Demographic Characteristics and Laboratory Data

	0%	0%	<20%	<20%	>70%	>70%
	Conventional (n=17)	ICSI (n=13)	Conventional (n=23)	ICSI (n=15)	Conventional (n=237)	ICSI (n=104)
Age	40.9±3.9	41.8±1.9	38.3±6.4	40.3±5.5	37.1±6.6	38.5±5.7
BMI	25.6±6.5	24.8±5.1	25.6±6.1	23.3±4.0	25.8±5.1	25.9±5.7
Day 3 FSH	9.2±5.6	8.6±4.4	8.3±3.7	9.1±5.4	6.8±3.7	7.0±3.4
Oocytes Retrieved	4.5±4.8	3.8±2.3	12.9±7.3	9.3±4.7	12.3±7.9	12.0±8.3
Day 1 Ongoing	0	0	1.5±0.7	1.3±0.6	10.0±6.2	9.5±6.5
Day 5 Ongoing	0	0	0.3±0.5	0.5±0.5	4.2±4.2	5.3±5.7
ET count	0	0	1.1±0.8	0.7±0.6	2.6±1.6	1.3±1.8
SA Initial Total Motile	15.9±13.5	9.5±5.6	16.4±14.8	11.6±7.9	18.7±24.2	15.8±22.5
SA Initial Motility	42.0±9.7	40.8±12.4	42.1±11.3	11.6±7.9	47.2±12.1	40.4±14.6
SA Initial Concentration	57.2±29.7	42.5±26.5	59.8±31.9	50.7±17.7	60.2±23.3	51.6±25.3

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DYNAMIC ANALYSIS OF HUMAN PARTHENOGENETIC ZYGOTES INDUCED BY ARTIFICIAL OOCYTE ACTIVATION. A. Tanaka, K. Yumoto, K. Iwata, C. Mizoguchi, M. Tsuneto, Y. Mio. Reproductive Centre, Mio Fertility Clinic, Yonago, Japan.

OBJECTIVE: The origin of the pronucleus (PN) in a single PN zygote (1PN), and whether its genome is normal still remains controversial. We recently established a novel method of discriminating between maternally- and paternally-derived PN using immunofluorescence staining and demonstrated the possibility that both the male and female genome could be packed in 1PN in some cases. However, currently analyzing karyotypes is an invasive technique, limiting its clinical application. Therefore, we tried to distinguish between normally-fertilized zygotes and 1PN zygotes by their morphology or developmental behavior. In this study, we used a microscope with time-lapse system to analyze the developmental time course and morphology of human 1PN zygotes, especially parthenogenetic zygotes induced by artificial oocyte activation.

DESIGN: Research study.

MATERIALS AND METHODS: This study used 32 MII oocytes donated between October 2014 and August 2015 by patients who gave informed consent for this study. Fresh or freeze-thawed MII oocytes were activated electronically and the oocytes were observed by EmbryoScope®. We compared the developmental time course and morphology between parthenogenetic zygotes and normal 2PN zygotes fertilized by assisted reproductive technology.

RESULTS: There was no difference in the diameter of the PN in parthenogenetic zygotes compared to the female PN in normal fertilized zygotes (28.9 $\pm$ 2.2 vs 26.4 $\pm$ 2.0 μ m, respectively). There were significant differences between normal 2PN and parthenogenetic zygotes for the time from intracytoplasmic sperm injection or electronic activation to the 2nd polar body (PB) extrusion (3.0 $\pm$ 1.7 vs 2.3 $\pm$ 0.5 h, respectively), from 2nd PB extrusion to syngamy (20.8 $\pm$ 4.1 vs 18.7 $\pm$ 2.9 h, respectively), from syngamy to 1st cleavage (3.0 $\pm$ 2.3 vs 3.9 $\pm$ 1.1 h, respectively), and from 1st cleavage to 2nd cleavage (9.8 $\pm$ 4.8 vs 13.6 $\pm$ 5.2 h, respectively). In addition, some parthenogenetic zygotes (6 of 21) developed to blastocysts.

CONCLUSIONS: The time required from electronic activation to 2nd PB extrusion and from 2nd PB extrusion to syngamy in parthenogenetic zygotes was significantly shorter than in normal zygotes. This may be because oocyte activation or decondensation of the sperm nucleus is not required in parthenogenetic zygotes. In addition, the time required from syngamy to 1st cleavage, and from 1st cleavage to 2nd cleavage in parthenogenetic zygotes was significantly longer than in normal embryos. Thus, differences in the time course of embryonic development in activated zygotes could be used to identify the characteristics of parthenogenetic zygotes, even though further studies are needed. Furthermore, although some parthenogenetic zygotes develop to blastocysts, the clinical use of zygotes with 1PN should be questioned.

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EFFECT OF OOCYTE ACTIVATION WITH CALCIUM IONOPHORE ON ICSI OUTCOMES IN PATIENTS WITH PREVIOUS FERTILIZATION FAILURE. S. A. Hebisha,[a] B. A. Aboelazm,[a] H. M. Adel,[a] A. A. Aboali,[a] A. I. Ahmed.[b] [a]Gynecology, Alexandria University - Faculty of Medicine, Alexandria, Egypt; [b]Obstetrics and Gynecology, MFM Division, Department of Medical Genetics, Perinatology Research Branch, Wayne State University, Detroit, MI.

OBJECTIVE: To evaluate the efficiency of chemical oocyte activation with calcium ionophore on fertilization and pregnancy outcomes after intracytoplasmic sperm injection (ICSI) in patients with previous fertilization failure.

DESIGN: Prospective controlled study.

MATERIALS AND METHODS: One hundred and eight patients with history of previous fertilization failure undergoing ICSI treatment with long agonist protocol were randomly divided into two groups: group A (n=54) and group B (n=54).

A total of 756 metaphase II (MII) oocytes were retrieved . In the oocytes of group A(n=350 oocyte), routine ICSI was applied; while oocytes in group B (n=406 oocyte) were entered in culture medium supplemented with 5 μM calcium ionophore (A23187) for 10 minutes and then washed at least five times with MOPS solution immediately after ICSI.

In both groups, the fertilization was evaluated after 16-18 hours.

RESULTS: The number of fertilized oocytes and embryos obtained were significantly different between two groups (p=<0.001*).

Fertilization rate was significantly higher in group B -where calcium ionophore was applied-compared to group A-control group-(32.2% vs. 9.1%, respectively, p=0.01*).

Cleavage rate also was significantly higher in group B compared to group A (26.7% vs 6.25% respectively, p=0.028*). Implantation rate was significantly higher in group B than in group A (17.64% vs. 2.41% respectively, p=0.035*).Pregnancy rate also was significantly higher in group B than in group A (21% vs. 3.7% respectively, p=0.042*).

CONCLUSIONS: Chemical oocyte activation with calcium ionophore resulted in a significant improvement in fertilization, cleavage, implantation and pregnancy rates after ICSI in infertile patients with previous fertilization failure.

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THE POLAR BODY DOES NOT ACCURATELY REPRESENT THE MITOCHONDRIAL MUTATIONAL LOAD WITHIN ITS CORRESPONDING OOCYTE. J. Kofinas,[a] L. Robinson,[b] M. Seth-Smith,[c] Y. G. Kramer,[d] D. H. McCulloh,[e] F. Wang,[b] J. Grifo,[f] D. L. Keefe.[g] [a]Division of Reproductive Endocrinology/Infertility, NYU, New York, NY; [b]Department of ObGyn, NYU, New York, NY; [c]NYU, New York, NY; [d]OBS-GYN, NYU Fertility Center, New York, NY; [e]Obstetrics and Gynecology, New York University Fertility Center, New York, NY; [f]NYU Langone Medical Center, New York, NY; [g]ObGyn, New York University Langone Medical Center, New York, NY.

OBJECTIVE: Mutations in mitochondrial DNA (mtDNA) cause a number of diseases in offspring. Since mtDNA mutations are transmitted exclusively through the oocyte, various manipulations of the oocyte cytoplasm have been proposed to prevent transmission of mtDNA diseases. A method to estimate mtDNA load at the oocyte level would help balance the risk/benefit of oocyte manipulations. We sought to determine whether polar bodies (PB) accurately represent the mitochondrial mutational load in their corresponding oocytes.

DESIGN: Translational science research.

MATERIALS AND METHODS: IRB approval was obtained for use of human discard material and a total of 10 oocyte-PB pairs were obtained. Immature oocytes received 2 hours post retrieval were placed in an incubator for 48 hours and those that matured were subsequently frozen. Prior to freezing, oocytes were processed with pronase and all excess cumulus cells were removed. The PB was then removed mechanically and frozen separately from the oocyte in PBS solution. A real time PCR assay was developed to measure mitochondrial copy number and the presence or lack thereof of the 4977 base pair common deletion (via the presence of the ND4 gene). The absolute mtDNA copy number minus the ND4 gene copy number divided by the absolute copy number yielded the deletion ratio. Paired t-test analysis was used for statistical analysis

RESULTS: In all ten oocyte-PB pairs, the mutational load in the PB exceeded that in the associated oocyte. The mean deletion ratio in oocytes was 30% and 61.8% in PBs (p= 0.001). The largest percent difference between the oocyte and PB mtDNA deletion ratio was 76%. Prediction of oocyte deletion ratio based on PB deletion ratio is shown with the equation [Oocyte deletion ratio=0.6064(PB deletion ratio) - 0.0708], however this relationship did not reach statistical significance.

Comparison of oocyte and PB deletion ratios

Oocyte Number	Oocyte deletion ratio	PB deletion ratio
1	0.34	0.62
2	0.45	0.68
3	0.66	0.90
4	0.57	0.62
5	0.21	0.97
6	0.06	0.24
7	0.56	0.64
8	0.15	0.56
9	0.02	0.62
10	0.02	0.33

CONCLUSIONS: It has been suggested that the PB can be used to estimate mitochondrial mutational load. Here we show that the PB overestimates the mutational load in the oocyte (as high as 76% in some cases). Higher deletion ratios in PBs tended to correlate with higher deletion ratios in associated oocytes. Larger numbers are needed for confirmation, but even from the sample size studied the PB deletion ratio clearly is not representative of its corresponding oocyte.

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CYTOKINE ANTIBODY ARRAY PROFILING IN HUMAN FOLLICULAR FLUID AS A POTENTIAL MARKER FOR OOCYTE QUALITY. M. Pavone,[a] J. M. Kelsh,[b] S. Malpani,[c] R. Confino,[a] S. Jasti,[b] S. M. Briley,[b] J. X. Zhang,[a] F. E. Duncan.[b] [a]Obstetrics and Gynecology, Northwestern, Chicago, IL; [b]Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; [c]Northwestern University, Chicago, IL.

OBJECTIVE: Advanced reproductive age and increased adiposity are associated with negative in vitro fertilization (IVF) outcomes, but the underlying mechanisms are unclear. The microenvironment in which an oocyte develops can significantly impact its quality. Therefore, the objectives of this study were to assess the cytokine profiles of human follicular fluid and to determine how they change with age and body mass index (BMI).

DESIGN: Translational.

MATERIALS AND METHODS: Follicular fluid from the first follicle retrieved at the time of IVF from a total of 26 patients age 27-44 years was run in duplicate on Human Cytokine C5 Antibody Arrays (Ray Biotech). Probed arrays were developed, intensities were quantified, and data were normalized and analyzed. Information including age, BMI, Anti-Mullerian hormone (AMH) levels, and infertility diagnosis was also collected.

RESULTS: The mean age of participants was 35.6 $\pm$ 5 years, mean BMI was 23.25 $\pm$ 2.9 kg/m^2, and mean AMH was 2.66 $\pm$ 1.8 ng/ml. There was a significant negative linear correlation between age and AMH, but no relationship between age and BMI was observed. 80 cytokines were analyzed, and 61 had intensity values above threshold. 39 cytokines showed a significant increase with age; these same cytokines showed a decrease with AMH, with 12 reaching statistical significance (MIP-1-beta, IL-3, IL-7, IL-12-P40, IL-15, TGFb1, TGFb3, VEGF, BDNF, Eotaxin2, PIGF, and Oncostatin M). 4 cytokines had a significant association with BMI; IL-8, MCP1, and Leptin were increased, whereas HGF was decreased. Interestingly, the cytokines that changed significantly with BMI were distinct from those that changed with age or AMH.

CONCLUSIONS: Specific cytokine profiles may be strong predictors of both chronological and reproductive age. Obtaining this information is non-invasive to the oocyte, and thus may have clinical utility. Studies are ongoing to determine whether such cytokines are correlated with IVF outcomes. Our findings also suggest that advanced reproductive age and increased BMI are associated with unique follicular fluid microenvironments that may negatively influence oocyte quality through different mechanisms.

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UNEARTHING ANEUPLOIDY: INVESTIGATING DOUBLE-STRAND BREAKS IN OOCYTES OF CAENORHABDITIS ELEGANS. F. Balmir J. L. Yanowitz. Obstetrics, Gynecology, and Reproductive Science, Magee-Womens Research Institute, Pittsburgh, PA.

OBJECTIVE: To better understand the components of double-strand break (DSB) machinery, in order to gain insight into the first event in meiotic crossover recombination.

DESIGN: Basic research animal study of *Caenorhabditis elegans.*

MATERIALS AND METHODS: We created double mutants and analyzed a matrix of interactions between partial loss-of-function alleles of genes involved in DSB formation in *C. elegans*. These include mutations in *lin-35*, *cep-1*, *dsb-2*, *rec-1*, *him-17*, *him-5*, *parg-1*, and *mre-11*. We have analyzed diakinesis oocytes by whole mount staining followed by confocal microscopy and 3D visualization allowing quantification of crossover