

Results: Table I. ZP thinning (TH) versus total ZP opening (TO).

	Group I (TH)	Group II (TO)	p
No. of patients	61	56	
Age	32.3 ± 3.8	31.6 ± 4.5	0.36
Embryos transferred	2.97 ± 0.9	2.96 ± 0.9	0.95
ZP thickness (μm)	17.3 ± 1.9	18 ± 2.6	0.22
Pregnancy rate per transfer	21.3%	25%	0.66
Implantation rate	11.6%	13.2%	0.74

Conclusion: There was no significant difference in AH efficacy when the procedure was executed with TH or TO of the ZP in thawed embryos. However, non-total opening (thinning) of the ZP may prevent the risk of early loss of blastomeres, embryo infections and the prophylactic use of antibiotics.

P-013

Transfer of Frozen/Thawed Embryos After One Day of Culture Optimizes Embryo Selection and Improves Pregnancy Rate. H. R. Santos, P. F. Vélez, V. G. García, C. G. Martínez, V. P. Galache, A. S. Hernández.

Objective: The worldwide trend in IVF is to extend in vitro culture of embryos to optimize natural embryo selection. This concept is mostly applied to fresh embryo transfer cycles. In this study we analyzed pregnancy rate after transfer of frozen/thawed embryos left for one day in culture.

Design: Retrospective clinical study.

Materials and Methods: Two groups of patients were analyzed. Group I included 74 patients having three or more excess embryos, frozen on the second day of development, and undergoing embryo transfer 24 hrs. after the thawing. Group II were 183 patients who had embryo transfer the same day of the thawing (day 2). Both groups were comparable for age, duration of infertility and protocol of hormonal replacement. Frozen embryos transfer from egg donation cycles were excluded.

Results: In group I, a total of 385 embryos were thawed, 296 (77%) survived and 243 (63%) were transferred (3.28 embryos per patient). Clinical pregnancy was established in 29 patients (39%). The implantation rate was 14% and the livebirth rate was 29%. In Group II, a total of 707 embryos were thawed, 587 (83%) survived and 580 (82%) were transferred (3.16 per patient). Clinical pregnancy was established in 34 patients (19%). The implantation rate was 6% and livebirth rate was 15%. The difference in pregnancy rate, implantation rate and livebirth rate between groups was p: 0.086, p: 0.05 and p: 0.01 respectively.

Conclusions: The transfer of embryos 24 hours after thawing increase pregnancy rate and improves significantly implantation and livebirth rates. The concept of extending in vitro culture can be also applied to frozen embryo transfer cycles. The in vitro culture may help frozen embryos to recover from the thermic changes and may further optimize embryo selection.

P-014

High-Order Multiple Gestations Can Be Limited by Controlling the Number of Completely Intact Embryos Transferred in Frozen-Thawed Embryo Transfer (FET) Cycles. M. S. Opsahl, M. E. Geltinger, S. H. Black, K. L. Blauer, S. R. Lincoln, E. F. Fugger. Genetics & IVF Institute, Fairfax, VA.

Objectives: IVF is an unquestionably important treatment for infertile couples. An undesirable but unavoidable risk of cotransferring several embryos is multiple gestations. Avoidance of all dizygotic multiple gestations cannot be achieved without a substantial reduction in cycle success rates. We evaluated our FET data for factors affecting the frequency of high-order multiple gestations.

Design: Retrospective study design from a large private infertility center.

Methods: Retrospective review of 1380 consecutive frozen-thawed non-donor embryo transfer cycles after conventional IVF or ICSI and 402 after donor IVF cycles. The number of 100%-intact embryos transferred was the most significant variable predicting a live-born delivery (stepwise logistic regression) and this variable was used to evaluate the probability of high-order multiple gestations.

Results: 186 pregnancies resulted in delivery of one or more infants after 1380 non-donor FET cycles; and 90 followed 402 donor FET cycles. No high-order multiple gestation occurred with ≤2 100%-intact embryos in non-donor cycles or with <2 100%-intact embryos in donor cycles regardless of the number of additional <100%-intact embryos transferred. No high-order multiple births occurred when only 2 donor 100%-intact embryos were transferred alone.

Donor cycles were more likely to result in pregnancy and high-order multiple births (p=0.04).

% Embryos transferred with 100% blastomere survival	Non-donor			Donor		
	Number of cycles	Live-born preg	Triples+ (% of preg)	Number of cycles	Live-born preg	**Triples+ (% of preg)
0	223	8.1%	0%	61	16.4%	0%
1	405	8.6%	0%	105	12.4%	0%
2	409	14.7%	0%	118	23.7%	10.7%
3	267	19.1%	3.9%	93	28.0%	3.7%
4	67	29.9%	10.0%	23	43.5%	20.0%
5	9	22.2%	0%	2	50.0%	0%
Total	1380	13.5%	2.1%	402	21.9%	6.7%

* In many cases one or more embryos with <100%-intact blastomeres were also transferred.

**Only one quadruplet pregnancy occurred.

Conclusions: Our data indicates that limiting the number of 100%-intact embryos transferred in FET cycles will decrease high-order multiple gestations particularly in donor FET cycles. For those couples who are particularly risk adverse, limiting non-donor FET cycles to ≤2 100%-intact embryos and donor FET cycles to one 100%-intact embryos plus any <100% embryos or 2 100%-intact embryos only will almost completely avoid the risk of high-order multiple gestation but at the expense of a reduction in liveborn infants. Overall, the risk of a high-order multiple gestations is well under 10% for both non-donor and donor FET cycles.

P-015

Cryopreservation of Human Blastocysts Produced Through Co-Culture After ICSI. S. J. Park, H. J. Cho, S. H. Yoon, W. Y. Son, W. D. Lee, J. H. Lim. Maria Infertility Clinic, Seoul, Korea.

Objectives: This study was to investigate the freezing-survival rate of human blastocysts produced through co-culture after ICSI and their viability in uterus after transfer.

Design: We have frozen only blastocyst stage embryos in all cycles with surplus blastocyst(s) from August 1995. Until October 1999, 1,581 cycles were received 4,117 of frozen-thawed blastocysts, 177 cycles of them were patients with blastocysts (n=49) obtained after ICSI. The survival rate and FHB(+) rate of frozen-thawed blastocysts were analyzed between IVF-ET and ICSI-ET during this period.

Materials and Methods: All zygotes derived from IVF or ICSI were co-cultured with cumulus cells in 10 μl YS medium containing 10/20% hFF and ET was performed on day 3 or 5. When the surplus embryos reached the expanding blastocyst stage (size: 160 μm & containing distinct ICM), they were cryopreserved. The pre-freeze equilibration of blastocysts was carried out in 5% glycerol and then 9% glycerol plus 0.2M sucrose. The cooling rates of blastocysts for cryopreservation were at -2°C/min to -7°C, -0.1°C/min to -8°C, -0.3°C/min to -38°C and plunging into -196°C LN₂ in turn. The frozen blastocysts were thawed in 36°C water bath and glycerol and sucrose were removed through 7 down-dose drops step by step. The frozen-thawed blastocysts were washed twice in PBS supplemented with 20% hFF, co-cultured with cumulus cells in a YS medium containing 20% hFF for 18 h and then transferred into uterus of natural cycle.

Results: The rates of blastocyst on day 5 in ICSI-derived embryos and IVF-derived embryos were 42.5% and 58.6%, respectively. The survival rate and the viability of frozen-thawed blastocysts generated after IVF and ICSI were as follows:

	Transfer of frozen-thawed blastocysts	
	IVF group	ICSI group
No. of replaced cycles	1,404	177
No. of thawed blastocysts	5,578	696
Survival rate (no.)	74.1% (4,133)	72.0% (501)
No. transferred blastocysts	3,650	439
Clinical pregnancy rate (no.)	35.9% (504)	32.2% (57)
Implantation rate (FHB no.)	19.2% (701)	20.7% (91)

Conclusion: The result in this study indicates that although the developmental competence of embryos derived from ICSI was lower than that derived from IVF, the freezing-survival rate of human blastocysts produced through co-culture after ICSI and their viability after uterus transfer should not be lower as compared with those after IVF.

P-016

The Egg or the Endometrium: Why Do PCO Patients Have Higher Rates of Miscarriage? A. B. Copperman, H. Applebaum, D. Osborne, T. Mukherjee.

Objective: Patients with polycystic ovarian syndrome appear to be at increased risk for pregnancy loss. Oocyte quality in women with polycystic ovaries may be diminished as a result of abnormal gonadotropin dynamics or a suboptimal follicular environment as a result of high follicular fluid androgen levels. Poor follicular development may also impair endometrial development, creating a hostile environment for the developing conceptus. We evaluated a large series of recipients who had implanted eggs from donors with a "PCO-like" response to gonadotropins to determine whether pregnancies from these donors' eggs conveyed a comparable miscarriage rate to women who received eggs from donors with a lower response.

Design: Retrospective analysis.

Setting: Mount Sinai Medical Center Ovum Donation Program Patients.

Methods: The records of 122 consecutive recipients were evaluated for a variety of factors including endometrial thickness, pattern, presence of anti-phospholipid antibodies, and number of eggs produced. In cases in which a donor's eggs were divided among two recipients, the donor was classified by the number of eggs produced (not the number of eggs allocated to each recipient). Using an ROC curve, we determined a cutoff value of 33 to classify donors as high responders. Recipients were then divided into Group I (recipients of donors making 33 or less eggs) and Group II (recipients of donors making more than 33 eggs).

Results: Donors produced 22 ± 11 eggs. There were no significant differences between Group I and Group II recipients with respect to age, endometrial thickness, peak estradiol, presence of male factor, and presence of antiphospholipid antibodies. The pregnancy rate in Group I recipients was 52/99 (53.5%) which was not different from the pregnancy rate in Group II recipients 13/23 (56.5%). This spontaneous abortion rate was significantly higher in Group II, 3/13 (23%) than in Group I, 3/52 (0.06%) ($p < 0.05$). The mean donor age was similar in patients who miscarried and patients who did not. The mean number of eggs produced was not significantly different in patients who miscarried (26.9) and patients who conceived but carried to term (23.4).

Conclusions: It appears that the higher rate of spontaneous abortion found in patients with polycystic ovaries may be related to defects in egg quality, rather than in impaired endometrial development. Further prospective data must be gathered in order to further clarify whether the oocyte quality defect is genomic or is a consequence of an impaired follicular apparatus.

P-017

Hydrosalpinx Fluid Effect on the Murine Embryonic Development in a Coculture System With Epithelial Endometrial Cells. ¹I. Carrasca, ²E. Cebral, ¹R. Benitez, ¹P. López, ¹D. Vantnian. ¹Institute of Maternal and Child Research, University of Chile, San Borja-Arriaran Clinical Hospital, Santiago, Chile. ²Center of Pharmacology and Botanic Studies. CONICET. Buenos Aires, Argentina.

Objective: The aim of the present investigation was to assess whether a coculture system protects the effect of the hydrosalpinx fluid (HF) on

murine embryo development evaluated though the number of blastocyst cells.

Design: Controlled prospective study.

Materials and Methods: Hydrosalpinx fluid was collected from patients with hydrosalpinx undergoing laparotomy. Embryos were obtained from superovulated CF1 mice and two-cell embryos were collected by flushing the oviducts with Human Tubal Fluid (HTF) with Hepes. Human endometrium was obtained by biopsies from 6 normal and 6 hydrosalpinx patients during the secretory phase of the menstrual cycle. Epithelial cells were separated after collagenase (0.1%) digestion and cultured until monolayers in Dulbecco's modified Eagle's Medium containing insulin (5 $\mu\text{g}/\text{mL}$) and fetal bovine serum (10%). A mean of 10 to 15 murine embryos were exposed in the absence or presence of different concentrations of HF (control 0% HF, 50% HF, 70% HF and 100% in HTF) on a simple culture (SCS), epithelial coculture (ECS) and hydrosalpinx epithelial coculture (HECS) systems. Embryonic development was evaluated at 72h and blastocyst cell number was determinate by Tarcowsky method. Data was analyzed using Wilcoxon test.

Results: In SCS, 91.9% of the embryos reached blastocyst stage, no significant differences were showed with the presence of HF. However, significant differences were observed in the blastocyst cell number: control: 94.2 ± 4.4 ; 50%: 70.7 ± 5.3 ; 70%: 56.3 ± 5.1 , 100%: 46.8 ± 4.8 , $p < 0.0001$. Embryos cultured in ECS, 97.1% reached blastocyst stage and high concentrations of HF caused a decrease on the embryonic development, 75.6% and 70.9% with 70% and 100% respectively ($p < 0.05$). Significant difference was observed between ECS and HECS on embryo development without HF, 97.1% and 79.5% respectively ($p < 0.05$). No differences were observed in HECS or ECS in the HF concentrations tested. However, these were clear in the blastocyst cell number: ECS (control: 90.2 ± 4.9 ; 50%: 65.7 ± 3.9 ; 70%: 47.6 ± 6.3 , 100%: 39.0 ± 7.5 , $p < 0.0001$) and HECS (the results were control: 66.6 ± 6.1 , and 44.6 ± 3.4 , 49.5 ± 3.0 and 34.3 ± 2.6 at 50%, 70% and 100% of HF, $p < 0.0001$).

Conclusions: High HF concentrations exert a dose-dependent deleterious effect in both, on the embryos and endometrium using the blastocyst cell number. Endometrium from hydrosalpinx biopsies have an intrinsic damage which does not increase with the exposure to high concentrations of HF. Supported by Sociedad Chilena de Fertilidad.

P-019

Factors Affecting the Survival and Pregnancy Rate in Frozen-Thawed Embryo Transfers. ¹J. W. Kim, ¹H. K. Byun, ¹H. W. Youm, ¹J. R. Jun, ¹Y. S. Park, ²J. Y. Jun. ¹Laboratory of Reproductive Biology & Infertility, ²Department of OB/GYN Samsung Cheil Hospital & Women's Healthcare Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Objective: The purpose of this study was to determine the important factors affecting survival and pregnancy rate in frozen-thawed embryo transfer cycles.

Design: A retrospective analysis of all individuals undergoing frozen-thawed embryo transfers from January, 1996 to December, 1999 at our institution was performed.

Materials and Methods: We performed retrospective analysis in 1099 consecutive cycles of frozen-thawed embryo transfers in relation to the insemination methods (conventional IVF and ICSI), the freezing stage of embryos (pronuclear stage, multicellular stage and combined stage), patient's age (<31, 31–35, 36–40, >40), infertility factors (male factor, tubal factor and other female factor) and the origin of injected sperm (ejaculated, epididymal and testicular) in ICSI cycles. After conventional IVF or ICSI, we transferred 4 or 5 embryos to the patients and the supernumerary PN stage zygotes or multicellular embryos were cryopreserved by slow freezing protocol with 1,2-propanediol (PROH) as a cryoprotectant. We compared the survival rate of thawed embryos and pregnancy outcome after frozen-thawed embryo transfers. The survival rate and pregnancy rate were analyzed by Chi-square test of ABstat program (rel 6.54, Anderson-Bell Co.).

Results: The survival rates of thawed embryos were 70.3% (2382/3386) in conventional IVF group and 70.8% (2440/3446) in ICSI group. After frozen-thawed embryo transfers, the pregnancy rate of ICSI group (26.8%) was significantly higher than conventional IVF group (22.6%). But, there was no significant difference in the survival and pregnancy rates according to the freezing stage (pronuclear stage, multicellular stage and combined stage), patient's age. However, the pregnancy rate of male factor infertility (31.4%) was significantly higher than the tubal (22.6%) and other female