

MATERIALS AND METHODS: Specimens of vaginal epithelium were obtained after prolapse surgery from premenopausal women (PRE, n = 8), postmenopausal women (POST, n = 8) and postmenopausal women using vaginal estrogen cream (EST, n = 8). After formalin fixation tissues were embedded in paraffin and sectioned and stained with Hematoxylin/Eosin or Periodic Acid Schiff. The slides were analyzed for epithelial thickness, number of epithelial layers and glycogen content. Measurements were done by 2 blinded assessors. Intraclass correlation coefficients (SPSS 19) were used to assess interobserver agreement. Means were compared using ANOVA.

RESULTS: The intraclass correlation coefficient between the two blinded observers for measurements of epithelial thickness in the was .921 ($P < .001$). The correlation between observers of the number of epithelial layers counted was not significant. Mean epithelial thickness was 167 μ m, 332 μ m and 298 μ m in POST, PRE and EST, respectively ($P < 0.05$). The reduction in glycogen in the POST samples was not seen in the EST samples.

CONCLUSION: Measurement of epithelial thickness is a more objective method of assessment of vaginal epithelial thickness than counting epithelial layers. Postmenopausal samples have the thinnest epithelia and the lowest glycogen content. In postmenopausal women the use of vaginal estrogen cream is associated with restoration of vaginal epithelial thickness and glycogen content.

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CORRELATION BETWEEN SERUM AMH, DAY 3 FSH AND RESPONSE TO CONTROLLED OVARIAN HYPERSTIMULATION (COH) IN A POPULATION OF INFERTILE MEXICAN PATIENTS.

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OBJECTIVE: To establish whether the values of AMH, a newly accepted marker of ovarian reserve, correlate with cycle day 3 FSH and ovarian response to COH in a population of infertile Mexican patients undergoing ART.

DESIGN: Prospective correlation analysis of IVF patients.

MATERIALS AND METHODS: A total of 70 consecutive cycles of IVF was performed serum AMH assay before the stimulation cycle using (Beckman Coulter Inc. Brea CA. U.S.A) ELISA KIT. Subsequently analyzed the correlation between the serum levels of AMH and the numbers of total oocyte and the different variables of reproductive outcomes. For the statistical analysis we used: Spearman correlation coefficient (r).

RESULTS: The correlation between AMH, FSH and ovarian stimulation variables are summarized in table1. When analyzed specifically for ages and numbers of oocytes retrieved, patients with 0-5 oocytes had a mean age 38.8 \pm 3.58 years, a mean FSH of 8.78 \pm 3.43IU/L and a mean AMH of 0.59ng/dl. Patients with 6-16 oocytes had a mean age of 27.69 \pm 6.94 years, a mean FSH of 5.22 \pm 1.65IU/L and a mean AMH of 2.27ng/dl. Finally, patients with >17 oocytes had a mean age of 27.6 \pm 7.67 years, FSH of 4.55 \pm 1.70 IU/L and mean AMH of 4.74 ng/dl.

Correlation between AMH and cycle day 3 FSH on Ovarian Stimulation Variables.

Variables	AMH (r)	P Value	FSH day 3 (r)	P value
Age	-0.50	<0.005	+0.55	<0.01
Fol >15mm d10	+0.70	<0.001	-0.45	<0.001
Estradiol d10	+0.62	<0.001	-0.30	<0.01
Total oocytes	+0.84	<0.001	-0.47	<0.001
Oocytes MII	+0.70	<0.001	-0.35	<0.01
Fertilization rate	+0.14	0.143	-0.20	0.25
Implantation rate	+0.18	0.183	-0.15	0.37

CONCLUSION: AMH is a better biomarker than the day 3 FSH profile in predicting response to COH. Particularly in women 39 years or older and still with normal FSH levels, AMH is a more accurate measure of the true ovarian reserve.

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DETERMINATION OF CARDIAC HISTOLOGIC INJURY IN A TRANSGENIC MOUSE MODEL OF HEART FAILURE EXPRESSING A NOVEL MITOCHONDRIAL PROGESTERONE RECEPTOR (PR-M). J. S. Yeh, C. E. Likes, III, K C. Hawkins, Q. Dai, T. M. Price. Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Duke University Medical Center, Durham, NC.

OBJECTIVE: 1. To develop an automated histological fibrosis quantification protocol.

2. To determine cardiac histologic injury in a transgenic mouse model of heart failure expressing a human mitochondrial progesterone receptor (PR-M).

DESIGN: Transgenic mouse study.

MATERIALS AND METHODS: PR-M is mitochondrial progesterone receptor isoform that increases cellular respiration. An inducible (TET-On) transgenic mouse model expressing human PR-M under the control of the cardiac myosin heavy chain 6 promoter was developed. PR-M expression was induced with oral doxycycline followed in 2 wks by surgical constant thoracic aortic constriction (cTAC) to increase after-load. Intact male and ovariectomized female mice were treated with progesterone in oil (2.5 mg/day SQ) starting 1 wk prior to cTAC. Mice were sacrificed 8 wks after cTAC and cardiac PR-M expression determined with realtime RT-PCR. Cardiac fibrosis and capillaries were identified with Masson's trichrome and immunostaining with lectin respectively, by an investigator blinded to gene expression and treatment. Histologic images were acquired via digital slide scanning. Morphometrics were computed with ImageJ64 (NIH) and Photoshop CS5 (Adobe Systems). Data from the histologic quantification were analyzed with unpaired Student's t-test.

RESULTS: Progesterone treated PR-M positive mice (N = 9) showed greater capillary density ($P=0.04$), greater capillary density to cardiomyocyte width ratio ($P=0.02$) and decreased cardiac fibrosis ($P=0.03$) after cTAC compared to progesterone treated PR-M negative mice (N = 17). The mean myocyte width was not different between the 2 groups ($P=0.33$).

CONCLUSION: A novel computerized fibrosis quantification method for cardiac tissue was developed. Compared to controls, progesterone treated transgenic mice expressing a human mitochondrial progesterone receptor (PR-M) had fewer histologic signs of cardiac injury. These observations support a role for progesterone via a mitochondrial progesterone receptor in cardiac function.

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AKAP-BRX INFLUENCES FOLLICLE STIMULATING HORMONE SIGNALING. M. Maguire, P. Driggers. Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; Uniformed Services University of the Health Sciences, Bethesda, MD.

OBJECTIVE: Protein A Kinase Anchor Proteins (AKAPs) are believed to play a key role in folliculogenesis by controlling specificity of cAMP signaling through regulation of protein kinase A activity. We previously reported that expression of Brx (a.k.a. AKAP-Brx, AKAP-lbc, AKAP13) was induced during granulosa cell (GC) growth and differentiation in mice and humans (Miller et al, Am J Obstet Gynecol 2000). However, it is not known whether AKAP-Brx may play a role in follicle stimulating hormone (FSH) signaling. The goal of these experiments was to investigate a possible role of AKAP-Brx in FSH receptor (FSHR) signaling in GCs.

DESIGN: Experimental studies in mice

MATERIALS AND METHODS: C57BL/6 mice were sacrificed between 6 and 8 weeks of age. Ovaries were harvested and granulosa cells were isolated and cultured according to previously published methods (Gong and McGee, Biol Reprod 2009). Granulosa cells were treated with recombinant FSH 1IU/ml for 12, 24, and 48 hours. RNA was extracted using Qiagen RNeasy Mini. cDNA was generated using Superscript III(Invitrogen). Levels of AKAP-Brx, luteinizing hormone receptor (LHR), and aromatase transcripts were compared by RT-PCR. To test whether AKAP-Brx was required for expression of LHR and aromatase, siRNA for AKAP-Brx, or a negative control, was transfected into primary cultures of murine granulosa cells.

RESULTS: FSH induced expression of AKAP-Brx, LHR, and aromatase within 48 hours. Control experiments confirmed that transfection of AKAP-Brx siRNA reduced AKAP-Brx RNA expression 5-fold. AKAP-Brx knockdown reduced aromatase expression 60%, but did not affect