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COMPARISON BETWEEN TP53 CODON 72 POLYMORPHISM GENOTYPE AND ANTI-MULLERIAN HORMONE LEVELS TO PREDICT POOR OVARIAN RESPONSE. J. G. Franco, Jr.,[a,b,c] L. D. Vagnini,[b] C. G. Petersen,[a,b,c] A. L. Mauri,[a,b] R. L. R. Baruffi,[a,b] J. B. A. Oliveira.[a,b,c] [a]Center for Human Reproduction Prof. Franco Jr, Ribeirao Preto, Sao Paulo, Brazil; [b]Paulista Center for Diagnosis Research and Training, Ribeirao Preto, Sao Paulo, Brazil; [c]Department of Gynecology and Obstetrics, Botucatu Medical School -Sao Paulo State University - UNESP, Botucatu, Sao Paulo, Brazil.

OBJECTIVE: Anti-Mullerian hormone (AMH) is a reliable tool for predicting poor ovarian response (POR). Recently, TP53 codon 72 (which encodes arginine [Arg] or proline [Pro]) genetic screening was suggested as a predictive tool for POR. Women with the Arg/Arg genotype are significantly less likely to have POR than those who are Arg/Pro or Pro/Pro. Conversely, Arg/Arg patients are significantly more likely to show a high ovarian response compared with Arg/Pro or Pro/Pro patients. The objective of this study was to determine whether TP53 codon 72 polymorphism genetic screening is as useful as the AMH level in predicting POR.

DESIGN: Prospective cohort.

MATERIALS AND METHODS: A total of 103 infertile women who underwent controlled ovarian stimulation were included. POR was defined as collection of $\leq$3 oocytes. Participant DNA was extracted from peripheral blood. TP53 gene codon 72 Arg/Pro SNPs (rs1042522) were genotyped using real-time PCR with Taqman Universal PCR Master Mix and Taqman SNP genotyping assays. AMH was enzymatically measured using an amplified 2-site immunoassay kit.

RESULTS: TP53 codon 72 polymorphisms (Arg/Arg x Arg/Pro + Pro/Pro) had an efficacy of 0.54 in identifying POR (sensitivity=0.66, specificity=0.43, positive predictive value [PPV]=0.20; negative predictive value [NPV]=0.86, and likelihood ratio [LR]=1.18). Fisher's exact test was not significant (P=0.60). AMH (cut-off: $\leq$0.2 ng/ml for POR) had an efficacy of 0.73 in identifying POR (sensitivity=0.50, specificity=0.97, PPV=0.81, NPV=0.90, and LR=21.2). Fisher's exact test was statistically significant (P<0.0001). Logistic regression revealed a significant correlation (P=0.0068) between POR and AMH (odds ratio [OR]=2.15, 95% CI=1.23- 3.75). TP53 codon 72 polymorphisms were not significantly correlated with POR (OR=0.57, 95% CI=0.19-1.65, P=0.30).

CONCLUSION: The results showed that AMH levels were more effective in predicting POR. The TP53 is not a promising genetic marker of POR.

FERTILITY PRESERVATION II

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FERTILITY PRESERVATION OF PRE-PUBERTAL CANCER PATIENT BOYS BEFORE AGGRESSIVE CHEMOTHERAPY. PELIMINARY RESULTS FROM IN VITRO CULTUE OF FRESH TESTICULAR TISSUE FROM THREE PRE-PUBERTAL PATIENTS. M. Huleihel,[a] M. Azab,[a] J. Kapelushnik,[b] E. Lunenfeld.[c] [a]Microbiology, Immunology and Genetics, Ben-Gurion University of the Negev, Beer-Sheva, Israel; [b]Pediatric Surgery and Pediatric Hemato-Oncology, Soroka Medical Center, Beer-Sheva, Israel; [c]IVF Unit, Soroka Medical Center, Beer-Sheva, Israel.

OBJECTIVE: To evaluate the possibility of inducing growth, proliferation and differentiation of testicular germ cells form prepubertal cancer patients (PBCP) in a novel three-dimensional Methylcellulose- Culture System (MCS).

DESIGN: Testicular biopsies (TB) from three PBCP were used for in vitro culture in MCS.

MATERIALS AND METHODS: Testicular cells (TC) were isolated from biopsies of three PBCP. The first patient is a 7 years-old boy. TB was performed before anti-cancer chemotherapy treatment. The second patient is a 7 years-old boy. The third patient is a 10 years-old boy. TB from both patients was performed after cancer recurrent and before anti-cancer chemotherapy. TC were isolated from the biopsies after enzymatic digestion. Isolated TC were added to MCS which contained RPMI and different substances. Developed cell clusters were examined for spermatogenic markers by qPCR or immunofluorescence staining (IF) analyses.

RESULTS: Isolated TC from *the three patients* before culturing in MCS were positively stained and differently expressed pre-meiotic markers (C-kit, GFR-a, and CD-9). These cells were also negative for the meiotic marker (CREM-1) and the post-meiotic markers protamine and acrosin. Culture of these cells, from the three patients, in MCS for 2-9 weeks showed development of duplicates, triplicates, aline and clusters/colonies of cells (at different sizes). Cells from the first patients that developed in MCS showed staining for CD-9 and GFR-a, and also for CREM-1, LDH and protamine. Cells and colonies developed in MCS, from the other two patients, are under evaluation for spermatogenic markers.

CONCLUSION: Our results show, for the first time, that MCS support the growth, proliferation and differentiation of spermatogonia from testicular cells of PBCP. These results may support the potential of in vitro culture systems in fertility preservation of in infertile survivors of childhood cancer.

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FEASIBILITY OF OVARIAN STIMULATION AND OOCYTE CRYOPRESERVATION FOR FERTILITY PRESERVATION IN FEMALE CHILDREN. G. Bedoschi,[a,b] V. Turan,[a,b] K. Oktay.[a,b] [a]Obstetrics and Gynecology, New York Medical College, Valhalla, NY; [b]Innovation Institute for Fertility Preservation and In Vitro Fertilization, New York, NY.

OBJECTIVE: To report the feasibility of ovarian stimulation and oocyte cryopreservation in post-pubertal children who are at risk for premature ovarian failure.

DESIGN: Case series.

MATERIALS AND METHODS: Children aged 13-15 years with Turner syndrome (n=3), germ-cell tumor (n=1), and lymphoblastic leukemia (n=1) underwent oocyte cryopreservation after ovarian reserve assessment and parental consent. Ovarian stimulation was performed with a short protocol using 150-225 units of gonadotropins. A GnRH antagonist was added at lead follicle size 14-mm. LH supplementation was provided due to the pituitary immaturity in all girls (HMG or recomb. LH).

Cycle characteristics and outcomes

Characteristic	Case1	Case2	Case3	Case4	case5
Age (y)	13	14	13	15	14
Diagnosis	Turner Syndrome	Turner Syndrome	Turner Syndrome	Germ cell Tumor	Acute lymphoblastic leukemia
Baseline FSH (mUI/ml)	5.7	5.3	5.6	5.6	7.8
Baseline LH (mUI/ml)	3.9	9.5	5.3	9.2	8.1
Baseline E2 (pg/ml)	15.1	65.2	33.5	66.0	28.15
AMH (ng/ml)	1.59	0.9	0.76	1.6	1.3
Antral follicle count	6	12	6	11	5
Starting gonadotropin dose	225	150 (1st cycle) / 225 (2nd cycle)	225	150	150
Total gonadotropin dose	2475	1800 (1st cycle) / 3750 (2nd cycle)	2025	1837.5	1550
# of days stimulated	11	10 (1st cycle) / 14 (2nd cycle)	10	11	12
Oocytes retrieved	19	11 (1st cycle) / 7 (2nd cycle)	16	8	21
M-II oocytes	9 + 1 IVM	8 (1st cycle) / 4 (2nd cycle)	7 + 5 IVM	4	10 + 1 IVM

RESULTS: Outcomes are summarized in table 1. Case #2 underwent 2 stimulation cycles 1 year apart. On average 13.8±5.6 oocytes were retrieved, of which 49.3% were mature. Of the immature, 16.6% were matured in vitro, increasing the mature oocyte yield to 59%. All children tolerated the procedures well and there were no complications.

CONCLUSION: Oocyte cryopreservation is a feasible technique in selected female children at risk for premature ovarian failure. During ovarian stimulation, LH supplementation is needed due to the relative immaturity of pituitary-ovarian axis and sensitivity to suppression by GnRH antagonists.

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ESTIMATED NUMBER OF MATURE OOCYTES NEEDED FOR FERTILITY PRESERVATION PATIENTS BASED ON THE NUMBER OF EUPLOID BLASTOCYSTS DIAGNOSED FOLLOWING PREIMPLANTATION GENETIC SCREENING (PGS). J. Barritt,[a] D. Hill,[a] M. Surrey,[a,b] S. Tormasi,[c] C. Welch,[c] S. Munne.[c] [a]ART Reproductive Center, Beverly Hills, CA; [b]Southern California Reproductive Center, Beverly Hills, CA; [c]Reprogenetics, Livingston, NJ.

OBJECTIVE: For oocyte fertility preservation patients there are no well-established guidelines for how many eggs should be frozen to attain a pregnancy in the future. The goal of this study is to determine how many mature oocytes (MII) are needed to be frozen to produce enough euploid blastocysts to achieve implantation and pregnancy, categorized by SART age group.

DESIGN: Retrospective data analysis at a private fertility clinic.

MATERIALS AND METHODS: Patients who underwent PGS cycles, using micro-array comparative genomic hybridization (aCGH) and blastocyst biopsy, were analyzed to determine number of MII oocytes collected, number of blastocysts produced, euploid embryos diagnosed and implantation rate.

RESULTS: See Table

Age Group	Cycles Analysed	Average MII Oocytes	Average Blastocysts	Average Euploid Embryos	Average MII's/Euploid Embryo	Implantation Rate
<35	50	11.7	7	4.7	2.5	31.1%
35-37	33	11.7	5.6	3.6	3.3	31.5%
38-40	31	11.5	5.2	2.3	5.0	25.5%
41-42	28	9.7	4.3	0.9	10.8	26.3%
>42	30	12	3.0	0.5	24	33.3%

CONCLUSION: Our results demonstrate that the number of MII oocytes needed to obtain a diagnosed chromosomally normal embryo is highly variable across the SART age groups. Increases of greater than 100% in the number of oocytes required between the ages 38-40, 41-42 and >42, are extremely important findings for patients. Additionally, we discovered that the implantation rates between age groups do not differ when diagnosed euploid embryos are transferred after trophectoderm biopsy and aCGH screening. Counseling of potential oocyte fertility preservation patients, as to the number of mature oocytes needed to achieve an expectation of a euploid embryo in the future, can now be accomplished for each SART age group based on the clinical results from >100 PGS cycles analyzed at a single fertility clinic.

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THE IMPACT OF LONG-TERM TAMOXIFEN TREATMENT ON OVARIAN RESERVE MARKERS IN WOMEN WITH BREAST CANCER: A PROSPECTIVE-LONGITUDINAL STUDY. K. Oktay,[a,b] G. Bedoschi,[a,b] M. Dickler,[c] S. Goldfarb,[c] V. Turan,[a,b] F. Moy.[a,b] [a]Obstetrics and Gynecology, New York Medical College, Valhalla, NY; [b]Innovation Institute for Fertility Preservation and In Vitro Fertilization, New York, NY; [c]Memorial Sloan-Kettering Cancer Center, New York, NY.

OBJECTIVE: Based on the recent ATLAS study (Lancet, 3/2013), tamoxifen (Tmx) treatment is now recommended for 10 years for women with ER+ breast cancer. This creates a dilemma for women who have not completed childbearing as Tmx is contraindicated during pregnancy. One possible strategy is to monitor ovarian reserve markers during Tmx treatment; the treatment is paused and pregnancy or fertility preservation is attempted before the reserve becomes depleted. However, reliability of ovarian reserve assessment by serum markers while on long-term Tmx treatment is unknown.

DESIGN: In this prospective study, we longitudinally studied the impact of Tmx primarily on serum AMH, and secondarily on Inhibin-B. E2 levels were also measured as Tmx is an ovarian stimulant and to control for the confounding effects of high E2 levels.

MATERIALS AND METHODS: 210 menstruating women with stage I-III breast cancer were enrolled prior to treatment and underwent longitudinal sampling at baseline, 4- and 8-months after initiating Tmx. Of those, 17 (mean age 39.5 ± 3.4 years, range 33-44) received Tmx without adjuvant chemotherapy. Patients with undetectable baseline AMH levels (n=3) were excluded.

RESULTS: Tmx treatment did not result in a statistically significant change in serum AMH, Inhibin B or Estradiol levels over 8 months follow up.

Impact of tamoxifen on ovarian reserve

Marker	Baseline (n=14)	4 Months (n=10)	8 Months (n=9)	P value
AMH (ng/ml)	1.3 ± 0.2	1.2 ± 0.3	0.9 ± 0.1	0.57
Inhibin B (pg/ml)	162.9 ± 51.6	135.5 ± 83.4	108.9 ± 61.3	0.84
Estradiol (pg/ml)	119.0 ± 28.0	166.7 ± 75.8	152.1 ± 39.0	0.75

When the analysis was limited to those from whom the complete longitudinal data were available (n= 9), the results remained the same.

CONCLUSION: This is the first study reporting the impact of long-term Tmx treatment on ovarian reserve markers. Tmx treatment does not appear to alter ovarian reserve assessment by serum AMH. This information has crucial value for breast cancer survivors, when making fertility decisions.

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RISK OF DIMINISHED OVARIAN RESERVE IN BRCA 1/2 MUTATION CARRIERS. E. T. Wang, M. D. Pisarksa, C. Bresee, Y. D. I. Chen, C. Alexander, B. Y. Karlan. Cedars Sinai Medical Center, Los Angeles, CA.

OBJECTIVE: BRCA 1/2 mutation carriers have significant reproductive pressures. Not only do they face the choice of risk-reducing salpingo-oophorectomy, emerging data indicates a lower oocyte yield in fertility preservation. We set out to determine if BRCA 1/2 mutation carriers have diminished ovarian reserve (DOR), based on serum anti-Mullerian hormone (AMH) levels, compared to non-mutation carriers.

DESIGN: Cross-sectional study.

MATERIALS AND METHODS: We studied 123 women, aged 24-45, recruited from the Gilda Radner Hereditary Cancer Program from 1991-2008. All participants underwent genetic testing to detect mutations in BRCA 1/2. Participants were limited to those with intact ovaries, age ≤45, and no history of breast/ovarian cancer. AMH was assayed using stored serum samples (AMH Gen II ELISA, Beckman Coulter). DOR was defined as AMH <1ng/mL. Linear and logistic regression models adjusted for age and body mass index (BMI) were performed to determine the association between BRCA 1/2 mutations and AMH.

RESULTS: Of 123 women included in this study, 75 women were BRCA 1/2 mutation carriers and 48 women were non-carriers. BRCA 1/2 mutation carriers were slightly younger (see Table 1). In multivariable linear regression analyses, BRCA 1/2 mutation carriers had a lower AMH compared to non-carriers (-0.20 ng/mL, P=0.03). Furthermore, BRCA 1/2 mutation carriers had >2-fold increased odds of DOR (odds ratio 2.64, 95% CI 1.11-6.27, P=0.03). There was no difference noted when BRCA1 and BRCA2 mutation carriers were analyzed separately.