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ORIGINAL ARTICLE

Sperm counts and sperm sex ratio in male infertility patients

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In recent years, investigators have noted a trend toward a declining proportion of male births in many industrialized nations. While men bear the sex-determining chromosome, the role of the female partner as it pertains to fertilization or miscarriage may also alter the gender ratio. We attempted to determine a man's secondary sex ratio (F1 generation) by directly examining the sex chromosomes of his sperm. We examined our male infertility clinic database for all men who had undergone a semen fluorescence *in situ* hybridization (FISH). Patient demographic and semen parameters were recorded. Chi-squared analysis was used to compare gender ratios (Y chromosomes/total chromosomes). Multivariable logistic regression was used to predict the odds of possessing a Y-bearing sperm after accounting for demographic and semen parameters. A total of 185 men underwent sperm FISH. For the entire cohort, the proportion of Y chromosome-bearing sperm was 51.5%. Men with less than five million motile sperm had a significantly lower proportion of Y chromosome-bearing sperm (50.8%) compared to men with higher sperm counts (51.6%; *P*=0.02). After multivariable adjustment, a higher sperm concentration, total motile sperm count and semen volume significantly increased the odds of having a Y chromosome-bearing sperm (*P*<0.01). As a man's sperm production declines, so does the proportion of Y chromosome-bearing sperm. Thus, a man's reproductive potential may predict his ability to sire male offspring.
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INTRODUCTION

In recent years, a trend toward a declining proportion of male births has been noted in several, but not all, industrialized countries.[1–4] Indeed, from 1940 until 2002, the proportion of male births has steadily declined in the United States from 51.33% to 51.17%.[5] The etiology of alterations in the sex ratio remains uncertain. Investigators have linked changes in the sex ratio in specific populations to environmental factors or ingested contaminants over discreet time periods.[6–11]

Investigators have postulated both pre- and post-fertilization influences. A Danish group used time to pregnancy as a marker for infertility and found a decline in the offspring gender ratio, hypothesizing that infertile men may have an impaired ability to sire male heirs.[12] James[13] reviewed the published literature and examined an additional 2.5 million Australian births and found that the sex ratio appears to decline with time to conception. To explain this phenomenon, investigators have postulated that the more powerful mobility that a Y chromosome-bearing sperm possesses over its X-bearing counterpart may be diminished, thus lowering the likelihood of a male birth.[14,15]

However, the relationship remains uncertain as a Dutch group found that the proportion of male births increases with time to pregnancy.[16] In contrast, Jacobsen *et al.*[17] found no association between abnormal semen analyses and an altered offspring sex ratio. Moreover, examination of a cohort of infertile couples in California also found no association between the offspring sex ratio and infertility diagnosis, duration or treatment.[18] In addition, Joffe *et al.*[19] examined time to pregnancy and again found no association with sex ratio.

Postfertilization influences on the offspring gender ratio may also be important. Either through genetic defects or maternal rejection, increased proportions of spontaneous abortions of male fetuses may occur.[20] It has been shown that the sex ratio of a population may change during times of major stressors.[8,9,21] A common explanation is that male fetuses have a greater likelihood of aborting in the case of stresses to the mother.[20,22]

As female influences are thought to be a powerful mechanism for gender selection, examining a postfertilization and postgestational outcome such as birth may be inadequate to assess the role of the male contribution to the sex ratio. As such, comparing markers of male fertility (i.e., time to pregnancy and semen parameters) to offspring sex ratios may not completely capture the role of the male in determining the sex of the offspring. Therefore, it may be enlightening to examine the relationship between a prefertilization outcome (i.e., sex chromosomes of a man's sperm) and male fertility as assessed by sperm production.

MATERIALS AND METHODS

Study population

After Institutional Review Board (IRB) at Baylor College of Medicine approval, we examined all men who had undergone evaluation with a sperm fluorescence *in situ* hybridization (FISH) test. Only men who

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were able to produce an ejaculated sperm sample were eligible for testing. Men from couples with recurrent pregnancy loss ($\geqslant$2 pregnancy losses), *in vitro* fertilization failure ($\geqslant$ 2 failed *in vitro* fertilization (IVF) cycles) or unexplained infertility (normal female and male fertility evaluation in couples who were unable to conceive after 1 year of unprotected intercourse) were offered sperm FISH testing. Patient age, year of evaluation, reason for sperm FISH, proportion of sperm aneuploidy, semen volume, sperm concentration and sperm motility were recorded. In addition, the numbers of X- and Y-bearing sperm were determined.

Semen analysis and sperm FISH analysis

Semen analysis was performed within 60 min of collection using a manual counting technique with a hemacytometer under ×400 magnification. The volume, concentration (million per ml) and motility were recorded. Volume, percent motility and concentration were multiplied to determine the total motile sperm count (TMC= volume×concentration×motility).

The rationale and methods of semen analysis and sperm FISH analysis have been previously described.[23,24] Briefly, sperm was first fixed to glass slides. Five-color FISH was then used to detect chromosomes X, Y, 13, 18 and 21 using direct-labeled chromosome-specific probes.

Statistical analysis

The median value for each parameter of a semen analysis (volume, concentration, motility and TMC) was calculated. Next, the population was stratified as either above or below the median value for volume, concentration, motility and TMC. The gender ratio (Y chromosome-bearing sperm/total sperm analyzed) for each category was determined and chi-squared analysis was performed for compared groups.

After the total motile sperm count was calculated, the population was stratified based on TMC cutoffs of 1, 5, 10, 20 or 45 million. The sex ratio (Y chromosome-bearing sperm/total sperm analyzed) for each TMC category was determined and chi-squared analysis was performed for compared groups.

An analysis on a per sperm basis was performed by assigning the relevant semen parameter for each sperm analyzed. Logistic regression was used to determine the relationship between the seminal parameters (volume, concentration, motility and TMC) and the odds of producing a Y chromosome-bearing sperm (*vs.* X chromosome-bearing sperm) after accounting for age, time of evaluation, days of abstinence and overall aneuploidy of the semen sample. Given that a high aneuploidy rate observed in sperm may impact the proportion of Y chromosome-bearing sperm, aneuploidy was included in our model. Removal of this factor did not measurably impact any measures of association nor alter the conclusions. Odds ratios were generated after transforming all semen analysis characteristics ×100 to facilitate reporting of the measures of association. Statistical significance was set at $P<0.05$. All P values are two-sided. Analyses were performed using Stata 10 (StataCorp LP, College Station, TX, USA).

RESULTS

In all, 185 men underwent a sperm FISH from 2003 to 2010 with a mean age (s.d.) of 37.9 (6.4) years. During those years, approximately 2100 men were evaluated for infertility in our Division of Male Reproductive Medicine and Surgery. Thus, the current cohort represents approximately 9% of all infertile men evaluated. Most men for whom data were available underwent testing for IVF failure (55%). An average of 669.5 sperm were evaluated per man. The mean semen volume was 2.9 ml, and the mean sperm concentration was 48.8 million per ml with a mean motility of 47.2%. The average total motile sperm count was 76.7 million with 42 (23.0%) men having less than five million total motile sperm in their ejaculate (**Table 1**).

The overall sperm Y/X ratio for the cohort was 51.4 : 48.6. Men with a semen volume or sperm concentration below the median had a lower proportion of Y-bearing sperm (**Table 2**). After stratifying by TMC, men with <1, 5, 10 and 20 million motile sperm had a significantly reduced proportion of Y-bearing spermatozoa compared to those with higher sperm production (**Table 3**; $P<0.05$). When the TMC cut point reached 45 million, there was no significant difference in the percentage of X- and Y-bearing sperm between men with more or less than the cutoff ($P=0.11$).

On age-adjusted models, there was a positive relationship between the odds of producing a Y chromosome-bearing sperm and semen volume as well as TMC (**Table 4**). After adjusting for age, date of FISH study, days of abstinence and degree of aneuploidy of sperm sample, a positive relationship existed with volume, concentration and TMC ($P<0.01$). No relationship existed between the proportion of Y-bearing spermatozoa and sperm motility.

DISCUSSION

The current study shows an inverse relationship between the production of Y chromosome-bearing sperm and sperm production among the infertile men, suggesting an impaired ability for infertile men to sire male heirs. TMC, sperm concentration and semen volume all showed a positive relationship with the proportion of Y-bearing sperm.

Other groups have examined whether infertility impacts the offspring gender ratio. Joffe *et al.*[19] examined data from four European datasets (n=49 506 births) and found no relationship between time to

Table 1 Characteristics of sperm FISH patients

Characteristic		*Value*
Patient number		185
Patient age (mean±s.d.), year		37.9±6.4
Patient age, year categories, *n* (%)	<30	9 (5)
	30–35	46 (25)
	35–40	68 (38)
	40–45	29 (16)
	45–50	15 (8)
	>50	14 (8)
Period of evaluation, *n* (%)	2003–2004	47 (26)
	2005–2006	71 (39)
	2007–2010	34 (19)
	2009–2010	28 (16)
Reasons for sperm FISH testing, *n* (%)	IVF failure	21 (55)
	Recurrent miscarriage	2 (5)
	IUI failure	8 (21)
	Unexplained infertility	7 (18)
Previous paternity at the time of infertility evaluation		0/38
Semen analyses, mean±s.d. (median)	Days abstinent	3.7±2.5 (3)
	Volume (ml)	2.9±1.6 (2.5)
	Concentration (million per ml)	48.8±48.2 (34)
	Motility (%)	47.2±21.9 (50)
	TMC (million)	76.7±83.7 (51.3)
Average sperm evaluated per man		669.5±254.9

Abbreviations: FISH, fluoroescence *in situ* hybridization; IUI, intrauterine insemination; IVF, *in vitro* fertilization; TMC, total motile sperm count.

Table 2 The proportions of Y-bearing sperm are listed after stratifying by the median values for semen volume, sperm concentration, sperm motility and TMC

Semen characteristics (Median cutoff)	n	*TMC (mean±s.d.)*	*Total sperm*	*Y sperm (%)*	P*
Volume					
⩽2.5 ml	95	1.7±0.6	61 332	50.9	<0.01
>2.5 ml	90	4.2±1.5	59 176	52.1	
Concentration					
⩽34 millions per ml	93	13.6±11.6	60 143	51.2	0.05
>34 millions per ml	90	85.2±44.4	58 456	51.7	
Motility					
⩽50%	94	29.7±15.8	60 496	51.4	0.82
>50%	89	65.7±7.5	58 103	51.5	
TMC					
⩽51.3×10^6	92	13.5±15.5	60 548	51.3	0.28
>51.3×10^6	91	140.5±75.6	58 051	51.6	

Abbreviation: TMC, total motile sperm count.
**P* value represents chi-squared test.

pregnancy and gender ratio. In the analysis, couples with >1 year of time to pregnancy were labeled infertile, but the specific infertility factors could not be assessed. In all the analyzed datasets, live births were assessed, but the individual parental contributions could not be separated. Examination of a US cohort (*n*=15 309 births) also failed to demonstrate any difference in offspring sex ratio between fertile and infertile couples.[18] Jacobsen *et al.*[17] linked semen data from men analyzed at the Sperm Analysis Laboratory in Copenhagen, Denmark over a 30-year period with birth registry data to determine if the offspring sex ratio varied based on semen parameters. The authors examined 25 738 births and found no association between semen characteristics and sex ratio, suggesting that there was no association between a man's sperm production and the offspring sex ratio.

Yet other groups have found a relationship between time to pregnancy and sex ratio. Smits *et al.*[16] queried 5283 women and found that a longer time to pregnancy was positively associated with the proportion of male births. In contrast, Zhou *et al.*[12] examined data from the 'Healthy Habits for two' study conducted in two Danish cites and noted a decline in the offspring gender ratio for couples (10 042 births) with a longer time to pregnancy (>12 months). James[13] examined approximately 2.5 million Australian births and also found that the sex ratio appears to decline with time to conception. Using offspring number as a surrogate for fertility, Edwards[25] reported a positive correlation between offspring number and the offspring sex ratio using German birth data from the nineteenth century.

The reason for different findings of the studies, including the current report, is unclear. Admittedly, any changes in sex ratio are likely to be small. Certainly, larger datasets will have more power to detect small differences in sex ratio which may explain some of the observed differences. In addition, population-based offspring samples may be misleading due to individual choices that couples have made about family size based on offspring gender. Moreover, using timing of conception as it relates to marriage or the number of offspring may be imprecise surrogates for fertility potential.[13,25] It may also be that examining the fertility potential of each member of a couple (i.e., male factor *vs.* female factor) may be important rather than examining fertility at the level of the couple.

In addition, given that societal stresses may impact sex ratio through unclear mechanisms, examining the sex chromosome composition of the gamete may be more enlightening for determining the parental contribution than looking at the sex of the resultant offspring.[8,9,21]. In fact, it is conceivable that societal stresses may first impact sperm quality which may in turn then affect sex ratio as suggested by Fukada and colleagues[9,26] who noted both a decline in sperm motility and sex ratio after the Kobe earthquake. It is important to note that while Robbins *et al.*[27] suggest that the proportion of Y-bearing sperm is related to the offspring sex ratio, to our knowledge, no studies have directly measured a relationship.

The current report suggests that men with impaired spermatogenesis have a diminished capacity to produce Y-bearing sperm and possibly male heirs. This supports the hypothesis that a common biological factor may act upon the male reproductive system and

Table 3 The proportions of X- and Y-bearing sperm are listed after stratifying by the total motile sperm count (TMC)

TMC category (millions)	n	*TMC (mean±s.d.)*	*Total sperm*	*Y sperm (%)*	P*
<1	21	0.4±0.3	9567	50.4	0.03
⩾1	162	89.1±83.4	109 032	51.5	
<5	42	1.6±1.4	21 958	50.8	0.02
⩾5	141	100.2±82.2	96 641	51.6	
<10	55	3.3±3.0	88 217	50.9	0.04
⩾10	128	109.0±80.7	30 382	51.6	
<20	67	5.5±5.3	37 562	51.0	0.04
⩾20	116	117.4±78.9	81 037	51.7	
<45	86	12.5±13.1	50 952	51.2	0.11
⩾45	97	134.3±75.7	67 647	51.6	

**P* value represents chi-squared test.

Table 4 Logistic regression models to estimate the odds of Y-bearing sperm[a]

Semen characteristics	*Age adjusted*		*Fully adjusted* [b]	
	OR (95% CI)	P	*OR (95% CI)*	P
Volume	5.14 (2.55–10.36)	<0.01	3.65 (1.59–8.38)	<0.01
Concentration	1.02 (1.00–1.05)	0.08	1.05 (1.01–1.08)	<0.01
Motility	1.04 (0.98–1.10)	0.20	1.02 (0.95–1.10)	0.56
TMC	1.02 (1.01–1.03)	<0.01	1.03 (1.01–1.05)	<0.01

Abbreviations: CI, confidence interval; OR, odds ratio; TMC, total motile sperm count.
[a] Odds ratios were generated after transforming all semen analysis characteristics ×100 to facilitate reporting.
[b] Adjusted for age, date of analysis, days of abstinence and aneuploidy of sample.

impair fertility and the offspring sex ratio.[28,29] Moreover, the current findings support the hypothesis which suggests that natural selection should favor parental ability to adjust the sex ratio of offspring according to the reproductive fitness of the parents.[30] Thus, men with impaired semen production should sire less male offspring who risk similar hardships with reproduction. Instead, subfertile men should sire daughters whose own reproductive success would be unlikely to be impaired from a paternal spermatogenic deficit.

Certain limitations warrant mention. While over 119 000 sperm were analyzed, the number of individual men for evaluation was limited. In addition, despite our criteria for sperm FISH being fairly stringent, many couples who were offered the test either refused or were unable to afford the study. Due to the possible biases in cohort assembly, it is possible that the results occurred by chance alone. Moreover, as most couples who obtained sperm FISH had IVF failure, it is uncertain if the findings are applicable to all men and further confirmatory studies are warranted. Nevertheless, our study represents an examination of the man's isolated contribution to offspring sex and suggests a relationship between sperm production and the sex chromosome composition of sperm.

AUTHOR CONTRIBUTIONS

MLE and LIL conceived the project. LM, KH and DJL performed FISH preparation, analysis and interpretation. MLE analyzed the data and MLE drafted the manuscript. All authors provided critical revisions for the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no conflict of interest.

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