

Ratio of X to Y-Chromosome Bearing Spermatozoa: Variation Within and Between Donors

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Abstract

The aim was to evaluate the ratio of X- to Y- Chromosome bearing sperm variation within and between the ejaculates from five sperm donors. Five separate ejaculates, and corresponding buccal swab samples, were obtained bi- or tri-weekly from each of the donors and the DNA extracted and quantitated using qPCR to determine the ratio of X- to Y- Chromosome bearing sperm in each ejaculate in relation to the respective buccal swab DNA. Since the sum of the percentages of X and Y chromosome bearing sperm in an ejaculate equals 100, the results of the percentages of Y chromosome bearing sperm are identically opposite to the percentages of X chromosome bearing sperm. Therefore, only the percentages of Y chromosome bearing sperm are expressed hereafter. The overall mean percentage of Y- Chromosome bearing sperm from one donor was significantly different ($p < 0.05$) from three others and the variation within each donor ejaculate was within its own 95% confidence interval of the mean for that donor. The variation of the Y-chromosome bearing sperm between donors was significant but not within each donor, suggesting that the ratio of X- to Y- Chromosome bearing sperm is consistent for each donor.

Keywords: Sex Ratio, Sperm, Ejaculates, Human.

INTRODUCTION

The percentages of X- and Y- chromosome bearing sperm in an ejaculate should theoretically be equal due to Mendelian segregation (Umehara et al, 2019; Rahman and Pang, 2020). However, our earlier studies revealed a wide variation in the percentage of Y chromosome bearing sperm vs. X chromosome bearing sperm among randomly selected men (Jeyendran et al, 2021; Jeyendran and Fishel, 2023). This is not surprising since a critical review of available literature revealed a wide variation in the ratio of X and Y- chromosome bearing sperm (Quinlivan and Sullivan, 1974; Martin et al, 1983; Clutton-Brock et al 1986; Bibbins et al, 1988; Rorie et al, 2014). However, to clarify whether the difference in the proportion of X or Y chromosome bearing sperm observed in an ejaculate was due to differences between donors or differences between ejaculates from the same donor, the following study was undertaken.

MATERIALS AND METHODS

Five separate ejaculates were obtained bi- or tri- weekly from five donors providing samples for a Sperm Bank

(Centro de Estudios Reproductivos, Santiago, Chile), by self-masturbation, which were then frozen following routine semen analysis. Corresponding buccal swabs were also obtained from each donor at the time of semen collection and stored dried. The DNA was extracted using a QIAGEN QIAamp DNA Mini Kit (QIAGEN, MD) from the frozen and then thawed sperm samples and from the dried buccal swabs samples according to the manufacturer's instruction.

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Name	Sequence	Labeled with
SRY Forward	TTCGAACCTCTGGCACCTTTC	
SRY Reverse	CATCGCTGTTGAATACGCTTAAC	
SRY-probe	TGACAAATGCAATCATATGCTTCTGC	CalFluor610
AMELX-X Forward	CCTGATTCTAAGATAGTCACACTCTATG	
AMELX-X Reverse	TTCTGCGGAGTCTCTCCTATAC	
AMELX-X Probe	TGTCTCTTGCTTGCCCTCTGCTGAA	FAM
PPIH Forward	TGGTGACAGTGATAGAAGGTAATG	
PPIH Reverse	GGACTCTCCTAAAGATGGTTGAC	
PPIH Probe	CCAAGGGTCTGTCCCTAGTTTATTTGCC	Quasar670

Quantitative PCR

To determine the percentages of both X and Y chromosome bearing sperm simultaneously in each sample, a quantitative PCR assay was developed using the sex determining region Y (SRY) on the Y-chromosome and the amelogenin gene on the X chromosome as the targets, and the peptidylprolyl isomerase H gene (PPIH) as the reference (Jeyendran et al, 2021; Jeyendran and Fishel, 2023).

The primers and probes for the three target genes were made by LGC BioSearch Technologies (Novato, CA) is listed below.

The real-time PCR reactions were performed on a BIO-RAD IQ5 Multicolor Real-Time PCR Detection System. Platinum™ Multiplex PCR Master Mix (Thermo Fisher) was used in the reaction with each primer concentration at 500 nM and probe at 125 nM in a multiplex set using the PCR programs recommended by Thermo Fisher. Ten ng of sperm genomic DNA was used as the input template in the reaction and data were analyzed on the same instrument.

The mean percentage of X- and Y- chromosome for each individual sample was calculated using the $\Delta\Delta C_t$ method. Briefly, the ΔC_t for the Y - chromosome in the pooled semen was calculated by subtracting C_t (SRY) from C_t (PPIH) and similarly, the ΔC_t for the unknown sample was calculated by subtracting C_t (SRY) from C_t (PPIH). We then calculated the $\Delta\Delta C_t$ by subtracting the ΔC_t (unknown sample) from the ΔC_t (Pooled). Finally, calculation of the Fold Gene Expression was made with the following equation: $(2^{-\Delta\Delta C_t})$. The Fold Gene Expression of the unknown sample will be greater or lesser than 1 when compared to the respective buccal swab Gene Expression (which is defined as 1.0, since the X:Y ratio is 1:1).

Since the sum of the percentages of X and Y chromosome bearing sperm in an ejaculate equals 100, the results of the percentages of Y chromosome bearing sperm are identically opposite to the percentages of X chromosome

bearing sperm. For practical reasons, only the percentages of Y chromosome bearing sperm are expressed hereafter.

The mean and $\pm$ 95% confidence interval of the Y-chromosome within and between donor ejaculates were calculated. The data were analyzed using the analysis of variance and the probability value less than 0.05 was considered significant.

RESULTS

There was a significant difference ($p > 0.05$) in the mean $\pm$ 95% confidence interval of the Y-chromosome among donors (Table 1). The mean of Y-chromosome bearing sperm in the ejaculates from donor 2 was significantly different ($p < 0.05$) from the ejaculates of donors 3, 4 and 5, but not from donor 1. The percentage of Y-chromosome bearing sperm from different ejaculates within the same donor remained within the 95% confidence interval of the mean for that donor.

DISCUSSION

We do acknowledge the small sample number in our study but the strength of the findings is due to the number of repeats per donor. The percentages of Y chromosome bearing sperm in the ejaculates from donor 2 were significantly different ($p < 0.05$) from the ejaculates of donor 3, 4, and 5, confirming our earlier findings of variation in the percentage of the X- and Y- chromosome bearing sperm between the ejaculates of individual sperm donors (Jeyendran et al, 2021; Jeyendran and Fishel, 2023). The percentage of Y-chromosome bearing sperm between the five ejaculates from each donor was within the 95% confidence interval, suggesting that an intrinsic factor constantly maintains the percentage of Y-chromosome bearing sperm in every ejaculate for that individual donor. Similar findings are also reported for bulls: the variation of sex-chromosome content observed was attributed to differences between individual bulls and not to differences between the ejaculates within a given bull (Checa et al, 2002).

A variety of procedures have been reported to promote fractions of sperm that are bearing either an X- or Y-chromosome (Oyeyipo et al. 2017; Yadav et al, 2017; Umehara et al. 2019) but to date, none of these procedures have yielded the desired clinical outcomes or proven clinically useful for the purpose of selecting the sex of the offspring. We speculate that one of the challenges of this process may be due to the intrinsically skewed percentage of X- and Y-chromosome-bearing sperm content in the ejaculates of certain individuals. We therefore suggest that, prior to undergoing a sex-based sperm selection

procedure, the ejaculate should be evaluated for the ratio of X and Y-chromosome bearing sperm to refine the estimate of successfully obtaining a child of the desired gender.

CONCLUSION

The variation of the Y-chromosome bearing sperm between donors was significant but not within each donor, suggesting that the ratio of X- to Y- Chromosome bearing sperm is consistent for each donor.

Table 1. Mean $\pm$ 95% confidence interval of the variation in the percentage of Y-chromosome bearing sperm in different ejaculates of the individual donor

	Percentage of Y-chromosome bearing sperm*					
Ejaculate	1	2	3	4	5	Mean $\pm$ 95% confidence interval
Donor 1	49	50	52	52	52	51.0 $\pm$ 1.8
Donor 2	54	51	51	54	53	52.6 $\pm$ 1.9**
Donor 3	50	51	51	49	50	50.2 $\pm$ 0.9
Donor 4	48	50	48	50	49	49.0 $\pm$ 0.9
Donor 5	49	48	50	51	48	49.3 $\pm$ 1.3

* Five different ejaculates were obtained bi or tri weekly from each donor

**Donor 2 is significantly different ($p < 0.05$) from Donors 3, 4, and 5

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