Use of suboptimal sperm increases the risk of aneuploidy of the sex chromosomes in preimplantation blastocyst embryos

Alison Coates, B.Sc.,[a,b] John S. Hesla, M.D.,[a] Amanda Hurliman, M.D.,[a] Breanne Coate, B.S.,[a] Elizabeth Holmes, M.S.,[a] Rebecca Matthews, Ph.D.,[a] Emily L. Mounts, M.S.,[a] Kara J. Turner, Ph.D.,[b] Alan R. Thornhill, Ph.D.,[b,c] and Darren K. Griffin, D.Sc.[b]

[a] Oregon Reproductive Medicine, Portland, Oregon; [b] School of Biosciences, University of Kent, Canterbury, Kent, United Kingdom; and [c] Illumina, Cambridge, United Kingdom

Objective: To compare autosomal and sex chromosome aneuploidy rates of embryos derived from sperm with abnormal and normal parameters.
Design: Retrospective cohort study.
Setting: Assisted reproduction center.
Patient(s): Three thousand eight hundred thirty-five embryos generated from 629 couples undergoing IVF.
Intervention(s): None.
Main Outcome Measure(s): Incidence of aneuploidy in the trophectoderm of blastocyst embryos derived from standard IVF embryos and intracytoplasmic (ICSI) males with normal and oligozoospermic semen samples, in couples with donor eggs (mean maternal age, 25.0 years) and their own eggs (mean maternal age, 35.4 years).
Result(s): The rate of sex chromosome aneuploidy was significantly (around threefold) higher in the oligozoospermic group compared with in both control groups (standard vs. ICSI insemination). This applied whether donor (young) or own (older) eggs were used. Significant differences were seen in the oligozoospermic samples for autosomes 1, 2, 11 (own eggs), and 18 (donor eggs) compared with both control groups; however, no significant difference was seen between each of the treatment groups for the overall rate of autosomal aneuploidy. No significant differences were seen between the two control groups (normozoospermic males, standard vs. ICSI insemination) in either of the egg group types for any chromosome pairs.
Conclusion(s): Severe male factor infertility is associated with a significant increase in the occurrence of sex chromosome abnormalities in blastocyst embryos compared with in embryos derived from normal semen samples. Aneuploidy rates in embryos derived from sperm with normal parameters were not significantly different whether ICSI or standard insemination was used to achieve fertilization. These results highlight severe male factor infertility as a possible referral category for preimplantation comprehensive chromosomal screening. (Fertil Steril® 2015;104:866–72. ©2015 by American Society for Reproductive Medicine.)
Key Words: Sex chromosome, aneuploidy, ICSI, autosome

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/coatesa-male-infertility-embryo-aneuploidy/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received February 12, 2015; revised and accepted June 24, 2015; published online July 14, 2015.
A.C. has nothing to disclose. J.S.H. has nothing to disclose. A.H. has nothing to disclose. B.C. has nothing to disclose. E.H. has nothing to disclose. R.M. has nothing to disclose. E.L.M. has nothing to disclose. K.J.T. has nothing to disclose. A.R.T. has nothing to disclose. D.K.G. has nothing to disclose.
A.C. presented the data live on the *Fertility and Sterility* journal club webinar.
Presented at the 63rd Annual Meeting of the Pacific Coast Reproductive Society (PCRS), Rancho Mirage, California, March 11 - 15, 2015; and winner of the PCRS Allied Health Professional Award for the oral presentation.
Reprint requests: Darren K. Griffin, D.Sc., School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, United Kingdom (E-mail: d.k.griffin@kent.ac.uk).

Fertility and Sterility® Vol. 104, No. 4, October 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.06.033

Studies have shown evidence of a significantly higher proportion of aneuploid sperm in the ejaculates of men with male factor infertility when compared with normal controls (1, 2). A review of the literature by Tempest and Griffin demonstrated a clear correlation between suboptimal semen parameters, principally oligozoospermia, and increased sperm aneuploidy rates for most chromosomes examined, although it

was noted that patient parameters were not always clearly defined (3). Sperm of poor quality are frequently unable to penetrate an egg, either in vivo or through standard insemination techniques in vitro, possibly leading to failure of fertilization. To overcome this barrier to conception, intracytoplasmic sperm injection (ICSI) was developed and has found widespread, worldwide use as the treatment of choice for male factor infertility (4, 5). Partly because of the increase in sperm aneuploidy associated with some infertile men, the rates of aneuploidy in the products of conception (POC) of nonviable pregnancies and the prevalence of birth defects and achievement of developmental milestones in children conceived through assisted reproduction have been studied. This has enabled the comparison of the outcomes of standard IVF, ICSI, and natural conception, and the results have been relatively reassuring (6–9). To date, however, there has not been a published study asking whether rates of aneuploidy in preimplantation blastocyst embryos are elevated in ICSI cases derived from oligozoospermic and/or normal sperm samples, controlling for any possible detrimental effect that the ICSI process itself may have on occurrence of aneuploidy.

In addition to the risk of perpetuating aneuploidy through the injection of disomic sperm, the technique of ICSI has, it has been suggested, the potential to be detrimental to embryo development in several ways (10). ICSI has been shown to compromise sperm nuclear decondensation, possibly leading to aneuploidy in the embryo (11). The ICSI process has the potential to disrupt the oocyte spindle apparatus when the ICSI needle passes through the oolemma into the center of the oocyte, possibly leading to abnormal patterns of chromosome segregation (12). Finally, the oocyte may be handled outside of the incubator for a longer period of time during ICSI compared with standard insemination, that is, during cumulus cell removal with hyaluronidase and injection of sperm. Slight temperature and pH changes during micromanipulation may increase the possibility of stress-induced aneuploidy. All of these interventions associated with the ICSI technique have the potential to impair chromosome segregation in the egg and subsequently the cleaving embryo.

If the genetic component of sperm is the predominant risk factor for aneuploidy in the blastocyst embryo, one may expect that aneuploidy would be the most prevalent in embryos derived from the sperm of more severely oligozoospermic males (as the potential for sperm aneuploidy appears to be directly correlated with the severity of abnormalities in semen parameters). Conversely, if the ICSI process itself induces aneuploidy, one would expect that all ICSI treatments would show an increase in embryo aneuploidy as compared with standard insemination cycles, regardless of sperm quality.

Testing the ploidy status of preimplantation embryos via trophectoderm (TE) biopsy and comprehensive chromosomal screening (CCS) is now a routine practice in many IVF centers throughout the world. Pregnancy rates per cycle are increased in older female age groups by identifying chromosomally abnormal embryos and selecting known euploid embryos for uterine transfer (13–15). The objective of this study was therefore to test the hypothesis that aneuploidy frequency (sex chromosome or autosome) in human embryos increases, either as a result of injection of suboptimal sperm or as a result of the ICSI procedure itself.

MATERIALS AND METHODS

Between August 2010 and March 2015, 629 couples underwent IVF using either ICSI or standard insemination. A total of 3,835 embryos resulting from these cycles were tested for aneuploidy by TE biopsy followed by CCS using array comparative genomic hybridization (aCGH) in a single tertiary fertility center.

All male patients underwent semen analysis before the IVF cycle commenced and again on the day of the oocyte retrieval to determine whether ICSI or standard insemination would be used to achieve fertilization. The parameters measured included sperm density/mL, forward progression, speed of progression, percent normal forms (16), and antisperm antibody binding. Patient history was also taken into account when deciding which method of insemination was to be used on the retrieval day. Reasons for performing ICSI on the day of egg collection were as follows: decreased sperm concentration, motility, and/or morphology; use of frozen sperm; many years of unexplained infertility without a pregnancy; or previous poor fertilization using standard insemination in an earlier IVF cycle. Those included in the study had their embryos biopsied at the blastocyst stage at their request to assess the chromosomal status before transfer to the uterus. Indications for CCS included advanced reproductive age of the female patient, history of repeated pregnancy losses, history of failed IVF cycles, history of previous aneuploid pregnancy, diminished ovarian reserve, or patient request.

Controlled ovarian stimulation protocols for these IVF cycles were carried out as described elsewhere (17). On completion of the retrieval procedure, oocytes were placed in Quinns Advantage Fertilization Medium (Origio) supplemented with 5% human serum albumin (HSA; Irvine Scientific) under oil (Ovoil, Vitrolife), and ICSI or standard insemination was carried out 4 hours after retrieval.

For standard insemination, 15,000 sperm were placed with each cumulus-oocyte complex on the day of oocyte retrieval. The ICSI procedure was performed on all mature eggs as described elsewhere (12). Once all eggs had been either inseminated or injected, they were returned to the incubator for overnight culture. All embryos were moved to Quinns Advantage Cleavage Medium (Sage, Origio) supplemented with 10% HSA from days 1 to 3 and subsequently moved to Quinns Advantage Blastocyst Medium (Sage, Origio) supplemented with 10% HSA from days 3 to 6.

All embryos to be biopsied were hatched on day 3 postretrieval with a Hamilton Thorne laser by making a small opening and then left in culture until day 5 or 6 of development. Embryos were considered suitable for biopsy on day 5 when at least 10% of the TE was protruding from the breach in the zona pellucida made on day 3. All embryos that had not fully expanded by day 5 were cultured until day 6 and biopsied before noon if they had reached full expansion by that time. Embryos were only biopsied if there was a visible inner cell mass (ICM) and multicelled TE protruding from