

Table 1 – Comparison of competency of embryos using livebirth outcome

Results	+LB, competent embryos	no LB, incompetent embryos	P value
N	38	142	
Age	32.5 +/- 4.3	35.5 +/- 2.5	P < .0001
# Embryo transferred	1.2 +/- 0.4	1.9 +/- 0.7	P < .0001
# Cells on Day2	3.95 +/- 0.8	3.93 +/- 0.4	P = 0.89
Morphological grade on Day 2	0.6 +/- 0.9	0.6 +/- 0.6	P = 0.68
# Cells on Day 3	11.1 +/- 14.8	8.4 +/- 3.7	P = 0.06
Morphological grade on Day 3	0.38 +/- 0.38	0.64 +/- 0.53	P = 0.0012
Day 3 Compare and Select score	1.44 +/- 0.17	1.27 +/- 0.39	P = 0.0002
Morphological grade on day 5 ICM	1.03 +/- 0.16	1.15 +/- 0.43	P = 0.011
Morphological grade on day 5 TE	1.19 +/- 0.7	1.42 +/- 0.13	P = .18
Day 5 Compare and Select score	2.03 +/- 0.2	1.92 +/- 0.26	P = 0.020

of inner cell mass (ICM) and trophectoderm (TE) on day 5 were analyzed.

Results: Among the embryos, 38 resulted in livebirth. Demographics and analytic parameters are shown in [Table 1](#). In addition, C/S score vs morphological grade on Day 3 ($P = 0.049$) and day 5 ICM ($P = 0.006$) by McNemar's test indicate increased sensitivity of C/S scores and that Day 5 ICM scores are more important than day 5 TE scores.

Conclusions: While morphological grading is still necessary in the process of embryo selection for transfer, mathematical C&S shows a higher sensitivity as a diagnostic tool for pregnancy outcome.

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PA-6

Normal euploid blastocyst grown from a thawed day 2 slow frozen embryo after 14 years in cryostorage

Z Haimowitz¹, AL Akopians², M Surrey^{1,2}, H Danzer^{1,2}, S Ghadir^{1,2}, J Barritt^{1,2}

¹ ART Reproductive Center, Beverly Hills, CA

² Southern California Reproductive Center, Beverly Hills, CA

Objective: Cryopreservation, extended embryo culture and preimplantation genetic screening (PGS) has given patients increased reproductive potential. Over the years, cryopreservation has changed from slow-freezing with ~40% survival to vitrification with significantly higher survival. Extended embryo culture to the blastocyst stage has become routine. PGS has changed from cleavage stage biopsies with FISH on limited chromosomes to trophectoderm biopsies with Next Generation Sequencing (NGS) of all 24 chromosomes. This case report describes a patient who after having one child in 2002 returned 14.3 years later and requested thawing, blastocyst biopsy and NGS evaluation of her previously cryopreserved Day 2 embryos.

Design: To report on the laboratory outcomes of a patient's request to evaluate her 14-year-old cryopreserved Day 2 cleavage stage embryos for genetic normality.

Materials and methods: All cryopreserved Day 2 cleavage stage embryos were originally developed from oocytes aspirated in 2002. Fertilization was achieved by ICSI and 8 cleavage stage

supernumerary embryos were cryopreserved via a 2-step Propandiol slow-freeze technique (Irvine Scientific, Irvine, CA). The embryos were thawed in 2016 using a slow thaw technique (Embryo Thaw – Irvine Scientific) and cultured for up to 5 days in single step media (CSCMC – Irvine Scientific). Trophectoderm biopsies were performed on hatching blastocysts of fair quality and with evaluation by NGS (Reprogenetics, Livingston, NJ).

Results: A 39-year-old female underwent an IVF cycle in 2002 with a transfer of a Day 2 embryo which resulted in a live birth of a healthy female. The 8 remaining embryos were frozen on Day 2 via slow-freezing. Fourteen years later she returned requesting PGS of her frozen embryos. Eight embryos were thawed, 7 survived and were cultured for up to 5 additional days. One out of 7 embryos developed into a hatching blastocyst of fair quality on Day 6 and 2 achieved this stage on Day 7, all 3 underwent trophectoderm biopsy. Four embryos were discarded due to poor quality. PGS results indicated 1 normal euploid male embryo, while the other 2 displayed aneuploidy.

Conclusion: A single euploid male embryo was diagnosed after thawing, extended culture and PGS testing of a 14.3 year stored slow-frozen Day 2 embryo. To our knowledge this is the longest cryopreserved embryo that has been successfully thawed, cultured to a blastocyst, had a trophectoderm biopsy and was screened as genetically normal by NGS. We await the patient's decision as to if she will proceed with a frozen embryo transfer.

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PA-7

Sperm DNA fragmentation – does age really matter?

D Renuka, L Saravanan, A Kabeer, B Kumaran, P Sangeetha, R Mohan, J Morris, N Yegyan, T Mahendran
ARC International Fertility and Research Centre, Chennai, Tamilnadu, India

Objective: Male fertility declines with increase in age where every single delayed year accounts to a considerable difference. Impaired fertilization, poor embryo and blastocyst development, lower implantation rates and higher instances of miscarriage are the adverse effects of damages in sperm DNA, thereby affecting fertility. Factors contributing to sperm DNA damage can be both internal and external. Intrinsic factors include protamine deficiency, mutations that affect DNA compaction, and advanced paternal age. High levels of reactive oxygen species (ROS) are detected in the semen of infertile men which directly or indirectly influences DNA fragmentation. In addition, certain external factors such as heat, chemotherapy, radiation, and others are also associated with an increase in DNA damage. Cigarette smoking, genital tract inflammation, varicoceles, and hormonal deficiencies have all been found to be the factors of DNA damage. To top it all, age is a constant factor influencing DNA fragmentation and chromosomal abnormalities in the spermatozoa of infertile patients and fertile men.

Materials and Methods: A research study conducted at ARC International Fertility & Research Centre in Tamil Nadu, India monitored Sperm DNA fragmentation in 192 infertile males and a comparison was made with the men's age, using Sperm Chromatin Structure Assay (SCSA) at Andrology Center in Tamil Nadu for detecting broad range of DNA damage.

Results: The study found that DFI was better below the age of 40, as a result of which patients considering a delay in childbearing for social reasons or those seeking fertility treatments could be effectively counselled. Group A with 96 infertile men below age of 40 yrs., it was found that 44% of the men were affected by sperm DNA fragmentation and group B with 96 infertile men above age of 41 yrs., 63% of men were affected, those with the change in HDS above normal limits, would result in negative pregnancy outcome.

Conclusions: The variation proves the difference in age could make in sperm DNA fragmentation. Hence, age is found to be an important factor to be considered for male infertility.

PA-8**Striving for one embryo-one baby: how the integration of vitrification and Preimplantation Genetic Screening (PGS) technologies has impacted society**

S Zozula¹, JB Whitney¹, F Garner², RE Anderson³, MC Schiewe¹

¹ Ovation Fertility, Newport Beach, CA

² Ovation Fertility/FCLV, Las Vegas, NV

³ SCCRM, Newport Beach, CA

Objectives: Optimization of embryo culture practices led to our highest success rates following fresh embryo transfer (ET) in 2010, with live birth rates (LBRs) per ET ranging from 75.3% for donor egg (DE) cycles to 53.2%, 55.1%, 36.8% and 20.7% for women <35yo, 35-37yo, 38-40yo and 41-42yo, respectively, transferring a mean of 2.0 to 2.6 embryos. These success rates were favorable, ranking in the upper 10th percentile nationally. However, multiple births were excessive at 28-70% when transferring 2 blastocysts. Through elective vitrification and adoption of blastocyst biopsy/PGS, we aimed to improve the quality of patient care by transferring fewer embryos, reducing miscarriages and increasing single healthy live births.

Study design: Retrospective analysis of the annual national report by the Centers for Disease Control (CDC) from 2010 to 2015. Our goal was to contrast industry trends regarding fresh DE-ET (control group) to autologous frozen ET cycles, in number of embryos transferred, implantation rate, and LBR/ET, and assess how our clinical practices improved the quality of patient care.

Materials and Methods: Our routine laboratory procedures involve: ICSI, group culture in Global™ medium + 7.5% protein supplement,

tri-gas incubation, transvaginal ultrasound-guided ET, and microSecure vitrification of blastocysts in non-DMSO I.C.E. solutions (Innovative CryoEnterprises, NJ) with or without blastocyst biopsy.

Results: Although the mean number of fresh DE embryos transferred decreased from 2.0 to 1.6 between 2010 to 2014, respectively, little change in LBR (55.8% to 56.8%) occurred in the USA. Conversely, the same mean cryopreserved embryo number yielded increased ($P < 0.01$) implantation success (<34.3% to 43.7%), more singleton LBs (<31.9% to 36.5%) and higher total LBRs/ET (38.4% to 46.6%). With emphasis on PGS in 2014, our SCCRM affiliate clinic transferred 1.1 to 1.2 blastocysts/ET in women <35 yo, 35-37yo, 38-40yo and 41-42yo ($n > 250$ cycles), achieving implantation rates of 77.9%, 61%, 60% and 84%, respectively, and 71%, 69.4%, 71.4% and 64.3% in 2015 (per CDC). Transferring 1.0 blastocyst/ET in women <35yo in 2015, we produced the highest healthy singleton LBR (61.3%) reported by the CDC for any center.

Conclusion: The global adoption of highly reliable vitrification practices has facilitated significant, progressive improvements in IVF-FET outcomes. In conjunction with blastocyst biopsy/PGS, single euploid embryo transfer has optimized embryo utilization rates, and significantly elevated LBRs/ET in women under 38yo. Although the same trend was not significant on a per cycle basis in older women (38-42yo), it appreciably reduced the trauma/depression associated with pregnancy loss and reduced the time needed to achieve the desired outcome of healthy singleton births. Overall, vitrification and PGS allowed us to enhance ethical standards in patient care and improve the healthy singleton LBR.

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