O-161 Tuesday, October 20, 2015 12:15 PM

AN FSH-LOWERING ACTIVIN DISRUPTING THERAPY PREVENTS EGG CHROMOSOME AND SPINDLE MISALIGNMENTS THAT PREDISPOSE TO ANEUPLOIDY, AND INCREASES FERTILITY, IN A MOUSE MODEL OF MIDLIFE REPRODUCTIVE AGING. L. R. Bernstein,[a,b,c,d] A. C. Mackenzie,[b] C. L. Chaffin,[e] I. Merchenthaler.[b] [a]Pregmama, LLC, Montgomery Village, MD; [b]Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD; [c]Gynecology and Obstetrics, Johns Hopkins School of Medicine, Baltimore, MD; [d]Veterinary Integrative BioSciences, Texas A & M College of Veterinary Medicine, College Station, TX; [e]Obstetrics, Gynecology, and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD.

OBJECTIVE: Women of advanced maternal age women (AMA, >age 35) have increased risk of oocyte & embryo aneuploidy, infertility, miscarriages, and trisomic pregnancies (collectively "egg infertility."). Egg infertility increases markedly with age due to elevated rates of egg aneuploidy. It is a significant public health problem, with 1 in 5 US women now attempting her first pregnancy after 35. Elevated FSH is one of the first signs of ovarian aging. We hypothesize that high FSH is a cause of egg infertility, that elevating FSH activity for the period of oocyte growth will increase egg infertility, and that lowering FSH for the period of oocyte growth will prevent egg infertility.

DESIGN: We developed SAMP8 mice as model with human-like midlife female reproductive aging characteristics, including elevated FSH, increased rates of oocyte spindle misalignments, and diminished fertility by midlife (age 7 months). A regimen to raise FSH activity with chronic PMSG treatment was given to one test group of midlife SAMP8 for 3 weeks, the period of oocyte growth. An FSH lowering regimen was developed using ActRIIB:Fc, an activin decoy receptor that sequesters activin and suppresses activin signaling. This was given for 3 weeks to a second test group. A third test group was comprised of untreated control mice.

MATERIALS AND METHODS: Chromosome and spindle misalignments of ovulated oocytes are highly predictive of impending aneuploidy, were scored in fluorescence microscopy. Fertility was compared between ActRIIB:Fc-treated and untreated midlife SAMP8 groups by quantitation of litter sizes after mating with young proven SAMP8 males.

RESULTS: PMSG increased rates of chromosome misalignments from 32/193 oocytes (16.5%) to 38/121 oocytes (31.4%; P=0.0013), and increased rates of spindle misalignments from 13/192 (6.77%) to 14/98 (14.3%; P=0.0331). ActRIIB:Fc lowered FSH in midlife SAMP8 to the level of young SAMP8. ActRIIB:Fc decreased chromosome misalignments from 32/193 (16.5%) to 11/159 (6.9%; P=0.0030), and decreased spindle misalignments from 13/192 (6.77%) to 4/155 (2.58%; P=0.0182). Rates of chromosome and spindle misalignments were lowered to those of young mice. ActRIIB:Fc restored nearly 40% of the fertility lost with age, increasing litter sizes from 5.06 to 6.29 pups/litter (P=0.0305, vs. 8.22 in young SAMP8).

CONCLUSIONS: These data provide supportive evidence that FSH may play a role in egg aneuploidy and infertility. Hormone normalization therapy ("HNT") to lower FSH and disrupt activin signaling shows promise as a novel therapeutic intervention to prevent oocyte aneuploidy and infertility, miscarriages, and trisomies.

Supported by: 1. Maryland Industrial Partnerships Grant (MIPS). 2. Technology Development Corporation of Maryland (TEDCO). 3. Max and Victoria Dreyfus Foundation Grant. 4. Bernstein and Pine Families. 5. Pregmama. 6. University of Maryland School of Medicine Funds. 7. Indiegogo campaign.

O-162 Tuesday, October 20, 2015 12:30 PM

GNRH AGONIST LEUPROLIDE ACETATE NEITHER ACTIVATES ANTI-APOPTOTIC GENES NOR PROTECTS HUMAN OVARY AND GRANULOSA CELLS FROM DNA DAMAGE AND APOPTOSIS INDUCED BY CYCLOPHOSPHAMIDE. G. Bildik,[a] N. Akin,[a] F. Senbabaoglu,[a] Y. Guzel,[b] U. Ince,[c] B. Balaban,[b] B. Urman,[d,b] O. Oktem.[d,b] [a]Reproductive Biology, Koc University Graduate School of Health Sciences, Istanbul, Turkey; [b]Women's Health Center Asssited Reproduction Unit Fertility Preservation Program, American Hospital, Istanbul, Turkey; [c]Pathology, Acibadem University, Istanbul, Turkey; [d]Obstetrics and Gynecology, Koc University School of Medicine, Istanbul, Turkey.

OBJECTIVE: Inconsistent results of randomized controlled trials (RCTs) and lack of a proven molecular mechanism of action with ovarian protection with co-administration GnRH agonists (GnRHa) with chemotherapy places GnRHa under scrutiny as a fertility preservation strategy. We aimed in this study to provide molecular evidence for-or-against the role of GnRHa in the prevention of cyclophosphamide induced damage in human ovarian tissue samples and granulosa cells.

DESIGN: A translational research study.

MATERIALS AND METHODS: Ovarian cortical pieces (n=15, age 14-37) and human mitotic non-luteinized (COV434, HGrC1) and non-mitotic luteinized (HLGC) granulosa cells were treated with 4-hydroperoxy cyclophosphamide (in vitro active metabolite of cyclophosphamide used at 50 and 100 μM) with and without GnRHa leuprolide acetate (50 ng/mL: peak intraovarian concentration of the drug) for 24 hrs. Cell proliferation (real-time quantitative assessment by xcelligence system),DNA damage (p-histone H2AX), apoptosis (cleaved caspase-3, YO-PRO-1), follicle counts, hormonal markers of ovarian function and reserve (estradiol, progesterone and AMH), and the expression of anti-apoptotic genes (bcl-2, bcl-xL, bcl-2L2, Mcl-1, BIRC-2 and XIAP) were compared among control, chemotherapy and chemotherapy+GnRHa groups.

RESULTS: GnRH receptor expression and its activation by GnRHa were validated with qRT-PCR and measuring intracellular cAMP level, respectively. Exposure to cyclophosphamide resulted in massive follicle loss, arrested cell growth, increased DNA damage/apoptosis and decreased hormone productions in the tissue samples and granulosa cells. The co-administration of GnRHa with cyclophosphamide did not prevent or attenuate any of these cytotoxic effects. Furthermore, GnRHa did not up-regulate the anti-apoptotic genes compared to control and cyclophosphamide treated samples.Mcl-1 and BIRC2 expressions were further decreased after cyclophosphamide+GnRHa (Table).

CONCLUSIONS: GnRH agonist leuprolide acetate does not offer any protection against cyclophosphamide induced damage in human ovary and granulosa cells via its cognate receptors.

The Impact of Cyclophosphamide (Cyc)±GnRHa on Ovarian Tissue Samples and Granulosa Cells

	Control	Cyc	Cyc+GnRHa	P value
Ovarian Tissue: Follicle reserve, anti-apoptotic gene expression and hormone productions				
Primordial/mm2	2.54±0.5	0.33±0.2	0.33±0.1	p=0.006 control vs. cyc+GnRHa
Preantral/mm2	0.6±0.2	0.12±0.05	0.14±0.1	p=0.3 cyc vs. cyc+GnRHa
Bcl-2	1±0.06	0.60±0.07	0.46±0.01	p
Bcl-xL	1±0.03	0.97±0.03	0.92±0.01	
Bcl-2L2	1±0.04	0.60±0.03	0.51±0.01	
Mcl-1	1±0.02	0.93±0.03	0.81±0.01	
BIRC2	1±0.04	0.75±0.01	0.59±0.01	
XIAP	1±0.01	0.86±0.02	0.72±0.05	
AMH(ng/mL)	1.2±0.09	0.3±0.03	0.3±0.02	p=0.004
E2(pg/mL)	788±98	185±16	148±43	control vs.
P(ng/ml)	1.76±0.4	0.3±0.02	0.31±0.06	cyc+GnRHa p=0.2 cyc vs. cyc+GnRHa
Luteinized Granulosa Cells (HLGCs): Hormone productions and apoptosis rate				
E2(pg/mL)	1560±112	271±28	207±31	p=0.009 control vs. cyc+GnRHa
P(ng/mL)	596±95	150±18	105±16	
Apoptosis (%)	3%	89%	91%	p=0.3 cyc vs. cyc+GnRHa
Mitotic granulosa cells: Proliferative index and apoptosis rate				
Proliferative index	1.2±0.2 3%		0.14±0.01 88%	p p=0.9 cyc vs. cyc±GnRHa (for proliferative index)
Apoptosis(%)				p p=0.8 cyc vs. cyc+GnRHa (for apoptosis rate)