amino acids are a powerful indicator of a number of metabolic and molecular processes and thus provide a snapshot of embryo function and viability.

**Methods:** In order to measure an ‘amino acid profile’ (AAP) the embryo must be cultured individually in a droplet of culture medium, typically with relatively low concentrations of amino acids, based on measurements made on the concentrations of amino acids within the reproductive tract and thus have no negative impact on development rates. Once this incubation period is complete, usually after ~24h, the spent culture medium is diluted and analysed by Reverse-Phase High Performance Liquid Chromatography. The amino acids within the medium undergo a chemical reaction in which they are derivatised, or fluorescently labeled, and then separated by chromatography and detected by a fluorescent detector in the HPLC system. The inclusion of internal reference standards, coupled with the highly accurate nature of HPLC enables minute changes in individual amino acid concentration to be reliably detected and subsequently related to embryo viability. The bulk of the research thus far has been carried out using retrospective approaches, however there are now data indicating the prospective applications of AAP.

**Results:** The evidence that AAP can act as a biomarker of embryo viability is accumulating and I will present data of a small study in which prospective selection based on AAP was carried out for the first time. Moreover it is now emerging that AAP can tell us additional information about the early embryo. Thus, using data collected from surplus human embryos as well as embryos from animal models, I will show that amino acid metabolism is correlated with molecular damage. Moreover I will demonstrate that AAP can tell us about origin of the embryo; embryos generated in vitro have a very different amino acid metabolic profile to their in vivo-derived counterparts. Furthermore, I will present new findings suggesting a relationship between maternal nutrition and embryo metabolism and viability and also evidence that suggests that the sex of an embryo is reflected in its amino acid profile.

**Conclusions:** There is strong evidence that differences between viable and sub-viable embryos are reflected by metabolic activity and the translational potential of such approaches to yield a biomarker of embryo viability is high. In addition to a large amount of published work supporting this notion, it is now evident that metabolism, in terms of AAP, gives us additional information about the phenotype of the early embryo. However, in order for the translational potential to be fully realised and integrated into clinical IVF, it is essential to test the concept in large-scale clinical trials.

**O-099 Non-invasive metabolomic profiling to select single embryos for transfer in clinical IVF**

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Elective Single Embryo transfer (eSET) combined with embryo cryopreservation has become an important strategy for reducing multiple pregnancies in IVF. Because only one embryo is transferred, the selection of the embryo with an optimum implantation potential is of great importance. Currently, embryo selection is mainly based on morphological criteria using light microscope analysis. SET increases the onus on morphology to select the best embryo for transfer, therefore new selection tools are needed to ensure success rates are maintained. New parameters to predict embryo viability, including non-invasive metabolomic profiling, have recently been studied. Metabolomics is the study of small-molecule metabolite byproducts left behind from cellular processes. By measuring byproducts of the embryonic metabolism you get a snapshot of the physiology of an embryo which translates to viability.

Recently, a number of studies have shown that metabolomic profiling of biomarkers of metabolism by Near Infrared (NIR) spectroscopy correlates with ongoing pregnancy, irrespective of embryo morphology in fresh IVF/ICSI cycles. The results indicate that NIR spectral analysis may allow discrimination of viable and non-viable embryos.

The metabolomic profiling data from cryo-thawed embryos show similar findings as the work using this method with fresh embryos: ie. higher viability scores result in higher pregnancy and life birth rates. Cryo-thawed embryos (of the same morphological grade) have a different metabolic activity which is correlated to implantation potential. Embryo morphology post thawing and viability score are not correlated, so these two quality parameters might be used in conjunction to each other.

In conclusion, metabolomic profiling by Near Infrared Spectroscopy is a rapid, objective and non invasive embryo assessment technique which may provide extra information about the implantation potential of fresh and cryo-thawed embryos at the time of transfer.

Currently, several randomized controlled trials are being performed to study the ongoing pregnancy and live birth rate comparing morphology alone to using metabolomic profiling as an adjunct to morphology for the selection of viable embryos.

**SELECTED ORAL COMMUNICATION SESSION SESSION 28: OVARIAN RESERVE 2 Tuesday 29 June 2010 10:00 - 11:30**

**O-100 Prediction of ovarian function after chemotherapy for breast cancer**

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**Introduction:** Increasing numbers of women are surviving breast and other cancers, and for younger survivors, future reproductive function is a major concern. Treatment with chemotherapy agents carries a high risk of ovarian failure, and the ability to predict this risk would be of considerable value. AMH is a product of granulosa cells of preantral and small antral follicles, and appears a closer marker of the ovarian reserve than other hormones. We sought to investigate whether AMH measured at cancer diagnosis predicts ovarian activity 4-5 years following chemotherapy.

**Materials and Methods:** Women with newly diagnosed breast cancer were recruited prior to starting chemotherapy to a 5 year prospective study of ovarian function. Chemotherapy regimens were generally anthracycline/cyclophosphamide based, following which most were treated with tamoxifen, which was changed to an aromatase inhibitor (AI) in 7 women in year 4 or 5. Return visits were scheduled to the early follicular phase if appropriate, and patients kept a menstrual diary throughout. AMH was measured by commercial immunoassay (Beckman-Coulter), sensitivity 0.05 ng/ml. FSH, estradiol and inhibin B were also analysed in all samples. Antral follicle count (AFC) and ovarian volume were measured by transvaginal ultrasound.

**Results:** Of the 42 women recruited, 35 remained in the study at 4 years and 33 at 5 years. Ten women maintained regular menstrual cycles throughout the 5 years and a further 3 women became amenorrhoic during chemotherapy, but resumed menses thereafter. By comparison 28 women became amenorrhoic and did not resume menses (menstrual diaries were not completed by 1 woman). Women with ongoing menses were younger (36.5 ± 1.6 vs 43.6 ± 0.9 yrs, p = 0.0002). Data from the 7 women taking AIs was also assessed separately demonstrating that all had become amenorrhoic before AI treatment started. Serum concentrations of estradiol, inhibin B and AMH were lower following chemotherapy than pre-treatment, and FSH was elevated. Overall there was no change in either AMH or inhibin B between year 2 and year 5, but in women in whom it was detectable, AMH showed a significant decline from year 2 to year 5 (0.66 ± 0.17 to 0.29 ± 0.07 ng/ml, p < 0.01, n = 6) whereas there was no change in inhibin B. Women with ongoing menses at 4-5 years follow-up were confirmed to have increased markers of ovarian activity ie higher AMH, inhibin B and E2 concentrations and lower FSH concentrations than amenorrhoic women.

Analysis of pre-treatment hormones showed that AMH was higher (2.5 ± 0.4 vs 0.7 ± 0.1 ng/ml p < 0.0001), and FSH was lower (5.2 ± 0.7 vs 13.1 ± 1.8 IU/L, p = 0.0008) in women with ongoing menses at 5 years. High AMH (>median, 0.96ng/ml) gave a relative risk of 2.1 of ongoing menses. Pretreatment Inhibin B and E2 did not differ between groups. Pretreatment AFC (19.8 ± 3.0 vs 17 ± 1.2, p = 0.0004) but not ovarian volume (4.2 ± 0.7 vs 2.9 ± 0.6ml) was also higher in women with ongoing menses.

Logistic regression analysis was performed to investigate relationships between pre-chemotherapy age, AMH, FSH and inhibin B with ongoing menses. This demonstrated that only AMH remained as a significant predictor of menses.
AMH-producing follicles are FSH-sensitive but devoid of LH receptors. Since pituitary gonadotropins influence AMH production. Since compared with women without T pathology (48.4% and 16.7%, p = 0.0082). Conversely, combined FSH + LH activities (hMG) administration led to a remarkable increase in serum AMH levels (median delta: + 0.34 ng/mL, ranges: +0.01 + 1.17 ng/mL, P < 0.003). In addition, a non significant trend to an increase in serum AMH levels was observed after isolated LH activity (hCG) administration (median delta: + 0.20 ng/mL, ranges: +0.14 + 1.05 ng/mL, P > 0.07). Incidentally, serum androgen levels failed to show any noticeable changes between BL and t24 in the 4 study groups. Conclusions: In contrast with isolated FSH activity, hMG administration, a ready-to-use combination of FSH + LH activities, induced a significant increase in serum AMH levels. Since at the same antral stage follicles do not express LH receptors in the GCs, such an effect is presumably mediated through a secondary, LH/hCG intra-ovarian androgenic increase, although peripheral androgen levels remained unchanged in all groups. In line with this hypothesis, AMH levels tended to increase after hCG administration. These results provide new documentation of a synergic interaction of FSH + LH activities in the AMH production by GCs of normo-ovulating women, which mechanisms require further clarification.

O-103 Anti-mullerian hormone (AMH) levels and expression in small, medium and preovulatory size follicles

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Introduction: Growing evidence indicates that Anti-mullerian hormone (AMH) has an essential role in folliculogenesis. In mammals, it is suggested to inhibit the recruitment of primordial follicles and modifies the growth of preantral and antral follicles by diminishing the sensitivity of follicles to FSH stimulation. However, the precise role of AMH in folliculogenesis and specifically its expression pattern in antral follicles has not been described in human ovarian follicles. Aim: This study investigates AMH secretion and mRNA expression pattern in human ovarian follicles in relation to different follicular size. Methods: AMH secretion and mRNA expression levels of different follicle sizes were analyzed from follicular fluid (FF) of antral follicles obtained during in vitro maturation (IVM) and in vitro fertilization (IVF) procedures. FF AMH levels and mRNA expression of mural granulosa cells were measured in small follicles (≤ 10 mm) and medium size follicles (11-15 mm) obtained in IVM procedures and large size follicles (>16 mm) obtained in IVF procedures. FF AMH levels were expressed as microgram per milliliter of AMH adjusted to its protein content. mRNA of cumulus and mural granulosa cells were analyzed by Real Time PCR, normalized to β-actin, and expressed in arbitrary units. Results: FF AMH levels in the small size follicles (n = 25) were significantly higher (1.56 ± 0.3 μg/ml), than FF AMH levels in the medium (n = 23) and large size (n = 12) follicles (0.3 ± 0.2 μg/ml and 0.2 ± 0.05 μg/ml respectively) P < 0.005. AMH mRNA expression of mural granulosa cells was 1.1 for small antral follicles, 0.43 for medium size follicles and 0.21 for large follicles. AMH mRNA expression in cumulus cells was 8.7 and 0.4 for mural granulosa cells of preovulatory follicles. Conclusions: Follicular fluid AMH secreted levels and AMH mRNA expression in human granulosa cells is significantly higher in small antral ovarian follicles than in preovulatory follicles. Its expression sharply decreases with foli..
icular size. Cumulus cells express higher amount of AMH mRNA than mural granulosa cells. This remarkable correlation between the mRNA levels in mural GCs and the secretion level in respect to follicle size suggests a transcriptional regulation of AMH decline during folliculogenesis.

**O-104**  **The nature of the independent relationship between AMH and age with respect to oocyte yields**

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**Introduction:** It has been established that anti-Müllerian hormone (AMH) is highly predictive of oocyte yield after controlled ovarian stimulation (COS). In a large prospective cohort we have previously shown that AMH and age are independent predictors of oocyte yield, despite the close negative correlation between AMH and age (Nelson et al. 2007). We have more recently shown that AMH can be used to direct strategic approaches to COS, and that distribution of antral follicles (AFCs) and the secretion level in respect to follicle size suggests a transcriptional regulation of AMH decline during folliculogenesis.

**Discussion:** These data confirm that age has an independent influence upon ovarian responses to FSH for a given AMH range. They also suggest that the biology underlying this phenomenon is related to the distribution of antral follicular sub-categories. Whilst AMH correlates strongly with FD2-5, the egg yield appears to be strongly influenced by the FD6-9 category. With increasing age amongst women with a maintained AMH and FD2-5 profile, the size of the FD6-9 follicle cohort appears to decline with age, leading to a reduced egg yield. The data suggest that there is increasing atresia of late stage antral follicles towards the end of normal reproductive life.

**O-105**  **Anti-Müllerian hormone confirms the classification of female functional androgenization**

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**Introduction:** Functional androgenization (FA) consists of various entities which have been termed either "polycystic ovary disease" (PCOD) or "non-classical congenital adrenal hyperplasia" (NC-CAH). In accordance with a recent comprehensive study, female FA can be subdivided into 5 groups corresponding to their predominant organ pathology classified as functional androgenization syndrome (FAS) I (ovary), II (adrenal gland), III (multi-organ disorder including ovarian dysfunction, obesity, hyperinsulinemia), IV (residual FA dysfunctions) and functional cutaneous androgenization (FCA) (skin). Group specific clusters were set up by primary variables like LH, testosterone (T), sexual hormone binding globulin (SHBG), free androgen index (FAI), dehydroepiandrosterone-sulphate (DHEAS), body mass index (BMI), enlarged polycystic follicular ovaries (EPOs), insulin and glucose levels. Anti-Müllerian hormone (AMH) is produced by granulosa cells of preantral follicles and is supposed to regulate early follicle growth. Several studies showed elevated AMH serum levels in women with "PCOD". Therefore AMH seems to be a promising variable for the classification of FA.

**Materials and Methods:** 196 patients (25.1 ± 5.7 yrs; BMI 27 ± 6.4 kg/m²) with FA were included. Data of 30 volunteers with regular menstrual cycles served as control (30.3 ± 5.2 yrs; BMI 21.1 ± 1.8 kg/m²). Patients were classified to the 5 FA groups as described above. In early follicular phase, primary variables were determined: sonographic ovarian morphology where EPO was defined by an ovary score ≥ 1.5 in at least one ovary, LH, T, SHBG, FAI and DHEAS. AMH was analysed using a specific ELISA (DSL). Further, an oral glucose loading test was performed with the analysis of glucose and insulin.

**Results:** AMH levels were significantly higher in FA patients (10.9 ± 6.6 ng/ml) vs. control (3.0 ± 2.0 mg/ml; P < .0001). In women diagnosed as FAS I, AMH was significantly increased compared to all other groups (P < .0001). Patients with FAS III showed significantly higher AMH levels vs. those with FAS II (P < .0001), IV (P = .004) or FCA (P = .002) or vs. control (P < .0001). AMH correlates significantly positively (P < .0001) with LH (R = 0.538) and T (R = 0.368). In regression and multivariate analysis, AMH was neither dependent on SHBG, DHEAS, BMI, glucose nor insulin.

**Conclusions:** AMH confirms the FA classification developed by our group. High AMH levels are found in groups FAS I and III where, by definition, EPOs are present and ovarian dysfunction constitutes the predominant pathology. In this study population, there was no correlation between AMH and metabolic parameters suggesting AMH to be a specific marker of ovarian function. In vitro, granulosa cells of women with "PCOD" showed a highly increased AMH secretion that was further enhanced by LH pointing to a pathological granulosa cell function in these women. As for reproductive medicine, women diagnosed as FAS I or FAS III are highly sensitive to gonadotropin stimulation and need a low dose approach in order to avoid ovarian hyperstimulation syndrome, whereas patients with FCA, FAS II or IV show an age-related ovarian reserve and may also be poor responders.

**Reference:**