Role of Gender Selection in ART

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Abstract

Study Question: We attempt to address an explicit request of infertile couples to select spermatozoa of a specific gender.
Summary Answer: Individual selection of spermatozoa post enrichment yielded the desired gender in the extreme majority of cases and this proportion was maintained in the resulting embryos.
What is known already: Several techniques have been developed to successfully select for X- or Y-bearing spermatozoa. Centrifugations methods and layering techniques, as well as the use of electrophoretic devices, have all been previously tested. The most effective method to date was through flow cytometry, which was capable of achieving a selection of 90% X- and 80%. Although successful, this method was expensive and required exposing spermatozoa to a fluorescent dye. We experimented with a method to allow spermatozoa to select themselves and generate embryos in couples being treated with ICSI.
Study design, size, duration: Over a course of 3 years, we processed by density gradient ejaculates from 21 couples being treated by ICSI. Fluorescent in situ hybridization was used to assess the proportion of X- and Y- bearing spermatozoa prior to and following selection. Single spermatozoa from the gender-enriched fraction were selected for injection according to an empirical head morphology. Preimplantation genetic diagnosis (PGD) results and pregnancy outcomes were recorded. Additionally, 26 couples were treated in 48 IUI cycles.
Participants/materials, setting, methods: A total of 47 couples were enrolled in our IRB study. A combination of gradient formulations was used for the ICSI and IUI treatments. During the ICSI procedure, an individual sperm selection was also implemented by carefully assessing spermatozoon. We confirmed the successful selection of the desired gender by performing cytogenetic analysis with 9 chromosome FISH on at least 1000 cells for each specimen.
Main results and the role of chance: A total of 47 couples (sperm concentration 66.7±27x10^6/ml, 46.4±5% motility, normal morphology). Prior to selection, average total aneuploidy for our subjects was 3.4±2%, compared to a normal value of <1.6%. After selection, our method consistently yielded 81.4% for X- and 78.0% for Y-bearing spermatozoa. In 21 couples treated by ICSI, GS enrichment was followed by pickup of individual spermatozoa selected according to their head morphometry. These couples achieved an average fertilization rate of 79.6% (164/206) and cleavage rate of 98.1%. Of these couples, 11 elected for PGS on the resulting embryos. PGS analysis evidenced that 3/3 (100%) couples selecting for male and 6/8 (75%) couples selecting for female obtained embryos of their desired gender. All embryos were cryopreserved. So far, 5 couples had their embryos thawed (n=11), 4 for female and 1 for male. Out of the remaining ICSI couples, of those that had an embryo replacement, 7 selected for female and 1 for male. Only 1 couple reported an ongoing pregnancy of female gender. No one else reported a pregnancy. In 26 couples with an average maternal age of 37.7±3yrs undergoing IUI, post sperm gender selection achieved 4 ongoing pregnancies. Thus far, 1 couple delivered the desired baby girl.
Limitations, reasons for caution: Our findings suggest that although it is possible to skew a spermatozoa population towards a specific gender, results are not definite despite taking into consideration sperm head morphometry. In this population with higher incidence of sperm aneuploidy, PGS on the conceptus can help identify euploid embryos of the desired gender.
Wider Implications of the findings: Multi-gradient gender selection methods, combined with individual spermatozoa identification by empirical head morphometry, can consistently help identify X- or Y-bearing spermatozoa. If proven reliable, this method to individually select spermatozoa of a particular gender may alleviate sex-linked disorders as well as aid with family planning in a morally accepted manner.
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Trial registration number: N/A

Background

Pre-conception gender selection has increasingly been sought after by couples looking to minimize the chances of passing on sex-linked genetic diseases or for family balancing. Several techniques have been developed to select for X- or Y-bearing spermatozoa, including centrifugation methods, layering techniques, and the use of electrophoretic devices. However, the most successful method to date was flow cytometry, which was capable of achieving a selection of 90% X- and 80% Y-bearing spermatozoa. Although effective, this method was expensive and required exposure of the spermatozoa to a fluorescent dye. The purpose of this study is to use a simple, inexpensive, and reliable method to allow for self-selection of spermatozoa and generate embryos of the desired gender.

Methods

Semen samples were collected in sterile containers from 34 couples being treated by ICSI. After processing by density gradient, fluorescent in situ hybridization (FISH) was performed to assess the proportion of X- and Y-bearing spermatozoa prior to and following selection. Single spermatozoa from the gender-enriched fraction were selected for injection according to empirical head morphology. Preimplantation genetic diagnosis (PGD) results and pregnancy outcomes were recorded for each patient. In addition, we performed our gender selection method on 34 couples who were treated in 59 IUI cycles.

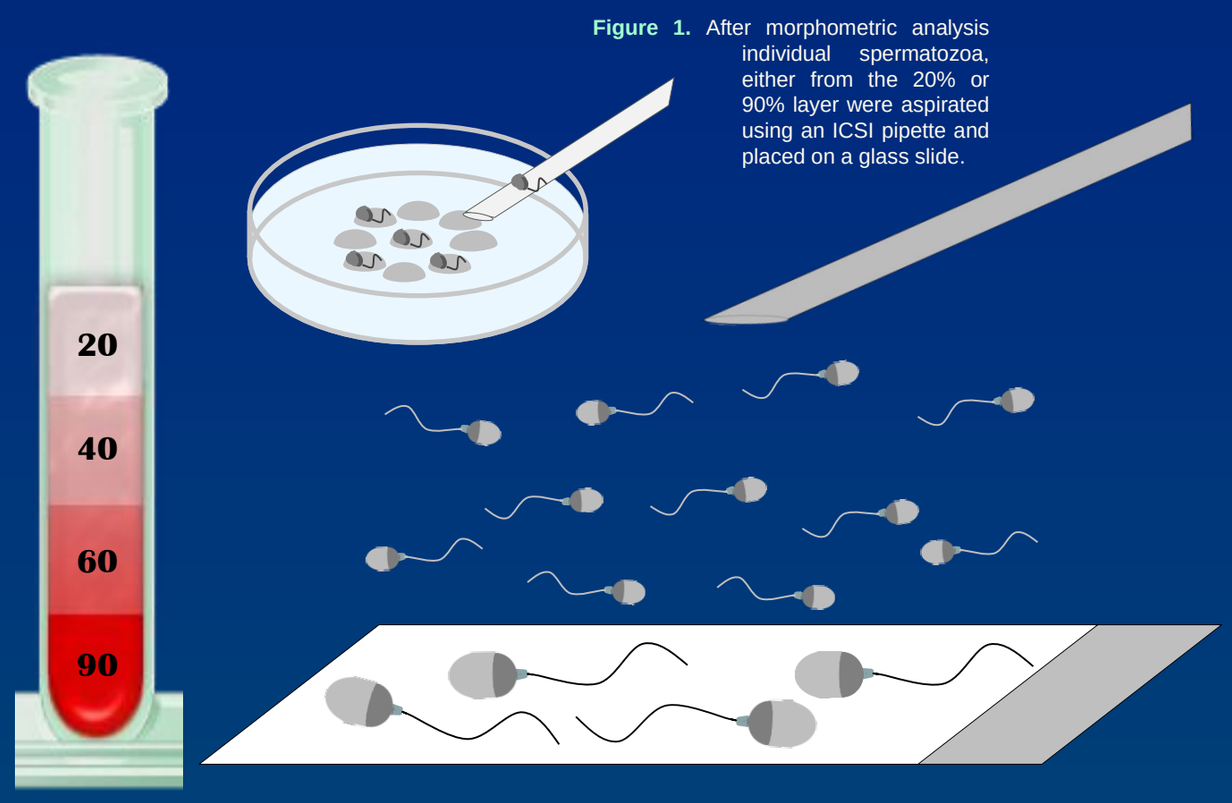

Figure 1. After morphometric analysis individual spermatozoa, either from the 20% or 90% layer were aspirated using an ICSI pipette and placed on a glass slide.

Results

A total of 68 couples (sperm concentration 66.7 ±27x10^6/ml, 46.4 ± 5% motility, normal morphology) were enrolled in our IRB study. FISH assessment on spermatozoa prior to selection yielded an average aneuploidy of 3.4 ±2%, compared to a normal value of <1.6%. After selection, our method was able to consistently yield 81.4% for X- and 78.0% for Y-bearing spermatozoa.

For 34 couples treated by ICSI, our gender selection enrichment method was followed by the individual aspiration of spermatozoa, selected according to their head morphometry (Figure 2). These couples achieved an average fertilization rate of 79.5% (279/351) and a cleavage rate of 98.1%. Twenty-four of these couples elected for PGD on their resulting embryos. PGD analysis evidenced that 7/7 (100%) couples who selected for male and 12/17 (70.6%) couples who selected for female obtained embryos of their desired gender. All couples had their embryos cryopreserved. Twelve of these couples had their embryos thawed thus far (n=19), 8 for female and 4 for male. Two couples selecting for female reported an ongoing pregnancy while one couple selecting for male reported an ongoing pregnancy. Out of the remaining couples who did not elect to have PGD performed prior to embryo transfer, 8 selected for female and 2 for male. One couple reported an ongoing pregnancy of female gender thus far. (Table 1).

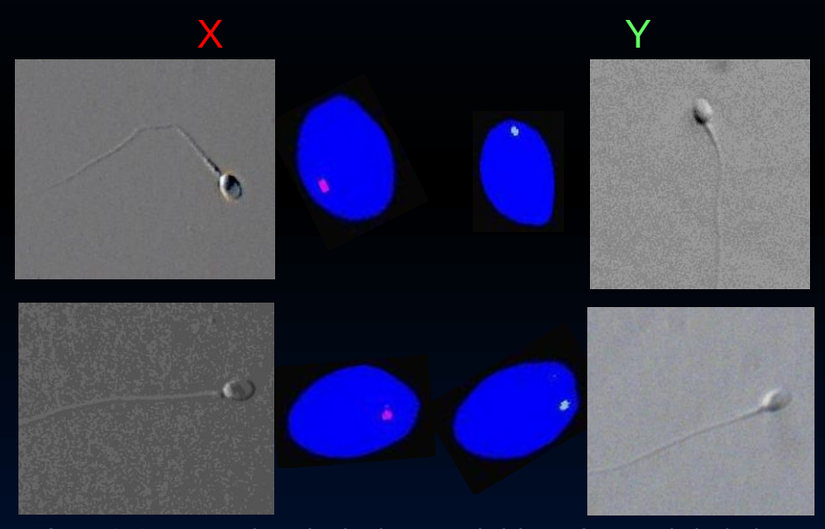

Figure 2. Sperm gender selection by manual pick up after morphological assessment and confirmed by FISH.

Table 1. Pregnancy outcomes for couples undergoing ICSI with or without PGD

ICSI with PGD		
n=24 patients, 24 cycles		
maternal age	38.9 ± 4yrs	
paternal age	41.0 ± 4yrs	
	♀	♂
Couples	17	7
Confirmed	12	7
Transfer	8	4
Pregnancy Outcome		
+βhCG	4	2
Clinical Pregnancy	2	1

ICSI without PGD		
n=10 patients, 15 cycles		
maternal age	39.5 ± 3yrs	
paternal age	41.0 ± 3yrs	
	♀	♂
Couples	8	2
Confirmed	–	–
Transfer	8	2
Pregnancy Outcome		
+βhCG	1	1
Clinical Pregnancy	1	--

In 34 couples with an average maternal age of 37.7 ±3yrs undergoing IUI, our gender selection method was able to achieve 7 ongoing pregnancies. Thus far, one couple has delivered the desired baby girl (Table 2).

Table 2. Gender selection IUI patient characteristics and pregnancy outcomes

Participants	
Couples	34
Cycles	59
Female age (M yrs ± SD)	37.7 ± 3yrs
Male age (M yrs ± SD)	38.2 ± 2yrs

Pregnancy Outcomes	**Female**	**Male**
Couples	24	10
Cycles	42	17
Clinical Pregnancy (+FHB)/Ongoing	4	3
Delivery	1	–

Conclusions

Multi-gradient gender selection methods combined with individual spermatozoa identification through the use of empirical head morphometry evaluation has helped us to identify X- or Y-bearing spermatozoa. Although our method has enabled us to skew a sperm specimen towards a particular gender, results are not definite. In this population of patients with a higher incidence of sperm aneuploidy, performing PGS on the conceptus can further help identify euploid embryos of the desired gender.