

**MATERIALS AND METHODS:** Cycles of IVF conducted between January 2010 and January 2013 at Maria Fertility Hospital in which late cleaved and rescue ICSI embryos were reviewed. The patients were divided in five groups: group A (n=17), Frozen-thawed late cleaved embryo transfer cycles, group B (n=5), Frozen-thawed rescue ICSI embryo transfer cycles in failed conventional insemination (CI), group C (n=9), Frozen-thawed rescue ICSI embryo transfer cycles in additional IVM, group D (n= 16), Fresh rescue ICSI embryo transfer cycles in failed CI, group E (n=66), Fresh rescue ICSI embryo transfer cycles in additional IVM. Cryopreserved embryos were transferred to patients either in natural cycle or in a hormonal cycle. Late cleaved and rescue ICSI embryos were frozen on day 4 using vitrification. Late cleaved and rescue ICSI embryos were thawed on 1 day before ET. Fresh embryo transfer was performed on day 3.

**RESULTS:** There were no differences in patient age, mean number of transferred embryos and endometrial thickness. Otherwise, pregnancy rates were improved in froze-thawed late cleaved embryo transfer (36.8%). In rescue ICSI cycle, the pregnancy rates were significantly higher in group B and C (60.0% and 22.2%) than in group D and E (6% and 3%).

**CONCLUSION:** Low pregnancy potentials of the late cleaved and rescue ICSI embryos can be overcome by frozen-thawed procedure. It seems that the frozen-thawed procedure of the late cleaved and rescue ICSI embryos enabled more appropriate synchronization between embryonic and endometrial development. Therefore the late cleaved and rescue ICSI embryo cryopreservation may be an effective alternative to fresh embryo transfer.

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**P-118** Tuesday, October 15, 2013

**KRIOBLAST™, A SYSTEM FOR KINETIC VITRIFICATION BY HYPERFAST COOLING: APPLICATIONS FOR REPRODUCTIVE & STEM CELLS.** I. I. Katkov,<sup>a,b</sup> V. F. Bolyukh,<sup>a,c</sup> Y. Liu,<sup>b</sup> D. Wu,<sup>b</sup> E. Y. Snyder,<sup>b</sup> S. Agarwal.<sup>d</sup> <sup>a</sup>CELLTRONIX, San Diego, CA; <sup>b</sup>Stem Cell Core, Sanford-Burnham Medical Research Institute, La Jolla, CA; <sup>c</sup>Electrical Engineering, National Technical University "KhPI", Kharkov, Kharkov Region, Ukraine; <sup>d</sup>Reproductive Medicine, UCSD School of Medicine, University of California at San Diego (UCSD), La Jolla, CA.

**OBJECTIVE:** Kinetic (very rapid) vitrification (K-VF) is a promising approach for cryopreservation (CP) of biological materials as it is simple, robust, and can achieve VF for practically any type of cells (Katkov et al, 2012).

**DESIGN:** Several methods of superfast K-VF, particularly for CP of oocytes, embryos, sperm and human embryonic stem cells have been proposed but practically all of them either require very small (in range 0.5-10 mcl) size of the sample or/and cannot avoid the Leidenfrost effect (LFE), which substantially impedes the rate of cooling.

**MATERIALS AND METHODS:** Here, we are reporting an entirely new system for hyper-fast cooling of one-two order of magnitude larger samples developed in CELLTRONIX, which we called "KrioBlast(TM)" that completely eliminates LFE and a need of potentially toxic and osmotically damaging vitrificants such as DMSO or PG used in the current methods of VF.

**RESULTS:** The system allowed us to vitrify up to 4,000 mcl of 15% glycerol solutions (used as a cooling rate marker), which theoretically corresponds to the critical cooling rate up to 600,000 C/min (Warkentin et al, 2008) or even higher. Particular applications for CP of reproductive and stem cells for reproductive and regeneration medicine, husbandry, and cryopreservation of wildlife genetic resources are discussed. Some promising preliminary data of hyper-fast of human sperm and embryonic stem cells are also presented.

**CONCLUSION:** The hyperfast method we proposed is scalable, automated, and robust to the human's error. This approach can "unify" the equipment, the accessories, and the vitrification media in one platform. This KrioBlast(TM) platform can be considered as a step toward the "Universal Cryopreservation Protocol" that will largely benefit Assisted Reproductive Technologies and Regenerative Medicine.

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**VITRIFICATION OF OOCYTES IS USEFUL FOR FERTILITY PRESERVATION IN COUPLE OF NON-OBSTRUCTIVE AZOO-**

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**OBJECTIVE:** Oocyte vitrification method has been improved over the past decade. The couples with severe male infertility are insufficient for fertilization of retrieved oocytes, and especially the couples suffered from non-obstructive azoospermia could not possible to fertilize with retrieved oocytes in IVF. In that case, vitrification of oocytes could be an effective method for future reference. The aim of this study is to find the outcomes of vitrified oocytes using donor sperm.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** Between Jan. 2009 and Dec. 2012, frozen oocytes from 6 patients of total 16 patients were thawed. And  $\geq 41$  yrs. and  $\geq 4$  cycles were excluded. Vitrification-thawed methods are followed our previous study, vol. 90 suppl. Sep. 2008 S282: F&S.

**RESULTS:** Clinical results are as follows.

Clinical Results.

No. of patients	6
Age(M $\pm$ SD)(yrs.)	29.2 $\pm$ 2.8
Infertility duration(M $\pm$ SD)	3.1 $\pm$ 2.0
No. of retrieved oocytes(M $\pm$ SD)	12.9 $\pm$ 4.8
No. of vitrified oocytes(M $\pm$ SD)	89(12.7 $\pm$ 5.0)
No. of survived oocytes(%)	85/89(95.5)
No. of 2PN(%)	65/80(81.3)
No. of embryos(%)	60/65(92.3)
No. of transferred embryos(M $\pm$ SD)	24(3.4 $\pm$ 1.0)
Pregnancy rate(%)	2/6(33.3)

**CONCLUSION:** Vitrification has been applied to human oocyte cryopreservation successfully so far. Furthermore this can induce more effective results in severe male factor like non-obstructive azoospermia in IVF.

**P-120** Tuesday, October 15, 2013

**TRANSFER OF DAY-3 EMBRYOS IN THE FRESH CYCLE AND SURPLUS BLASTOCYST IN THE THAWING CYCLE: ITS SUMMARY PREGNANCY CHANCE.** F.-T. Kung, Y.-J. Lin, F.-J. Huang, K.-C. Lan, P.-Y. Lin, H.-J. Chiang. OBGYN, Kaohsiung Chang Gung Memorial Hospital, Niao Sung District, Kaohsiung, Taiwan.

**OBJECTIVE:** To examine the pregnancy potential of thawing blastocysts which were originally frozen on day 5 after extended culture of day-3 surplus embryos in fresh cycles and to calculate the subsequent summary pregnancy rate.

**DESIGN:** Prospective observational study.

**MATERIALS AND METHODS:** All of surplus embryos on the day 3 of embryo transfer were kept cultured till day 5. Only the embryos able to grow up to blastocyst stage were frozen by using vitrification method. Those whose original fresh transfers were canceled with any reasons and who did not return for transfer yet were excluded from the study. Endometrium was prepared by clomiphene citrate stimulation or hormone replacement, or naturally. The summary pregnancy rate was defined as the number of pregnancies after transfer of sibling embryos both in fresh and thawing cycles divided by the number of patients.

**RESULTS:** A total of 107 fresh cycles had available blastocysts for freezing. Among them, 46 (43%) cycles in 46 patients had at least one thawing embryo transfer. Originally, the clinical pregnancy and implantation rates were 28.3% and 13.7%, respectively, in those 46 fresh cycles. Subsequently, 102 blastocysts were thawed and 99% of them survived. The mean of storage time was 9.5  $\pm$  10 months. Thirteen (28.3%) patients succeeded in pregnancies with an implantation rate 14.8%. There were no differences between success and failure in pregnancy groups in age, body mass index, embryo storage time, endometrial thickness and pre-freezing blastocyst quality. The summary pregnancy rate up to now was 56.5%.