

**Material and Methods:** The Ethics Committee of Rabin Medical Center approved the study protocol, and written consent was obtained from every adult patient or the parents of all minors. The study included 13 frozen-thawed ovarian samples from girls and women (aged 10-27 years). One tissue sample was fixed immediately after thawing (thawed control). Seventy slices were cultured on either matrigel or on alginate matrices. The samples were incubated for two weeks in a culture medium containing a serum-free combination (human serum albumin and insulin, transferin and selenium) and follicle stimulating hormone. Growth evaluation consisted of follicular counts and classification (after histological preparation), immunohistochemistry for proliferating cell nuclear antigen (PCNA) expression and measurement of  $17\beta$ -Estradiol ( $E_2$ ) production in spent media samples.

**Results:** The number of developing follicles (from primary stages onwards) was significantly higher ( $p < 0.003$ ) in samples cultured on alginate matrices compared with matrigel (53.9% compared with 17.2%, respectively). By contrast, the percent of primordial follicles decreased in follicles cultured on alginate matrices (9.4%) compared with those cultured on matrigel (33.1%), although the difference was not significant. Positive immunohistochemical staining for PCNA in granulosa cells was significantly higher in those cultured on alginate compared with matrigel ( $P < 0.0001$ ).  $E_2$  production was similar in spent media samples from follicles cultured on alginate as on matrigel (average range: 125-366 pg/ml per slice).

**Conclusions:** Our study shows for the first time that culturing human primordial follicles in slices on three dimensional alginate matrices is a promising putative *in vitro* technology. This method seems to activate more cultured primordial follicles than the commonly used matrigel coating. Additional studies are still needed to clarify the role of alginate matrices in culturing human primordial follicles.

#### O-232 Cryo-survival, fertilization and early embryonic development of vitrified oocytes derived from mice of different reproductive age

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**Introduction:** Female fertility declines with reproductive aging. One of the reasons is due to the declined quality of oocytes. Recent advances made in oocyte vitrification technology provide the possibility of female fertility cryopreservation. Although many reports have indicated high survival, fertilization and early embryonic development as well as pregnancy rates achieved with vitrified oocytes, the cryo-survival of vitrified oocytes derived from mice of different reproductive age remains unclear. The objective of this study was to examine the effect of female reproductive age on oocyte cryo-survival, fertilization and the subsequent embryonic developmental following vitrification using the mouse model.

**Materials and Methods:** Female mice (CD-1) of 8-10, 16-20, 32-36 and 44-48 weeks (wks) of age were stimulated respectively by 10.0 IU pregnant mare's serum gonadotropin (PMSG) followed 48 hours later by 10.0 IU human chorionic gonadotropin (HCG). Mature oocytes were collected 14 hours post-HCG injection. The mature oocytes were vitrified using McGill Cryoleaf with a Vitrification kit (MediCult, Denmark) as instructed. After cryo-storage of at least 7 days, the vitrified oocytes were warmed using a Warming kit (MediCult, Denmark). The survived oocytes were inseminated by intracytoplasmic sperm injection (ICSI) with a Piezo system and were cultured *in vitro* for 120 hours in Embryo Maintenance Medium (Coopersurgical/SAGE, USA) at 37°C in 5%  $CO_2$  in humidified air. The oocyte survival, cleavage and blastocyst development rates were compared, and the differences were analyzed by Chi-Square test.

**Results:** There was no significant difference in oocyte cryo-survival rates among the four groups examined ( $98.8 \pm 2.1\%$ ,  $98.0 \pm 3.3\%$ ,  $100.0 \pm 0.0\%$  and  $90.4 \pm 7.9\%$ , respectively). However, the rates of fertilization and the rates of embryos developed to blastocyst stage in the group of 32-36 wks ( $69.7 \pm 20.8\%$  and  $64.2 \pm 17.4\%$ ) and in the group of 44-48 wks ( $63.6 \pm 9.2\%$  and  $4.1 \pm 8.3\%$ ) were reduced significantly ( $P < 0.05$ ) compared with the group of 8-10 wks ( $90.7 \pm 13.7\%$  and  $71.8 \pm 8.8\%$ ) and the group of 16-20 wks ( $91.9 \pm 10.5\%$  and  $66.4 \pm 10.7\%$ ).

**Conclusion:** As shown by results obtained in the present study, although cryo-survival of the oocytes may not be affected by the increase in reproductive age, a reduction in fertilization rate and poor embryonic development have been found to be

associated with the vitrification procedure. Our results suggest that an age limit may need to be considered for oocyte vitrification in human reproductive medicine.

#### O-233 Mouse oocyte and embryo vitrification with and without dimethyl sulphoxide: comparison of survival and embryo development

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**Introduction:** Oocyte and embryo cryopreservation are important components of fertility preservation and efficient infertility treatment. Vitrification is an emerging method for cryopreservation of oocytes and embryos. Most vitrification solutions contain ethylene glycol (EG) and dimethyl sulphoxide (DMSO) as permeating cryoprotectants, which protects cells from intracellular ice crystal formation when used at high concentrations. This high concentration of DMSO has raised concern regarding cell toxicity and compromised survival and function. To investigate new material and technology safety and efficacy, animal model systems are essential. The purpose of this study was to compare two different vitrification protocols, with and without DMSO, for oocytes and embryos and document cryopreservation survival and subsequent embryo development.

**Materials and Methods:** Mouse metaphase II oocytes and pronuclear zygotes were collected from 35 day old mice superovulated with 5 IU pregnant mare's serum gonadotrophin and 5 IU human chorionic gonadotrophin. Zygotes were grown *in vitro* to 2-cell, 8-cell, and early blastocyst before cryopreservation. Treatments included: 1) control (Cont; no solution exposure or cryopreservation); 2) vitrification with DMSO (DMSO+) with cell exposure to equilibration solution (7.5% EG + 7.5% DMSO + 20% Serum Substitute Supplement in H199-Hepes media), then exposure to vitrification solution (15% EG + 15% DMSO + 20% Serum Substitute Supplement + 0.5M Sucrose in H199-Hepes); 3) vitrification without DMSO (DMSO-) with cell exposure to solutions with double the amount of EG and no DMSO. Vitrification was performed with CryoTop containers and warming was similar between DMSO+ and DMSO- treatments. Oocytes were warmed and assessed for survival immediately after warming solution exposure (T0) and after 4h of culture (T4). Two-cell embryos, 8-cell embryos, and early blastocysts were warmed, culture in KSOM + 0.1% BSA for 96h, 72h and 24h, respectively, and assessed for survival, cleavage, and expanded/hatched blastocyst development. All results were analyzed using Chi-square and considered significant at  $p < 0.05$ .

**Results:** Survival of oocytes following vitrification at T0 in DMSO+ ( $n = 500$ ; 97%) and DMSO- ( $n = 500$ ; 94%) and at T4 in DMSO+ (92%) and DMSO- (91%) solutions were not significantly different. Survival rates were not significantly different for 2-cell embryos cryopreserved in DMSO+ ( $n = 63$ ; 100%) and DMSO- ( $n = 55$ ; 100%); 8-cell embryos cryopreserved in DMSO+ ( $n = 59$ ; 100%) and DMSO- ( $n = 61$ ; 97%) and early blastocyst cryopreserved in DMSO+ ( $n = 43$ ; 100%) and DMSO- ( $n = 43$ ; 100%). For control zygotes ( $n = 156$ ) cleavage and expanded/hatching blastocyst development rate was 98% and 94%, respectively. Cleavage rates ranged from 100% to 91% in all vitrification treatments and were not significantly different. Expanded/hatching blastocyst development rates were similar between DMSO+ and DMSO- vitrification of 2-cells (83% vs 73%, respectively), 8-cells (73% vs 76%) and early blastocyst (81% vs 77%).

**Conclusions:** Concerns regarding DMSO toxicity precipitated investigation of its removal from vitrification solutions. In mouse oocytes and embryos, vitrification without DMSO performed as well as with DMSO for cell survival, embryo cleavage, and embryo development. Continued investigations of pregnancy rates, birth rates and offspring health in model systems and impact on human oocytes and embryos are needed.

#### O-234 Antioxidants for female subfertility the results of a Cochrane systematic review

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**Introduction:** Assisted reproductive therapies (ART) do not cure subfertility they simply seek to potentially overcome its barriers. It is proposed that supplementary measures including the modification of environmental and biochemical factors, for example taking dietary supplements such as oral antioxidants may improve fertility treatment outcomes (Ebisch, 2007).

The term “Antioxidant” refers to a group of organic nutrients (vitamins and minerals). Antioxidants are mostly unregulated and are readily available at cost to highly motivated consumers, therefore antioxidant therapy may be self initiated and unsupervised. There is also a perception by consumers that antioxidant therapy is not associated with harm and only associated with benefit. It is important to establish whether or not this therapy truly is capable of relieving the causes of subfertility which is a radically different focus compared to other conventional non curative invasive therapy such as ART.

**Objective:** The findings of a Cochrane systematic review (SR) reflecting the role of oral antioxidant supplementation for subfertile women is presented.

**Material and Methods:** The SR authors collated all the available evidence fitting pre-specified eligibility criteria. Bias was minimized through the use of explicit, systematic Cochrane methodology.

Included trials were truly randomised controlled trials considering women taking any type of oral antioxidant supplementation who were part of a couple with female factor subfertility or unexplained subfertility referred to a fertility clinic and who may or may not have been undergoing assisted reproduction techniques (ART), compared to placebo.

We searched the Cochrane Menstrual Disorders and Subfertility Group trials register (searched to October 2009), The Cochrane Library (Issue 1, 2008), MEDLINE (1966 to October 2009), EMBASE (1980 to October 2009), Current Contents (1993 to October 2009), Biological Abstracts (1969 to October 2009), Social Sciences Index (1980 to October 2009), PsycINFO (1972 to October 2009) and CINAHL (1982 to October 2009). ISI Web of Knowledge, Current Controlled Trials Open SIGLE and from citation lists.

#### Selection criteria

Randomised comparisons per woman of any type, dose, single or combined oral antioxidant supplementation versus placebo for the outcomes: live birth, pregnancy (biochemical and or clinical), miscarriage, were sought. Any adverse event data (including withdrawals) associated with oral antioxidant supplementation, or as reported by the trial for example multiple birth rates were also extracted.

#### Data collection and analysis

Seven studies were included and five excluded. Peto Odds ratios with 95% CI's were calculated for the outcomes which were dichotomous.

**Results:** Seven studies of 1710 women were incorporated. The small numbers of studies in each comparison and the clinical heterogeneity precluded Meta analysis for many outcomes.

#### Single oral antioxidant supplementation versus placebo

Pregnancy: one included study, n = 804, OR 0.78 (0.56 to 1.07), P = 0.12

Miscarriage: two included studies n = 862, OR 0.99 (0.20 to 4.95), P = 0.99

Any adverse event: one included study n = 804, OR 0.65 (0.26 to 1.62), P = 0.36

#### Combined oral antioxidant supplementation versus placebo

Pregnancy: three included studies n = 187, OR 2.05 (1.05 to 4.01), P = 0.04

Miscarriage: two included studies n = 81, OR 0.85 (0.17 to 4.24), P = 0.84

**Conclusions:** The analysis of the available evidence suggests that there is a positive treatment effect with the use of combined antioxidants for the outcome pregnancy.

However the application of these of these findings is limited because there was no live birth data reported, and live birth is undeniably the primary ethical outcome of interest for all subfertility stakeholders. In addition there was very little available evidence reporting adverse events.

Despite this however a substantial proportion of women with compromised fertility persist with antioxidant therapy. More research is therefore mandated.

#### O-235 Ultrastructure and intracellular calcium response to ionophore A23187 in human oocytes after vitrification

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**Introduction:** The sensitivity of human oocytes to cryodamage may compromise their developmental competence following cryopreservation. We demonstrated that slow-freezing with 1.5 mol/l propanediol and 0.3 mol/l sucrose induces cortical granules (CG) exocytosis, redistribution of pericortical organelles, and morphofunctional damage of pericortical mitochondria, and suggested a causal link between decreased developmental potential and malfunctioning of Ca<sup>2+</sup> signalling at fertilization in thawed oocytes. Herein, we studied the ultrastructure of vitrified metaphase II (MII) human oocytes and the response to calcium ionophore A23187 in fresh (F), vitrified (V) and slow-frozen (SF) MII human oocytes.

**Material and Methods:** Supernumerary oocytes were donated by consenting IVF patients. MII oocytes were analysed fresh, after slow-freezing or vitrification. Slow-freezing was carried with 1.5 mol/l propanediol and 0.3 mol/l sucrose, followed by thawing in 0.3 mol/l sucrose and decreasing concentrations of propanediol. Vitrification was performed by progressive exposure to 7.5% ethylene glycol (EG) and 7.5% dimethylsulfoxide (DMSO), exposure to 15% EG, 15% DMSO, 0.5 mol/l sucrose, loading in cryotips and submersion in nitrogen within 1 min of treatment. Thawing was performed in decreasing concentrations of sucrose. Thawed oocytes were cultured 2h before analysis. V oocytes were fixed for electron microscopy and their ultrastructure was compared with that reported in F and SF oocytes. F, V and SF oocytes were loaded for 30 min with 10 μM Fluo-4 AM in DPBS, and then cultured for 30 min. Individual oocytes were held with a holding pipette in DPBS-HSA at 37°C on a Nikon TE 2000 equipped with a Nikon DS-U2 camera. A23187 10 mM was added 10 s after beginning of acquisition and the fluorescence was recorded for 180sec and analysed by means of Nikon NIS-Element Imaging AR 3.0. Relative fluorescence intensities (RFI%) were calculated by normalizing the intensity after addition of Ca<sup>2+</sup>-ionophore A23187, against oocyte basal levels.

**Results:** The complement of CG in V oocytes was higher than in SF oocytes but markedly reduced compared to F oocytes (CG/10mm surface profile, means ± SD: V, 4.89 ± 2.34; SF, 3.35 ± 1.94; F, 10.3 ± 3.9). In V oocytes the incidence of mitochondrial damage, vacuolization, and organelle redistribution from the pericortical cytoplasm were slightly increased compared to F oocytes but markedly reduced compared to SF oocytes (oocytes with mitochondrial damage: V, 15%; F, 0%; SF, 71%; oocytes with vacuoles: V, 33%; F, 12.5%; SF, 64%; oocytes with organelle redistribution: V, 10%; F, 0%; SF, 52%). A23187 increased the intracellular Ca<sup>2+</sup> in all oocyte groups but the peak average increase in SF and V oocytes was higher than in F oocytes (RFI% peaks: SF, 107 ± 42; V, 103 ± 36; F, 70 ± 16). Moreover, the ability of SF oocytes to recover Ca<sup>2+</sup> to basal levels was reduced compared to both V and F oocytes (RFI at 180 sec/RFI peak X 100: SF, 70; V, 35; F, 25).

**Conclusions:** Different methods of oocyte cryopreservation may induce ultrastructural damage to a certain extent. The deterioration of mitochondria, development of vacuoles and redistribution of organelles from the pericortical cytoplasm might be responsible for the low developmental competence of cryopreserved oocytes. Findings here reported indicate that the incidence of these damages after vitrification is significantly lower than after slow-freezing. A consistent exocytosis of CG observed both in V and SF oocytes indicate that cryopreservation induces a premature Ca<sup>2+</sup> increase. Moreover, Ca<sup>2+</sup> data indicate that cryopreservation may alterate the response to A23187, supporting the hypothesis that a low developmental competence of thawed oocytes can be due to a malfunctioning of Ca<sup>2+</sup> signalling at fertilization.

## SELECTED ORAL COMMUNICATION SESSION

### SESSION 59: ETHICS & LAW

Wednesday 30 June 2010

10:00 - 11:45

#### O-236 Restricted consent in embryo donation for stem cell research

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