CONCLUSIONS: The association between slower embryo development and aneuploidy is more pronounced with advancing age. This finding is consistent with prior studies using lower resolution to interrogate chromosome copy number, but this is the first and study to demonstrate this association using targeted NGS. While differences in the incidence of aneuploidy at discrete checkpoints of blastocyst development were observed, the use of time-lapse imaging in future studies may advance clinical understanding of the point in time when embryos become amenable to TE biopsy and the developmental threshold beyond which they are more likely to be aneuploid. Clinical studies should aim to determine whether the implantation potential of euploid embryos is impacted by their rate of development.

**P-113** Tuesday, October 31, 2017

**LOWER EMBRYONAL MITOCHONDRIAL DNA CONTENT IS ASSOCIATED WITH BETTER QUALITY EMBRYOS.** A. M. Klimczak,[a] L. E. Pacheco,[a] N. Massahi,[b] J. P. Richards,[c] W. G. Kearns,[b] A. F. Saad,[a] J. Crochet.[c] [a]Ob/Gyn, University of Texas Medical Branch, Galveston, TX; [b]AdvaGenix, Rockville, MD; [c]Center of Reproductive Medicine, Webster, TX.

OBJECTIVE: Currently, there is no consensus on whether mitochondrial DNA (mtDNA) content is associated with embryo quality. Our objective was to determine if mtDNA content is a predictor of embryo quality.

DESIGN: Retrospective chart review.

MATERIALS AND METHODS: We performed a retrospective chart review of subjects whose embryos underwent preimplantation genetic screening (PGS) in our center between 2013 and June 2016. A total of 259 subjects with 1510 blastocyst biopsies were included. Embryo grade was defined based on the Gardner blastocyst grading system as follows: 1—High (3-6 AA and 4-6 AB), 2—Mid (any BB, 1-3 AB, and 1-2 AA), and 3—Poor (any AC, CA, BC, CB, or CC). 'Embryonal mtDNA content' was defined as a ratio of mitochondrial DNA to nuclear DNA. Our primary outcome was to compare embryonal mtDNA content to embryo grade. For our secondary outcomes we compared mtDNA to age and repeated the analysis after excluding embryos with aneuploidy. Statistical analysis was performed using a multinomial logistic regression model of prediction and linear regression. $P<0.05$ was considered significant.

RESULTS: From a total of 1510 blastocyst biopsies, the majority of embryos consisted of Grade 1 (N=951; 62%), followed by Grade 2 (N=331; 21%) and Grade 3 embryos (N=228; 15%). Embryos with high mtDNA content were found to be of poorer quality (Grade 3) relative to grades 1 and 2. (RR 1.03 [95% CI 1.01-1.05]; P=0.003). Using a logistic model, mtDNA best predicted lowest and highest grades, but not mid-grade (Grade 2) embryos (data not shown). There was no correlation between mtDNA content and the subjects' age (R-square = 0.0018). After excluding embryos with aneuploidy, a total of 717 euploid embryos were identified. In this subpopulation, a non-statistically significant trend was observed where poor quality embryos had higher mtDNA content (OR 1.16, [95% CI 0.27-5.01]; P= 0.834).

CONCLUSIONS: Our study is the largest to evaluate the association between mtDNA content and embryo quality. A higher quantity of embryonal mtDNA content suggests a poorer quality embryo, possibly due to greater oxidative stress. Conversely, lower mtDNA content suggests a higher quality embryo. In euploid embryos, mtDNA content may not be predictive of embryo quality. Further clinical trials are needed to further elucidate this relationship.

TABLE.

| | | |
|---|---|---|
| Subjects | 259 | |
| Age (Median, range) | 35 [31-38] | |
| BMI (Median, range) | 25 [22-28] | |
| Ethnicity (N, %) | | |
| White | 157 (60) | |
| Black | 22 (8) | |
| Hispanic | 34 (13) | |
| Asian | 46 (17) | |
| | | Mitochondria DNA Content (Median, range) |
| All Embryos (N, %) | 1510 (100) | 0.000741 [0.0004375-0.00144] |
| High-1 | 951 (62) | 0.000624 [0.000363-0.00097] |
| Mid-2 | 331 (21) | 0.000623 [0.00037-0.00112] |
| Poor-3 | 228 (15) | 0.000656 [0.0003915-0.0009345] |
| Euploid Embryos (N, %) | 717 (100) | |
| High-1 | 521 (72) | |
| Mid-2 | 140 (20) | |
| Poor-3 | 56 (8) | |

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**P-114** Tuesday, October 31, 2017

**THE VALUE IN RETAINING NON-PRONUCLEAR (0PN) ZYGOTES FOR EXTENDED CULTURE.** M. K. Catala,[a] K. R. Sieren,[a] K. Silverberg,[b,a] M. D. VerMilyea.[a,b] [a]Ovation Fertility, Austin, TX; [b]Texas Fertility Center, Austin, TX.

OBJECTIVE: To determine the occurrence and ploidy status of usable embryos derived from oocytes not displaying two pronuclei (0PN) at the time of fertilization assessment.

DESIGN: Retrospective study in a private in vitro fertilization laboratory.

MATERIALS AND METHODS: A total of 3,370 blastocysts were identified as being morphologically suitable for fresh embryo transfer, vitrification or trophectoderm biopsy for genetic testing of ploidy status followed by vitrification (i.e. usable blastocysts). Usable blastocysts were obtained on Day 5, 6, or 7. Mature oocytes were fertilized using ICSI and pronuclear assessment was performed and recorded using an inverted microscope 16-18 h post insemination. Embryos with 2 pronuclei were separated from all other embryos arising from 0PN, 1PN, 3PN and ≥4PN zygotes. Embryos were group cultured in a continuous single culture medium (Irvine Scientific). Chromosomal copy number was assessed for suitable blastocysts with the Illumina MiSeq platform.

RESULTS: Over the span of 18 months, 830 fresh oocyte retrievals resulted in 7,258 zygotes with two haploid pronuclei (2PN). These 2PN zygotes developed into 3,349 usable blastocysts (46%). 2.9% of the oocyte retrievals had usable blastocysts derived from oocytes not displaying pronuclei (0PN) at the time of fertilization assessment. 24 usable blastocysts (from 0PN zygotes) from 24 different patients were vitrified. 14 (58%) of these blastocysts were untested for chromosomal copy number confirmation. 10 blastocysts derived from 0PN zygotes were screened by PGS. Four embryos (44%) were reported as normal while two embryos were reported as abnormal XO and three embryos as complex abnormal XY. No transfer of a usable blastocyst derived from a 0PN has yet occurred.

CONCLUSIONS: Our results demonstrate that usable blastocysts derived from zygotes with abnormal pronuclear (0PN) formation can develop into a chromosomally normal embryo. However, these embryos may also have a high possibility of multiple chromosomal gains or losses. These results are similar to previous reports of developmental potential, chromosomal analysis and ongoing pregnancies from zygotes containing abnormal pronuclei[1-3]. Patients should be counseled regarding the chromosomal abnormalities associated with untested usable blastocysts derived from atypical zygotes. Further investigation of ploidy status of usable blastocysts derived from abnormal zygotic embryos with a single pronucleus (1PN), three pronuclei (3PN) or higher (≥4PN) may provide further information regarding the retention value of abnormal pronuclear zygotes.

Ploidy status of Usable 0PN Blastocysts

| Embryo | Trophectoderm Diagnosis |
|---|---|
| 1 | Complex +5, +8, +16; XY |
| 2 | Abnormal XO |
| 3 | Complex -2, -3, +6, -8, -12; XY |
| 4 | Complex +3, -5, +19, -15, -16, -17, -18. -19, +20, +21; XO |
| 5 | Abnormal XO |
| 6 | Normal XX |
| 7 | Normal XX |
| 8 | Normal XY |
| 9 | Normal XY |
| 10-24 | Unknown |

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**P-115** Tuesday, October 31, 2017

**ULTRA-LOW (2%) OXYGEN TENSION POSITIVELY AFFECTS BLASTOCYST QUALITY.** A. F. Ferrieres-Hoa,[a] A. Gala,[b] D. Haouzi,[c] K. Roman,[a] S. Hamamah.[d] [a]ART/PGD Department, CHRU Montpellier, Montpellier Cedex 5, France; [b]Chu Montpellier, Montpellier, France; [c]U1203 - IRMB - CHRU Montpellier, Montpellier, France; [d]ART/PGD Department, Montpellier, France.

OBJECTIVE: Improving the ex-vivo culture conditions of early embryos is still the main challenge in ART. The oxygen concentration is one of the criteria that influence protonated culture. In the uterus, early embryos are exposed to an ultra-low (1.5%) $O_2$ atmosphere. Moreover, embryo damage caused by reactive oxygen species (ROS) is related to the $O_2$ tension during in vitro culture. Currently, the traditional 6%/20% of $CO_2/O_2$ or 6%/5% $CO_2/O_2$ conditions are the most currently culture parameters used for early human embryo culture. However, few studies investigated the in vitro culture conditions under more physiological $O_2$ tension. The present study investigates the effect of ultra-low (2%) $O_2$ tension on blastocyst development and useful blastocyst (UB) formation rate.

DESIGN: This is a single-center observational study, performed from January 2016 to April 2017. The endpoint for the study was whether $O_2$ tension could enhance blastocyst development. The relationship between the oxygen tension and standard morphological evaluation according to the Gardner grading system was also examined: at day 5/6, full (grade 3), expanded (grade 4), hatching (grade 5) or fully hatched (grade 6) blastocysts with at least a grade B trophectoderm quality were considered as UBs.

MATERIALS AND METHODS: Day 1 embryos were cultured in 6% $CO_2$ and 5% $O_2$ for three days; and then in 6% $CO_2$ and 2% $O_2$ atmosphere from day 3 until day 5/6 (ultra-low $O_2$ group, n=779 embryos, n=137 patients, mean women age 33.1 years $\pm$ 4.78) or in 6% $CO_2$ and 5% $O_2$ (controls, n=169 embryos, n=42 patients, mean women age 33.1 years $\pm$ 4.66).

RESULTS: Blastocyst formation rate was 51 % (397/779) in the ultra-low $O_2$ group and 52.1% (88/169) in the control group (p= 0.8). The UB rate was significantly higher in the ultra-low $O_2$ group (282/397; 71.0%) than in the control group (40/88; 45.5%) ($p<0.0001$). Clinical pregnancy rate per transfer was higher but not significantly in the ultra-low $O_2$ group (29/89, 32.6% versus 6/23, 26.1%, p 0.76). There was no significant difference in mean patient's age between the two groups.

CONCLUSIONS: Our study demonstrates that ultra-low $O_2$ tension is associated with higher blastocyst quality compared to low $O_2$ tension, supporting the hypothesis that more physiological culture conditions improve early embryo development.

*Supported by:* This work was partially supported by a grant from the Ferring Pharmaceutical Company. The authors declare ni conflict of interest.

**P-116** Tuesday, October 31, 2017

**IDENTIFICATION AND CHARACTERIZATION OF AMYLOID-LIKE SUBSTANCE IN MOUSE OOCYTES AND EMBRYOS.** R. N. Pimentel,[a] F. H. Wang,[b] L. G. Robinson,[b] R. A. Radjabi,[c] P. A. Navarro,[d] D. L. Keefe.[e] [a]OB/GYN, NYU Research Scientist, Specialist in Human Reproduction, Goiania, Brazil; [b]NYULMC, New York, NY; [c]Reproductive Endocrinology and Infertility, NYULMC, New York, NY; [d]Department of Obstetrics and Gynecology, Faculty o, Ribeirao Preto, Brazil; [e]ObGyn, New York University Langone Medical Center, New York, NY.

OBJECTIVE: Mammalian oocytes are unique among germ cells in that they age precociously. The cellular and molecular mechanisms driving oocyte aging remain poorly understood. During meiosis in yeast, an RNA-binding protein, with a predicted prion domain, assumes an amyloid-like configuration to translationally repress cyclins and ensure homologous chromosome segregation. These findings suggest that prion domains enable formation of amyloid-like effectors in diverse settings. Since amyloid-like molecules can accelerate aging in long-lived, post mitotic cells, such as neurons, we sought to identify and characterize amyloid-like substance in oocytes, cleavage stage embryos, and blastocysts, using a murine model.

DESIGN: Experimental pilot study.

MATERIALS AND METHODS: Metaphase II mouse oocytes and *in vivo* fertilized embryos (1 cell, 2 cells, 4 cells, 8-12 cells, morulas and blastocysts) (n=20 samples for each stage) (Embryotech Laboratories, Inc.,Wilmington, MA) were fixed and immunostained for evaluation of amyloid-like substance, using an anti-amyloid antibody (Fibrils OC Antibody [AFOC] Millipore). Imaging was performed using a confocal microscopy (Zeiss 880 laser scanning confocal microscope with a 63X Plan-Apochromat N.A. 1.4 objective). Image analysis was used to quantify immunostaining across early development.

RESULTS: In all 140 samples immunostaining for amyloid-like substance appears throughout the zona pellucida, as well as in the cytoplasm and nucleus of oocytes, polar bodies and preimplantation embryos. In oocytes, a large amount of amyloid-like substance was distributed heterogeneously throughout the cytoplasm. Less staining was identified in the polar body. In 1 and 2 cell-stage embryos, distribution within the cytoplasm was more homogeneous and more intense immunostaining was visualized in the nucleus. In 4 cell stage embryos, the amyloid-like substance was proportional between nucleus and cytoplasm. In morula stage embryos, a clear predominance of immunostaining for amyloid-like substance was observed in nuclei compared to cytoplasm. In blastocyst stage embryos, a greater concentration of amyloid-like substance appeared in inner cell mass, with nuclear staining more prominent than cytoplasmic. Trophoblast cells were devoid of amyloid-like substance. Descriptive data regarding the presence and distribution of amyloid plaques are presented.

CONCLUSIONS: We demonstrate for the first time the presence and distribution of immunostaining for an amyloid-like substance in mammalian oocytes and embryos. Since amyloid-like substances can produce cellular pathology, futures studies should investigate the role of amyloid-like substance in oocyte aging.

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*Supported by:* Support from FAPESP, Brazil (Process 2015/21907-0) (PN) and the Stanley H. Kaplan Endowment Fund.

**P-117** Tuesday, October 31, 2017

**IMPORTANCE OF MULTINUCLEATION AT THE TWO-CELL STAGE IN EMBRYO DEVELOPMENT.** S. Arrones-Olmo, A. Genoves, I. Martinez, I. Cuevas. Reproductive Medicine Unit, Hospital General Universitario de Valencia, Valencia, Spain.

OBJECTIVE: To study the impact of multinucleation at the 2-cell stage human embryos in vitro development, implantation and abortion rate.

DESIGN: A retrospective morphokinetical analysis of 323 Known Implantation Data (KID) embryos was performed. Analysis consisted on comparing KID and abortion relative to 2-cell stage multinucleated versus non-multinucleated embryos. We included here clinical and biochemical abortions.

MATERIALS AND METHODS: Embryos were cultured at MIRI-TL® incubator with Single Culture Media (Irvine®). Data were analysed by Student's t-test using SPSS 15.0 v. statistics software.

RESULTS: From the 323 KID embryos, 76 were multinucleated at the two cell stage (23,5%). Among them, 26 were KID+ and 50 KID- (34,2% vs. 65,6% respectively). The other 247 embryos (76,5%), showed no multinucleation; 61 were KID+ (24,7%) and 186 were KID- (75,3%). No significant differences were found between implantation status (KID+/KID-) of multinucleated and non-multinucleated embryos. Regarding abortion, no