Sperm Selection by Microfluidic Separation Improves Embryo Quality

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Title: Microfluidic Separation Reduces Sperm DNA Damage and Improves Embryo Quality

Background/Significance
More than 70 million couples worldwide are infertile and up to 40 million are actively seeking infertility care. In the year 2013, a total of 160,521 assisted reproductive technology (ART) procedures were performed in the United States (Sunderam *et al,* 2015). Isolation of motile and morphologically normal sperm is an integral part of assisted reproduction. Traditional sperm processing for assisted reproduction involves centrifugation and "swim up" techniques that employ a density gradient to isolate motile sperm. However, studies have suggested that centrifugation induces reactive oxygen species and DNA damage (Malvezzi *et al*, 2014; Wang *Assist Reprod Genet,* 2014). Increased sperm DNA damage has been associated with poor outcomes in assisted reproduction, including lower fertilization rates, impaired embryo progression, and decreased pregnancy rates (Virro *et al*, 2004; Benchaib *et al* 2007; Simon *et al* 2014).

In contrast, microfluidic-based sperm sorting has the capability of selectively isolating highly motile, morphologically normal sperm with high DNA integrity from an unprocessed semen sample (Shirota *et al* 2016; Asghar *et al,* 2014; Tasoglu *et al* 2013). In semen samples from healthy male volunteers split into standard processing via centrifugation and swim-up procedure compared with microfluidic sperm sorting, a significantly higher percent motility and lower rate of sperm DNA fragmentation was detected with microfluidic sperm sampling (Shirota *et al* 2016). While the microfluidic sperm sorting technique has thus proven to be an efficient and reliable means of sperm preparation compared with the centrifugation and swim-up procedure, its use in clinical practice has not been rigorously studied. We aim to compare traditional preparation and microfluidic sperm sorting on assisted reproductive technology outcomes including oocyte fertilization and embryo quality in subjects electing to undergo in vitro fertilization for infertility.

Preliminary Studies:
The microfluidic sperm sorting device has been shown to isolate highly motile, morphologically normal, and high DNA integrity sperm from unprocessed human semen. We have utilized the microfluidic sperm chip to select sperm for intracytoplasmic sperm injection (ICSI) in 26 cases of patients with poor prior ART outcome after standard sperm processing and selection. In these poor prognosis cases, we had 73% fertilization rate and 59% good quality day 3 embryos. Furthermore, among those patients who have received an embryo transfer, 58% (7 out of 12) have had a positive pregnancy test. While we cannot compare these data statistically to the prior failed cycle (due to concerns regarding regression to the mean), these data compare favorably to age-matched controls. However, given that this is a highly selected population, we believe a randomized controlled trial is necessary to rigorously explore the utility of sperm selection with the microfluidic sperm chip.

Hypothesis:
Subjects randomized to sperm preparation by microfluidic sperm sorting will obtain higher quality day 3 embryos than those randomized to o traditional sperm processing.

Study Aims:
1) Primary Aim: To compare day 3 embryo quality resulting from fertilization of oocytes by sperm selected by microfluidic sperm sorting compared to traditional sperm preparation methods.
2) Secondary Aim: To compare fertilization rates, blastulation rates, and pregnancy rates for subjects randomzied to sperm selection by microfluidic sperm sorting compared to traditional sperm preparation methods.

Design:
This is a randomized controlled trial of couples undergoing in vitro fertilization for unexplained infertility. Couples will be randomized to sperm selection by the clinical standard of centrifugation

and density-gradient processing compared to the microfluidic sperm sorting chip. Subjects and investigators performing outcome assessment will be blinded to treatment assignment.

Inclusion Criteria:
The target population includes couples planning in vitro fertilization (IVF) with or without intracytoplasmic sperm injection for unexplained infertility at the UCSF Center for Reproductive Health with a history of poor embryo quality as defined by < =40% high quality D3 embryos in a prior IVF cycle. All eligible couples will be asked to join the study.

Exclusion Criteria:
Male partner with severe oligoasthenospermia (concentration < 5 x 10^6 spermatozoa/mL; motility< 10%)
Female partner with anovulation (PCOS, FHA)
Female partner age >41
Female partner AFC< 7
Female partner with obstructed fallopian tubes (assessed in all patients prior to IVF)
Use of oocyte donor

Either Partner:
Cancer diagnosis in either partner
Any significant disease or psychiatric disorder that would interfere with consenting process

Treatment History:
History of >1 prior cycle cancellation due to poor response

Treatment Plan:
Embryo co-culture
Use of adjunctive non-gonadotropin medications to improve embryo quality: growth hormone, sildenafil

Study Procedures:
Couples meeting eligibility criteria will be offered enrollment. Randomization will be performed by the study coordinator upon initiation of controlled ovarian stimulation. Neither study clinician nor subject will be aware of treatment allocation. Ovarian stimulation will be performed with standard treatment chosen by primary physician. The day of ovulation trigger will be determined by the primary physician. The andrology lab will be notified of allocation to conventional sperm preparation versus microfluidic sperm sorting on the day of ovulation trigger. Oocyte retrieval is performed 36 hours after ovulation trigger. On the day of oocyte retrieval, the male partner will produce a semen sample per usual clinic protocol. A semen analysis will be performed on all samples for assessment of volume, motility, concentration, and morphology prior to semen processing according to WHO criteria using a light microscope, consistent with usual clinic protocol. Following the semen analysis, the semen of those assigned to microfluidic sperm sorting will be pipetted into the inlet chamber of the sorting chip. The microfluidic sperm sorting device is a flow and chemical-free microfluidic single use “chip” with an inlet sample chamber connected to an outlet collection chamber by a microfluidic channel. The sorted sperm are collected from the outlet.

The semen of those assigned to standard processing will undergo conventional centrifugation and density gradient separation. ICSI or IVF will be performed by an embryologist blinded to treatment allocation with the processed sperm according to the primary physician’s orders. After 16-18 hours, fertilization will be evaluated by the existence of two pro-nuclei. Fertilized embryos will be cultured to day 3 and evaluated by an embryologist blinded to treatment allocation for embryo quality. Cell number will be assessed in addition to blastomere symmetry and fragmentation (grade 1-6, highest quality to lowest). Those whose treatment plan involves a day 3 embryo transfer (determination made by primary physician) will undergo embryo transfer per clinic standard on day 3. Otherwise, embryos will be cultured to the blastocyst stage prior to

transfer. Blastocysts will be graded by Gardner Criteria, per clinic protocol. All women who undergo an embryo transfer will have a serum pregnancy test (beta HCG) 14 days later. Those with a positive serum test will repeat testing 48 hours later. Pregnancy ultrasounds will be scheduled at 5 weeks 5 days per clinic standard of care.

Device:
FERTILE: microfluidic sperm sorter
The device is labware. It is not an implant and is not purported to support or sustain human life. It is a flow and chemical free microfluidic single-use chip with an inlet chamber for receiving untreated semen samples and an outlet for collection of normal, healthy sperm for use in assisted reproductive technology procedures in andrology and embryology laboratories. It does not present any potential for serious risk to the health, safety, or welfare of participants.

Statistical Methods:
As this is a randomized controlled trial, known and unknown confounders are assumed to be evenly distributed between treatment groups. However, at the completion of enrollment, baseline characteristics will be assessed between groups and chi squared or t-tests will be performed, as appropriate to ensure groups are evenly distributed with respect to the most important clinical parameters such as age, duration of infertility, gravity/parity, prior infertility treatment, and ovarian reserve (AFC). The primary outcome, day 3 high quality embryo proportion will be defined as proportion of all viable embryos on day 3 with at least 6 cells and fragmentation/symmetry scores of 1-2. This proportion will be compared between groups with a t-test. An ANOVA will be performed to obtain and age and AFC-adjusted comparison of day 3 high quality embryo proportion between groups. Secondary outcomes will include fertilization and pregnancy rates. Fertilization rate will be defined as number of 2PN embryos per oocyte retrieved. Pregnancy rate will be defined as clinical pregnancy (ultrasound demonstrating gestational sac with yolk sac) per transfer. These secondary outcomes will be evaluated with a t-test.

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