Percentage of SOX17 Positive Human Oogonias Undergoing Mitosis at Different Developmental Stage

Age	No. of specimens	No. of VASA+cells	SOX17+/ VASA+(%)	VASA+SOX17+ PHH3+(%)
7GW	2	172	91.43%	33.72%
15GW	2	814	93.95%	6.14%
16GW	4	714	94.42%	3.92%
17GW	2	682	90.99%	3.81%
20GW	6	802	81.60%	3.74%
21GW	4	894	90.98%	2.68%
22GW	6	1876	81.29%	4.05%
23GW	4	954	86.39%	4.19%
24GW	4	206	95.13%	2.91%
26GW	2	558	97.81%	0.36%
28GW	2	870	93.54%	0.23%

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Supported by: National Natural Science Foundation of China NO:81270750; Grant of Sun Yat-sen University NO:16ykjc10.

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TELOMERE LENGTH AND ANDROGEN RECEPTOR OR FSH RECEPTOR MESSENGER RNA AS BIOMARKERS FOR OOCYTE AMD EMBRYO QUALITY IN ART CYCLES.

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OBJECTIVE: The determine the association among telomere length, androgen receptor (AR), FSH receptor (FSHR), and LH receptor (LHCGR) expression in cumulus cells with oocyte quality and embryo development.

DESIGN: A prospective observational study.

MATERIALS AND METHODS: Cumulus cells were obtained from cumulus-oocyte-complex (COC; n=484), which were retrieved in vitro fertilization cycles for PCOS (n=84) and control patients (n=400). The COCs from control group patients were divided into young (n=287) and advanced age (n=131) groups according to 38 years of age. The telomere length relative to a single copy marker gene (36B4) was determined by real time quantitative PCR. Messenger RNA of AR, FSHR, and LHCGR were measured by real time quantitative RT-PCR.

RESULTS: The telomere length in cumulus cells is correlated with oocyte and embryo quality in young and PCOS groups. The telomere length in cumulus cells from PCOS patients is shorter than that in young group. The AR expression is correlated to telomere length and embryo quality in young age group. By contrast, the FSHR expression is associated with telomere length and embryo quality in PCOS group.

CONCLUSIONS: The telomere length could be a biomarker for oocyte quality in young and PCOS groups. The function of AR might relate to telomere length in cumulus cells, especially in young age group. The relationship among telomere, AR, and FSHR deserves further investigation.

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Supported by: This work was supported by the Chung Shan Medical University Hospital (grant number: CSH-2014-C-012 to TH Lee)

P-97 Tuesday, October 31, 2017

MICRO-RNA SEQUENCING OF INDIVIDUAL HUMAN OOCYTES.

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OBJECTIVE: MicroRNAs (miRNAs) are important regulators of gene expression during oogenesis. Differences in miRNAs stored in the ooplasm have been correlated with oocyte competence, but to date miRNA profiling of single oocytes has proven difficult. The aim of this study was to validate single oocyte small RNA-sequencing in order to characterize the miRNA content of the human oocyte.

DESIGN: Research study.

MATERIALS AND METHODS: We collected 3 samples consisting of a pool of 5 human MII oocytes and 3 samples consisting of single human MII oocytes. Germinal vesicle oocytes were obtained after retrieval following their elimination from standard IVF treatment cycles, and matured *in vitro*. Total RNA extraction was carried out using the quick-RNA MicroPrep (Zymo Research, USA). Following library preparation using the SMARTer smRNA-Seq Kit (Clontech laboratories Inc., USA), deep sequencing was carried out on Illumina NextSeq with a 74 single-read module. Data were filtered using cutadapt and only 15 nt sequences or longer were retained. Annotation of known microRNA sequences was run using Bowtie1 and featureCounts. The discovery of new potential miRNA sequences was performed using miRDeep2. Target genes of highly expressed miRNAs were predicted with miRWalk 2.0. GO and KEGG pathway analyses were performed using ClueGo Cytoscape plug-in.

RESULTS: A mean of 240 known and 391 potentially novel miRNA sequences were identified overall: 235 and 338 in pooled oocyte samples and 244 and 444 in individual oocytes, respectively. Principal Competent Analysis showed that pooled oocyte samples had more similar miRNA profiles than individual oocytes. Eleven of the top 20 highly expressed miRNAs were in common between pooled and individual oocytes. These miRNAs were predicted to target 3917 genes, and enriched pathways controlling oocyte maturation including MAPK, mTOR, Neutrophin and FOXO metabolic pathways. Biological processes related to nuclear membrane disassembly, regulation of cytochrome C from mitochondria and blastocyst development were enriched. Furthermore, molecular functions were associated with phosphotransferase activity and RNA and DNA regulation. Among the targeted genes, 49 are known to be involved in oogenesis.

CONCLUSIONS: There were no differences in the number of miRNA identified in pooled or individual oocytes. The dissimilarity of single oocyte miRNAs may reflect individual oocyte variability, which could be important biologically, and which may be smoothed out and lost in pooled samples. This work describes a novel methodology for exploring the miRNome of individual human oocytes, and could be used to investigate miRNAs associated with reproductive failure during ART procedures.

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REGULATION OF ANTI-MULLERIAN HORMONE (AMH) BY OOCYTE SPECIFIC GROWTH FACTORS IN HUMAN CUMULUS GRANULOSA CELLS.

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OBJECTIVE: Regulation of AMH production by granulosa cells is poorly understood. The purpose of our study is to determine the role of oocyte-secreted factors including growth differentiation factor 9 (GDF9) and bone

morphogenetic protein 15 (BMP15) on AMH production in primary human cumulus cells.

DESIGN: Prospective in vitro studies using human primary cumulus granulosa cell cultures.

MATERIALS AND METHODS: Follicular aspirates of women undergoing in vitro fertilization (IVF) at a University IVF Center were used. Cumulus cells were mechanically separated from the oocyte, seeded on 24-well culture dishes pre-coated with extracellular matrix at a density of $3x10^4$ cells/well and cultured for 24 hours before treatment. Cells were treated with a combination of GDF9, BMP15, recombinant FSH, and specific signaling inhibitors for 48 hours. Cells were harvested for protein and RNA isolation. AMH mRNA and protein levels were quantified by real-time PCR and Western blot, respectively.

RESULTS: Stimulation with GDF9 or BMP15 separately had no significant effect on AMH mRNA levels. In contrast, simultaneous stimulation with GDF9 and BMP15 (G+B) resulted in a significant increase in AMH mRNA and protein expression. Increasing concentration of G+B (0.6, 2.5, 5 and 10 ng/ml each) stimulated AMH in a dose-dependent manner showing maximal effect at 5 ng/ml. Western blot analyses revealed an average 16-fold increase in AMH protein levels in cells treated with G+B when compared to controls. FSH co-treatment tended to decrease the stimulation of AMH expression by G+B but without reaching statistical significance. Treatment with G+B had a tendency to increase the activity of a reporter construct carrying a 2kb section of the AMH promoter. Finally, the effect of G+B on the expression of AMH was significantly decreased by inhibitors of the SMAD2/3 signaling pathway.

CONCLUSIONS: These findings show, for the first time, that AMH production is regulated by oocyte-secreted growth factors in primary human granulosa cells. We demonstrated that only the combination of GDF9 and BMP15 potently stimulates AMH production supporting the idea that these factors form heterodimers. Inhibition of SMAD signaling blocks the stimulatory effect of G+B, suggesting that these heterodimers are likely to activate the BMPR2-ALK4-ALK6 receptor complex.

Supported by: NIH grant R56HD086054 and RO1HD057110 (CS)

OOCYTE MATURATION

P-99 Tuesday, October 31, 2017

MITOCHONDRIAL FUNCTION AND MT-DNA CONTENT ARE ASSOCIATED WITH THE POOR QUALITY OF OOCYTES FROM PATIENTS OF ADVANCED MATERNAL AGE. R. L. Krisher, R. Pasquariello, A. Ermisch, S. McCormick, W. B. Schoolcraft. Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: To investigate the effects of advanced maternal age (AMA) on mitochondrial function (mitochondrial membrane potential, MMP) and mtDNA content in in vitro matured human oocytes.

DESIGN: Research study.

MATERIALS AND METHODS: Immature oocytes discarded from stimulated IVF treatment cycles were either collected immediately at the germinal vesicle (GV) stage or matured in vitro for 24 hours. MII oocytes were stained (10 μM JC-1) and imaged using confocal microscopy (high MMP, red; low MMP, green). MMP was quantified using the average ratio of red:green pixel intensity in four cortical regions of each oocyte. Single GV and mature (MII) oocytes, paired by patient, were analyzed for mtDNA copy number quantification using a qPCR based assay by comparison to a standard curve obtained by cloning mtDNA regions of the human MT-COX2 gene.

RESULTS: Oocytes were grouped based on maternal age (Young, 26-32 yr; AMA, 35-42 yr). For MMP determination, a total of 51 mature oocytes were collected from 14 patients and three egg donors (Young, 29 oocytes; AMA, 22 oocytes). Oocytes from AMA patients had lower ($P<0.05$) MMP (0.90 ± 0.09) than those from Young patients (1.16 ± 0.08). Interestingly, higher MMP was associated with maturation success, as oocytes from cohorts with >50% maturation *in vitro* had higher MMP ($p<0.01$; 1.17 ± 0.08) than cohorts with less than 50% maturation (0.77 ± 0.08). For mtDNA quantification, a total of 47 oocytes were collected from 12 patients and 2 egg donors (Young, 12 GV and 11 MII oocytes: AMA, 12 GV and 12 MII oocytes). mtDNA copy number in GV oocytes was not different between AMA (223,573 $\pm$ 22,941) and Young (212,966 $\pm$ 16,666) patients and was higher ($P<0.05$) than MII oocytes in both patient groups. However, MII oocytes from AMA patients had higher ($P<0.001$) mtDNA copy number (179,579 $\pm$ 19,494) than young MII oocytes (121,654 $\pm$ 12,307).

CONCLUSIONS: Mature oocytes collected from patients of advanced maternal age have reduced mitochondrial function (MMP) and increased mtDNA copy number. These results suggest that in poor quality oocytes, dysfunctional mitochondria may result in suboptimal energy production and a compensatory upregulation of mitochondrial biogenesis, leading to the compromised clinical outcomes associated with AMA.

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IN VITRO MATURATION (IVM) OF HUMAN OOCYTES WITHOUT HCG ADMINISTRATION AND EMPLOYING A PRE-IVM PERIOD OF MEIOTIC ARREST IS A VIABLE CLINICAL TREATMENT OPTION. R. L. Krisher, R. Kile, L. A. Munkwitz, C. B. Pospisil, A. Schneiderman, L. Reed, A. Shreeve, S. McCormick, W. B. Schoolcraft. Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: In vitro oocyte maturation (IVM) has not been widely adopted because blastocyst development and pregnancy rates are typically reduced. The objective of this study was to evaluate a novel IVM system for clinical use in ART.

DESIGN: Clinical trial.

MATERIALS AND METHODS: Oocyte Handling Medium pre-maturation (OHM pre-mat) and maturation (OHM mat) media used in this study were approved for an investigational device exemption by the FDA, and the study was IRB approved. Consenting patients were treated with 150 IU FSH (Menopur, Ferring) per day until the largest follicle was ~12 mm in size, and the retrieval scheduled for the following day. Cumulus oocyte complexes (COC) were aspirated directly into OHM pre-mat, and cultured in OHM pre-mat for 2 hr. At that time, COC were placed into OHM mat for 30 hours, when oocytes were partially stripped of cumulus cells to determine maturation status. Immature oocytes were returned to the incubator for further maturation assessment, and mature oocytes were fertilized by ICSI. Zygotes were cultured in OEC sequential culture medium for 5-7 days and good quality (GQ) blastocysts were biopsied for CCS testing and vitrified.

RESULTS: To date 7 patients have participated in the study (average age 34.9 yr, range 27-41). Patient diagnoses varied: 3 advanced maternal age, 2 polycystic ovaries, 1 tubal occlusion, 1 unspecified. Following FSH stimulation (average = 6.0 days (range 4-8), a total of 74 immature oocytes were recovered (10.6 per patient) and 35 oocytes matured successfully (47%; average 5.0 per patient, range 0-11). Following ICSI, 66% of mature eggs fertilized (n=23, 3.8 per patient), and 43% of those developed to GQ blastocysts (26% GQ blastocysts by D5). Of the 7 patients in the study, 5 produced good quality blastocysts, and 4 patients had at least one euploid embryo. Of the 8 embryos tested, 6 were euploid (80%). All four patients with euploid embryos have undergone FET (three single ETs, one with 2 embryos) resulting in singleton pregnancies (57%, 4/7 patients). One healthy baby has been born and three pregnancies are ongoing.

CONCLUSIONS: Oocyte recovery without hCG followed by a brief (2hr) period of meiotic arrest prior to IVM can be successfully applied to the treatment of infertility. Although maturation success is low, development to blastocyst is equivalent to standard IVF cycles and a high percentage of these embryos are euploid. These preliminary results demonstrate that the IVM embryos produced using this system are of high quality. Preliminary indications suggest that this novel IVM system may be a viable alternative for the treatment of infertility, with reduced costs, high embryo quality, fewer surplus embryos, and good success rates.

P-101 Tuesday, October 31, 2017

EFFECT OF MELATONIN ON DEVELOPMENTAL COMPETENCE OF DENUDED HUMAN OOCYTES DURING IN VITRO MATURATION. R. Matsunaga,[a] S. Watanabe,[a] W. Mita,[a] M. Miura,[a] Y. Kobayashi,[a] N. Yamanaka,[a] M. Kamihata,[a] A. Kuwahata,[a] M. Ochi,[a] T. Horiuchi.[b] [a]Ochi Yume Clinic Nagoya, Nagoya, Japan; [b]Department of Life Sciences, Pref. University of Hiroshima, Shobara, Japan.

OBJECTIVE: Melatonin is known to be a free radical scavenger and to exist at high concentrations in follicular fluid. It has been previously reported