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 to men with extreme OTA samples as well as patients undergoing TESE/ICSI.

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 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 sperms) (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 sperms.

Study funding/competing interest(s): No funding

Trial registration number: No

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O-117 Euploid embryos are far more likely to undergo blastulation than aneuploid embryos when based on single blastomere array Comparative Genomic Hybridization (CGH)

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Study question: Is blastulation rate associated with euploid embryo status?

Summary answer: Euploid embryos appear to be three times more likely to undergo blastulation than aneuploid embryos undergoing blastomere biopsy and array CGH for Pregestational Genetic Screening (PGS).

What is known already: Recent studies on trophoeotderm biopsies show a higher rate of euploid embryos at the blastocyst stage than the euploid rate previously reported from cleavage stage blastomere biopsies. However, it has historically been thought that aneuploid embryos are just as likely to undergo blastulation as euploid embryos.

Study design, size, duration: Retrospective cohort study was performed on 44 cycles between January 2011 and December 2012 that underwent IVF and day three single cell blastomere biopsy with array CGH and PGS.

Participants/materials, setting, methods: Subjects underwent IVF-ICSI and CGH at a university hospital based IVF center. All cleavage-stage embryos underwent single cell blastomere biopsy and fixation for microarray CGH and results were reported for all embryos on day 5. The percent of both euploid and aneuploid embryos were compared utilizing Fisher’s exact test.

Main results and the role of chance: Mean patient age among the 44 cycles was 37.2 (range 28-42). A total of 463 embryos were produced in 44 cycles from which 382 (82.5%) six to eight embryos were biopsied. Overall blastulation rate for all biopsied embryos was 32.0% which was not significantly different from a matched control group that did not undergo biopsy. The number of euploid and aneuploid embryos after biopsy was 106 (27.7%) and 276 (72.3%), respectively. 84 (79.2%) of the euploid embryos and 66 (23.9%) of the aneuploid embryos progressed to blastocyst stage. (p < 0.0001). However only 58/106 (54.7%) of the euploid embryos formed fully expanded or hatching blastocysts by day 5, and therefore would not have been able to undergo tropheochoderm biopsy.

Limitations, reason for caution: Limitations include retrospective design and somewhat small study group. Additionally mosaicism in single cell biopsy could affect our outcomes.

Wider implications of the findings: Our results suggest that euploid embryos are far more likely to undergo blastulation than aneuploid embryos. However there are still many aneuploid blastocysts that would be transferred in cycles without PGS. One advantage of day three biopsy is that only half of the euploid embryos in this study advanced enough for tropheochoderm biopsy on day five.

Study funding/competing interest(s): The study had no funding.

Trial registration number: Not applicable.

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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 <
The ongoing clinical pregnancy rate per embryo transfer was 58.8% (group-B) vs. 33.3% (group-A) in fresh cycles (p = 0.08); 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 germinal 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 germinal mosaicism?

Summary answer: Our data set has revealed a higher incidence of chromatin abnormalities versus chromosome abnormalities in unutilised 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 evidence for germinal 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. Successful WGA was obtained for 95% (61/64) of oocytes. The rest failed to amplify due to poor quality DNA. All the GVs assessed 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 MII’s and MII-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 MII-PB complexes analysed, 5/11 showed reciprocal errors and 4/11 MII-PB complexes showed non-reciprocal errors and 2 MII-PB complexes showed both types of errors. Interestingly, 2/17 metaphase I oocytes, showed gains and/or losses providing evidence for germinal mosaicism.

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 dominant for female carriers. An interchromosomal effect was evident 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 < 0.001) 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.