($p<0.05$) in grafts (40.39 ± 0.57 μm; n=112) when compared to that of the fresh tissues (28.33 ± 1.09 μm; n=15).

Conclusion: It is confirmed that the fetal primordial follicles survived after vitrification and resumed the growth.

** Supported by a grant (HMP-98-M-5-0054) of the Good Health R&D Project, Ministry of Health & Welfare, Republic of Korea.*

P-382

Effect of Cryopreservation on Sheep Ovarian Tissue Comparing Two Cryoprotectants: Dimethylsulfoxide (DMSO) and 1,2-Propanediol. [1,2]A. Gutiérrez, [1]M. A. Corona, [1]M. A. Vargas, [2]P. Méndez-Sashida, [2]M. S. Flores, [2]E. Gallardo. [1]Biotecnología y Reproducción Especializada, Rancho Santa María de Bolaños, Cd. Manuel Doblado Guanajuato, México and [2]Instituto de Medicina Reproductiva del Bajío, Hospital Aranda de la Parra, León Guajauato, México.

Objectives: Ovarian tissue cryopreservation is now becoming an important issue because of a steadily improvement on long-term survival rates for young people with malignant diseases with the additional need of preserving their fertility. The objectives of this study were: (1) to determine the feasibility of freezing and thawing ovarian tissue and (2) to compare the results of post-thaw effects with two different cryoprotectants: DMSO and propanediol.

Design: Prospective and experimental study assessing the viability of ovarian tissue after freezing and thawing with DMSO or propanediol. It was performed in 15 sheep undergoing bilateral ooforectomy for further autotransplantation.

Materials and Methods: Bilateral oophorectomy were performed in 15 Kathadin sheep. The ovarian tissue was placed in α-minimum essential medium (α-MEM). The ovarian cortex was dissected using a scalpel to obtain pieces no thicker than 2 mm. The cortex was then divided into three pieces, one fixed in formaldehyde and sent to histologic evaluation and two frozen in either DMSO (1.5 M) or propanediol (1.5 M). Samples were gently rolled to promote rapid equilibration for 30 minutes at 4°C in 1ml cryogenic vials. Then, the samples were frozen under a slow freezing