MATERIALS AND METHODS: Acclimatized 52-weeks old female SD rats were randomly assigned to a cell therapy group or non-treated group. Rats in cell therapy group were received a single tail vain injection of $5X10^5$ hPD-MSCs. At various time-points following transplantation (e.g. 1, 2, 3, and 5 weeks), we measured body weight and organs weight in hPD-MSCs treated vs control animals. The ovarian structures and follicle numbers were also confirmed histologically. Level of hormones, circulating miRNAs, mRNAs and mtDNA copy numbers were detected by ELISA and quantitative real-time RT-PCR.

RESULTS: Body weight significantly decreased after hPD-MSCs injection. Human DNA (Alu) sequences were found in different organs (lung, liver, spleen and ovary) after hPD-MSCs therapy. The mean total number of secondary and antral follicles was significantly increased in ovaries at 1 week after hPD-MSCs injection. Genes associated with follicle formation and growth, including *Bmp13*, *Cyp19a1*, *Gdf9*, *Mki67*, *Sirt1* and *Zp3*, were markedly increased in ovaries, starting from the second week after cell therapy. Levels of miR-21, miR-34a, miR-145 and miR-191, known as associated with ovarian reserve and aging, were changed in serum. In addition, hPD-MSCs therapy led reduction of mtDNA content and improved mitochondrial condition by increasing levels of *Atp5a1*, *Esr2* and *Tomm7*.

CONCLUSIONS: Our study verified that the hPD-MSCs injection via tail vein led to the improved folliculogenesis through gene expression involved in follicle assembly in the aged rats. We also observed that hPD-MSCs therapy mended aging phenotypes through weight loss, improved mitochondrial fitness and change of circulating miRNAs involved in ovarian reserve and aging process. Our findings suggest the future therapeutic potential of hPD-MSCs transplantation for women in advanced age to improve their ovarian function and keep youthfulness.

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GONADOTROPINS AND OOCYTE-SECRETED FACTORS COORDINATED REGULATION OF DDIT4 AND CYR61 IN PRIMARY HUMAN CUMULUS GRANULOSA CELLS.

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OBJECTIVE: Functional analysis of microarray data obtained in human primary cumulus granulosa cells (hGCs) revealed for the first time the expression and regulation of DDIT4 and CYR61 by gonadotropins and growth factors (1). In cancer cells, DDIT4 has been shown to promote proliferation and cell survival while CYR61 promotes cell apoptosis. Because oocyte-secreted factors influence GC growth, the purpose of this study was to determine the role of the OSFs growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) on DDIT4 and CYR61 expression in primary hGCs.

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

MATERIALS AND METHODS: Follicular aspirates from 20 women undergoing in vitro fertilization at a University clinic were used. Cumulus cells were mechanically separated from the oocyte, seeded at a density of $6x10^4$ cells/ml on culture dishes pre-coated with extracellular matrix and cultured for 24-72 hours before treatment. Cells were treated with a combination of GDF9, BMP15, recombinant follicle stimulating hormone (FSH), and SMAD inhibitors for 48 hours. Cells were harvested for analysis. DDIT4 and CYR61 mRNA and protein levels were quantified using real-time PCR and Western Blot respectively. Data were analyzed by two-way ANOVA and $P<0.05$ was considered significant.

RESULTS: Treatment with GDF9 or BMP15 alone, or in combination (G+B) had no effects on DDIT4 and CYR61 mRNA and protein levels, compared to the untreated control group (C). FSH-stimulation of DDIT4 mRNA expression was significantly potentiated in the presence of G+B (DDIT4 mRNA fold increase relative to control: FSH: 2.4±0.3 vs.FSH+G+B: 4.6±1.2, $P<0.01$). Similarly, FSH-inhibition of CYR61 mRNA expression was significantly potentiated in the presence of G+B (CYR61 mRNA relative expression: C: 26.2±6.4 FSH: 21.2±6.0 vs.FSH+G+B: 7.0±2.0, $P=0.002$). The regulation of DDIT4 and CYR61 was confirmed by Western Blot ($P<0.05$). G+B inhibition of CYR61 followed a dose-dependent pattern with maximal inhibition observed at 5ng/ml for each GDF9 and BMP15. The addition of SMAD2/3 and SMAD1/5/8 inhibitors had no effect on the potentiation of DDIT4 stimulation by G+B. In marked contrast, the addition of either of these inhibitors augmented the expression of CYR61 strongly even in the presence of FSH and G+B ($P<0.0001$).

CONCLUSIONS: Our data highlight the expression and regulation by OSFs of two genes involved in proliferation and survival. The findings provide a novel mechanism by which the oocyte, via the secretion of GDF9 and BMP15, potentiates the protective effects FSH on GC survival and proliferation.

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MODELING FRAGILE X-ASSOCIATED PRIMARY OVARIAN INSUFFICIENCY (FXPOI) VIA ECTOPICALLY EXPRESSED CGG_{99} REPEATS.

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OBJECTIVE: Women heterozygous for an expansion of CGG repeats in the 5'UTR of *FMR1* are at risk for Fragile X-associated Primary Ovarian Insufficiency (FXPOI). We seek to determine whether these expanded CGGs, independent of *Fmr1*, are sufficient to drive ovarian insufficiency. A second objective is to elucidate whether dysfunction arises via expression of CGG-containing mRNAs or a peptide product translated from these RNAs.

DESIGN: Prospective study with murine models

MATERIALS AND METHODS: Heterozygous females from two mouse lines expressing either CGG RNA-only (RNA-only) or CGG RNA and its translated polyglycine product FMRpolyG (FMRpolyG+RNA) with expanded from the *Rosa26* locus[1] were used. Fertility was assessed by continuous breeding and superovulation studies. Other phenotypic data were collected, and histology examined in aging mice (early life through 8 months). Morphology was correlated with gene expression changes, assayed by qRT-PCR on whole ovary tissue. Data were analyzed in Prism 6 with appropriate statistical tests and post-hoc comparisons applied.

RESULTS: Our data suggest that CGG RNA and FMRpolyG+RNA both contribute to ovarian dysfunction, albeit differently. Immunostaining showed FMRpolyG was present in oocytes and granulosa at postnatal day 4. Superovulation revealed that young RNA-only and FMRpolyG+RNA mice ovulated fewer oocytes/female compared to controls. Continuously breeding FMRpolyG+RNA mice exhibit declining fertility with age. The reductions in litters and cumulative pups/female were not seen in RNA-only mice. Cessation of breeding in FMRpolyG+RNA females is preceded by significant weight gain compared to control mice, and histology reveals a lack of ovulation as well as hyperplastic stroma and disorganized theca in aged ovaries. Gene expression from whole ovary tissue collected at 8 months shows increased *Vcam1* and decreased *StAR* and *Cyp11a1*, consistent with morphology. Unexpectedly, ovarian cysts lined with ciliated epithelia were noted in a subset of FMRpolyG+RNA and RNA-only ovaries aged beyond 6 months. The origin of these lesions is not yet known.

CONCLUSIONS: Globally expressed FMRpolyG+CGG RNA leads to declining fertility and increasing weight in females. Ovaries show a loss of ovulation and altered steroidogenic capacity with age. These data suggest changes in the hypothalamic-pituitary-ovarian axis rather than in mating behaviors lead to infertility. CGG RNA-only mice do not exhibit this fertility decline or increased weight. Diminished response to superovulation suggests that CGG RNA-alone may affect ovarian function, albeit less robust than when expressed in conjunction with FMRpolyG. Future work will include tissue-restricted expression of CGG repeat alleles to evaluate intraovarian effects of expression.

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