cryopreservation method of very low count human spermatozoa. Current sperm cryopreservation methods are associated with loss of a considerable proportion of spermatozoa. A simple and useful sperm cryopreservation method can be beneficial not only for azospermic man but for cases of extreme oligozoospermia as well.

**Study design, size, duration:** Our prospective study included 20 samples from very severe male factor patients and 10 TESE samples. Using cryolock (AL-RAD,Israel) a devise usually used for oocyte or embryo vitrification, the efficiency of two cryoprotectants and two freezing protocols was studied. Each experiment designed on the basis of results achieved previously.

**Participants/materials, setting, methods:** sperm was mixed with Test yolk buffer (TYB) or HEPES- glycerol – glucose solution (final ratio 1:1 vol/vol). 5 µl mixture aliquot was then placed on two cryolocks. One plunged directly into liquid nitrogen (LN) and the other exposed to LN vapors. Thawing: cryolock tip was immersed directly into warm medium.

**Main results and the role of chance:** In all twenty OTA samples, sperm were detected post thawing. Better survival rate was found when using TYB as cryoprotectant and exposure to liquid nitrogen vapor compared to HEPES-glycerol-glucose solution followed by directly plunging into liquid nitrogen (95% vs. 35% respectively) P < 0.0001.

Based on this, very few spermatozoa (10-50 sperm) (15 samples) and TESE samples were frozen using only TYB as cryoprotectant and exposure to LN vapor. After thawing, sperm were identified in all 15 samples and at least one motile spermatozoon was detected in 14/15 samples (93%).

Moreover, In 9 out of 10 frozen/thawed TESE samples (90%) sperm cells were found in the culture dish under the microscope, and in four (44%) of them, at least one motile spermatozoon was detected post thawing.

**Limitations, reason for caution:** No limitation and no special equipment are needed.

**Wider implications of the findings:** The present data suggest that Cryolock can be a useful tool for freezing not only female gametes or embryos but also for cryopreservation of very small number of human sperm cells. These findings can benefit men with extreme OTA samples as well as patients undergoing TESE/microTESE. However, further studies concerning various technical aspects are needed to improve the yield of this method for cryopreservation of individual sperm cells.

**Study funding/competing interest(s):** no funding

**Trial registration number:** Not applicable.

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**SELECTED ORAL COMMUNICATION SESSION**

**Session 31: PGD/PGS**

**Tuesday 9 July 2013 10:00 - 11:30**

**O-118**  Preimplantation genetic screening on day 3 embryos using array comparative genomic hybridization in patients with advanced maternal age: a prospective double blinded randomized controlled trial

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**Study question:** Does cleavage stage preimplantation genetic screening (PGS) using Array Comparative Genomic Hybridization (aCGH) improve the clinical outcome of in vitro fertilization (IVF) techniques in patients of advanced maternal age (AMA)?

**Summary answer:** PGS using aCGH by analyzing embryos at cleavage stage improved the efficiency of the IVF techniques, determining a significantly increase of live birth rate in patients of advanced maternal age.

**What is known already:** Initial PGS studies demonstrated a high prevalence of aneuploidy in human embryos. This provided an apparent opportunity to improve IVF outcomes by screening embryos for aneuploidy before transfer. However, several prospective, randomized controlled trials (RCTs) have failed to show any improvement in live birth rate using fluorescence in situ hybridization (FISH)-based PGS on cleavage stage embryos. The main reason for this poor clinical performance could be attributed to the technical limitations of the FISH technology.

**Study design, size, duration:** A prospective, double blinded, RCT including female patients aged 36-43 years, randomized into two groups: ICSI and day-5 double embryo transfer (group-A); ICSI, day-3 embryo biopsy and PGS with day-5 double embryo transfer when possible (group-B). Primary endpoint: live birth rate (baby born per transferred embryos). Secondary endpoints: ongoing pregnancy rates, implantation rate.

**Participants/materials, setting, methods:** 84 patients were enrolled, 19 did not meet the inclusion criteria, 65 were randomized: 34 in group-A and 31 in group-B. Mean female age was 39.7 ± 1.5 years. Single cells from 201 embryos were first lysed, DNA amplified by whole genome amplification (WGA) and processed by aCGH using 24Sure, BlueGnome.

**Main results and the role of chance:** A total of 46 cycles (34 fresh, 12 frozen) were performed for group-A (66 fresh, 23 frozen embryos transferred) and 37 cycles (31 fresh, 6 frozen) for group-B (27 fresh, 8 frozen embryos transferred). A significant increase in live birth rate was found in the PGS group compared with the control group (14/27(51.9%) vs. 13/66(19.7%) in fresh cycles (p = 0.002); 17/35(48.6%) vs. 15/89(16.9%) if considering cumulative cycles (p < 0.0001).
The ongoing clinical pregnancy rate per embryo transfer was 58.8% (group-B) vs. 33.3% (group-A) in fresh cycles (p = 0.00); 56.5% (group-B) vs. 28.9% (group-A) in cumulative cycles (p = 0.02). The ongoing clinical pregnancy rate per patient was 32.4% (PGS group) vs. 32.3% (control group) in fresh cycles (NS); 35.1% (PGS group) vs. 28.3% (control group) in cumulative cycles (NS).

Limitations, reason for caution: The study was limited to the analysis of a population of AMA patients. Furthermore, the negative predictive value of aCGH performed on a single blastomere biopsied from embryos at cleavage stage could not be assessed in this study.

Wider implications of the findings: Our results show that PGS using aCGH is beneficial for patients with advanced maternal age, offering an additional selection tool for choosing the most competent embryo(s) for transfer over simple morphological and developmental criteria. PGS allows to enhance embryo selection, identifying and selecting for transfer chromosomally normal embryos, thus increasing live birth rate for IVF patients on a per transfer base.

Study funding/competing interest(s): BlueGnome Ltd, Cambridge (UK)
Trial registration number: ISRCTN37972669

O-119 Non-reciprocal errors and germlinal mosaicism detected by the application of array-CGH to oocytes and polar bodies unexposed to sperm
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Study question: In women of average maternal age 1) what is the incidence of chromosome versus chromatin abnormalities in oogenesis. 2) how common are non-reciprocal errors. 3) how common is germlinal mosaicism?

Summary answer: Our data set has revealed a higher incidence of chromatin abnormalities versus chromosome abnormalities in unflitified human oocytes. 64% of the MII-PB (Metaphase II - 1st Polar Body) complexes show non-reciprocal errors. Only 2/29 immature oocytes have shown both chromosome and chromatin gains and chromatin losses providing possible evidence of germlinal mosaicism in the oocytes from certain women predisposing them to produce aneuploid oocytes.

What is known already: Studies have revealed two predominant aneuploidy causing mechanisms in female meiosis either whole chromosome non-disjunction or premature separation of chromatids. The outcome of clinical application of array CGH (Comparative Genomic Hybridisation) for polar body testing suggests almost all anomalies are chromatin in women of advanced maternal age. Previous research studies gave more mixed results. Clinical studies also show that polar body testing is the least reliable approach compared with using blastomere or trophectoderm (TE) biopsy; the reason is not clear.

Study design, size, duration: Ongoing application of array CGH to oocytes unexposed to sperm with the aim of obtaining data from at least 100 research oocytes.

Participants/materials, setting, methods: In total, 64 unfertilised oocytes at different stages of oocyte maturation were subjected to whole genome amplification (WGA) using SureplexTM and array-CGH using 24sureTM Bluegnome arrays. Thirty-two women undergoing IVF, average maternal age 36 years (range 29-45) donated their oocytes at The Centre for Reproductive and Genetic Health. Eighteen metaphase I oocytes (MI), 11 metaphase II oocytes (MII) without 1st Polar Body (PB), 22 MII-PB complexes and 13 germinal vesicles (GV) were included in the study.

Main results and the role of chance: Successful WGA was obtained for 95% (61/64) of oocytes. The rest failed to amplify due to poor quality DNA. ALL the GV’s were analysed were found to be euploid. Both chromosome and chromatin abnormalities were present in 11% (2/17) metaphase I oocytes. The overall aneuploidy rate for the MI’s and MI-PB complexes was 34% (11/32). The average maternal age of the women donating MII oocytes was 36 years, whereas the average maternal age of women that had abnormal MII’s was 38 years. Out of the 11 abnormal MI-PB complexes analysed, 5/11 showed reciprocal errors and 4/11 MI-PB complexes showed non-reciprocal errors and 2 MI-PB complexes showed both types of errors. Interestingly, 2/17 metaphase I oocytes, showed gains and/or losses providing evidence for germlinal mosaicism.

Limitations, reason for caution: As yet, our numbers are small.

Wider implications of the findings: Our data set has revealed the common incidence of non-reciprocal errors in MII oocytes and 1st PB’s; this may be one reason why the PB approach to PGS (Preimplantation Genetic Screening) is the least reliable. Non-reciprocal gains of chromatids provide possible evidence of germlinal mosaicism as does the finding of errors in MII oocytes from certain women predisposing them to produce aneuploid oocytes, which may be of some clinical significance.

Study funding/competing interest(s): Funding from the Leverhulme Trust, UK; no competing interests.

Trial registration number: Not applicable

O-120 Analysis of gametes and embryos from translocation carriers reveals sex-specific differences in chromosome segregation patterns and the existence of an interchromosomal effect
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Study question: Which are the most prevalent meiotic segregation patterns producing abnormalities in embryos generated by male and female carriers of a translocation? Does the presence of such a chromosomal rearrangement lead to an increased risk of aneuploidy affecting structurally normal chromosomes (interchromosomal effect)?

Summary answer: The alternate segregation pattern dominated for reciprocal and Robertsonian translocation carriers, regardless of their sex. Of abnormal segregations, the adjacent-1 segregation pattern was most common in abnormal embryos of male reciprocal translocation carriers, whereas adjacent-2 was more prominent for female carriers. An interchromosomal effect was evident only in cleavage stage embryos of Robertsonian translocation carriers.

What is known already: Chromosome rearrangements represent a common form of genetic abnormality, affecting one person in 500. During meiosis, pairing of homologous fragments of the normal and derivative chromosomes leads to the formation of complex structures. Different segregation patterns exist for these structures, potentially leading to losses/gains of chromosomal material in the resulting gamete. It is still unclear whether an interchromosomal effect, increasing the risk of aneuploidy for structurally normal chromosomes, affects gametes and embryos.

Study design, size, duration: Embryos generated from 51 reciprocal translocation carriers (36 male and 15 female) and 27 Robertsonian translocation carriers (16 male and 11 female) (average female age 34 years) were examined. The aneuploidy rate was also calculated for embryos from an age-matched control group of karyotypically normal patients undergoing routine aneuploidy screening.

Participants/materials, setting, methods: A total of 417 cleavage stage embryos and 109 blastocysts were analysed via array comparative genomic hybridisation (aCGH). Segregation patterns were determined according to the losses/gains of chromosome fragments identified from the aCGH analysis. Aneuploidy rate in first and second polar bodies of 36 oocytes from carriers of translocation were analysed to study ICE. Statistical analysis took place via the Chi square test.

Main results and the role of chance: The alternate segregation pattern was the most frequently observed in embryos of both male and female carriers of reciprocal (28% and 38%, respectively) and Robertsonian translocations (61% and 55%, respectively). Of the segregation patterns leading to abnormalities, adjacent-1 was significantly (P = 0.02) more common in embryos of male reciprocal translocation carriers (28%), whereas for female carriers, adjacent-2 was more dominant (17%) (P = 0.04). No differences in segregation pattern frequency were observed for male and female carriers of Robertsonian translocations. A significant increase (P < 0.0001) in the aneuploidy rate of chromosomes not involved in the translocation was found in cleavage stage embryos generated from Robertsonian translocation carriers (regardless of the gender), when compared to a matching control group.
Limitations, reason for caution: aCGH is not able to distinguish between normal and balanced embryos. However, this did not prevent the determination of abnormal segregation patterns, nor did it preclude assessment of a possible interchromosomal effect.

Wider implications of the findings: The gender of reciprocal translocation carriers influences the segregation pattern during meiosis. The observed sex-specific differences have implications for the frequency and types of unbalanced embryos generated, important considerations for patient counselling. The interchromosomal effect was restricted to cleavage stage embryos of Robertsonian translocation carriers (not seen in oocytes or blastocysts). This might be a consequence of altered chromosome positioning in interphase nuclei, disrupting the migration to the spindle and alignment of structurally normal chromosomes.

Study funding/competing interest(s): none

Trial registration number: Not applicable.

O-122  Re-analysis of whole day-5 embryos using the same arrayCGH platform used for day-3 diagnosis showed a high confirmation rate


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Study question: Is day-3 PGS by arrayCGH giving representative of the whole embryo regarding concordance between day-3 single cell analysis and day-5 re-analysis of the whole embryo using the same arrayCGH platform?

Summary answer: The high confirmation rate obtained between day-3 diagnosis and day-5 re-analysis using the same arrayCGH platform suggests that day-3 diagnosis is representative of the whole embryo and therefore an accurate diagnosis can be performed with day-3 arrayCGH for PGS.

What is known already: Aneuploidies found in preimplantation embryos are the result of meiotic (chromosomally abnormal gametes) or mitotic errors during embryo development leading to a mosaic embryo. Mosaicism has been described at cleavage stage but also at blastocyst stage. It has been widely discussed that day-3 embryo biopsy could not have a role for PGS arguing that the analysis of a single cell is not representative of all the embryo due to embryo mosaicism at cleavage stage.

Study design, size, duration: Prospective blinded study to re-analyse embryos previously diagnosed as chromosomally abnormal by day-3 arrayCGH. The only excluding criterion was the presence of a partial gain/loss on day-3 diagnosis. A numerical code was given to the included embryos. Size: 50 embryos from our PGS program. Duration: October 2012 to December 2012.

Participants/materials, setting, methods: Day-5 embryos selected for the study were washed with PBS, placed individually into PCR tubes and amplified using Sureplex (BlueGnome Ltd., Cambridge, UK). The same 24-hrs arrayCGH protocol used on day-3 was then performed (BlueGnome Ltd., Cambridge, UK). After scanning and analysing day-5 samples, day-3 and day-5 results were compared.

Main results and the role of chance: In all of the 50 re-analysed embryos an abnormal day-5 diagnosis was obtained. Results can be divided according to day-5 diagnosis. In 37/50 (74%) cases, day-5 showed exactly the same day-3 aneuploidies. In 6/50 (12%), not all but at least one day-3 aneuploidy was present in the whole embryo on day-5. In 4/50 (8%), day-5 showed the complementary aneuploidy for a given chromosome (monosomy vs. trisomy, or trisomy vs. monosomy). In 2/50 (4%), all day-3 aneuploidies were observed on day-5 but an additional chromosome loss was also observed (monosomy for chromosome 19 and 21, respectively). Finally, one embryo was assigned as euploid by the software but it showed the same result obtained on day-3 (monosomy 18) in mosaic (log2 ratio was -0.34).

Limitations, reason for caution: A low degree of mosaicism could be not detected by arrayCGH technology on day-5 biopsies. It is estimated that aneuploidy should be detected if presented in at least 25%-30% of embryo cells. It is still unknown the percentage of abnormal cells in a blastocyst to be considered “chromosomally normal”.

Wider implications of the findings: The same protocol for day-3 arrayCGH diagnosis can be used for day-5 arrayCGH re-analysis. This strategy is very less time consuming compared with day-5 embryo re-analysis using FISH and enables to obtain information of all chromosomes. Moreover, the high confirmation rate obtained in this work demonstrates that day-3 arrayCGH is an option for PGS clinical practice although embryo mosaicism can be present at cleavage stage but also at blastocyst stage.

Study funding/competing interest(s): none

Trial registration number: none