Andrology-Open Access
ISSN: 2167-0250
OPEN ACCESS Freely available online
Short Communication

Inequality of X- and Y-Sperm in Human Ejaculates

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DESCRIPTION

The study objective was to determine the percentages of X and Y-sperm simultaneously in an ejaculate and validate our earlier study using only SRY. A real-time Polymerase Chain Reaction (qPCR) assay was developed and used to calculate the percentages of X and Y-sperm in individual ejaculates from fourteen randomly selected men. There was a significant difference ($p<0.05$) in the mean ± SD between the proportion of X and Y-sperm (55.12 ± 11.72 *vs.* 44.88 ± 11.72). Six (43%) of the fourteen ejaculates had more than 10% difference between the percentage of X and Y-sperm, confirming the variation in the percentages of X and Y sperm in ejaculates [1]. The percentages of X and Y-sperm in any sample should be equal due to meiotic division during spermatogenesis, however, critical review of publications revealed a wide variation in the ratio of X and Y-sperm in humans and various other species [2]. These studies did not definitively determine the underlying reasons for this variation but included motility and related variables and genetic instability. Additionally, the uterus and oviduct may be involved as well, since they may select only functional sperm for fertilization (Holt and Fazeli 2010). Oyeyipo et al. (2017) investigated what occurred when spermatozoa were exposed to different pH values (5.5 - 9.5), increased temperatures (37°C - 45°C) and Reactive Oxygen Species (ROS) levels (50 - 1,000 µM), and the live and dead cells were then separated using a modified swim-up technique. From these conditions, incubation in acidic media, increased temperatures and elevated H_2O_2 resulted in X chromosome-bearing spermatozoa enrichment. It is therefore not surprising our earlier blinded, randomized study of the variation of Y-bearing sperm within human ejaculates with Sex-determining Region Y (SRY gene) using real-time quantitative Polymerase Chain Reaction (qPCR) revealed a wide variation in percentage of Y sperm among 50 randomly selected men. To clarify the results in relation to the X sperm, the homologous amelogenin gene was used to reconfirm the earlier findings by measuring the percentages of both X and Y sperm simultaneously in any sample [3].

Ejaculates from 14 men were frozen following routine semen analysis. Buccal swabs were also collected from each of these men and stored dry until use. The Institutional Review Board exempted this study under 45 CFR 46.101(b) (4). Informed consent was obtained from each participant. Frozen sperm samples were thawed, and DNA extracted from each sample and from the corresponding buccal swabs using the QIAGEN, QIAamp DNA Mini Kit. Additionally, the 14 semen samples were pooled, and a DNA extract made from the pool [4].

To determine X and Y sperm percentages simultaneously in each sample, a quantitative PCR assay was developed using the SRY sex-determining region Y (Y-chromosome) and the amelogenin gene (X-chromosome), using the Peptidyl-Prolyl cis-trans Isomerase H gene (PPIH) as a reference gene for normalization of data, since it is a housekeeping gene and therefore present in all samples. Primers and probes were made by LGC Bio Search Technologies (Novato, CA). Sequences were:SRY(reverse):CATCGCTGTTGAATACGCTTAAC;SRY(probe):TGACAATGCAATCATATGCTTCTGC (label:CalFluor610).

AMELXX(forward):CCTGATTCTAAGATAGTCACACTCTATG;AMELX(reverse):TTCTGCGGAGTCTCTCCTATAC;AMELXX(probe):TGTCTCTTGCTTGCCTCTGCTGAA;(label:FAM); PPIH(forward):TGGTGACAGTGATAGAAGGTAATG;PPIH(reverse):GACTCTCCTAAAGATGGTTGAC;PPIH(probe):CCAAGGGTCTGTCCCTAGTTTATTTGCC;(label: Quasar 670).

qPCR was performed on a BIO-RAD IQ5 Multicolor Real-Time PCR Detection System, with Platinum™ Multiplex PCR Master Mix (Thermo Fisher); each primer concentration was 500 nM and probe 125 nM in a multiplex using thermal cycling parameters described, with 10 ng sperm genomic DNA as the input template. The X and Y sperm mean percentage for each sample was calculated using the ΔΔCt method [5-7].

There was significant difference ($p<0.05$) in the mean ± SD between the proportion of X and Y sperm (55.12 ± 11.72 *vs.* 44.88 ± 11.72). Variation in the percentages of X and Y sperm was seen among the ejaculates of the 14 individuals; six (42.86%) had more than 10% difference between the percentage of X or Y sperm Table 1. Our pooled ejaculate when compared to buccal cells had an almost identical ratio (52:48).

These results confirm our earlier findings of variation in the ratio of X to Y-sperm in ejaculates of 50 individuals. As revealed

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Received: 11-Jul-2023, Manuscript No. ANO-23-25537; **Editor assigned:** 14-Jul-2023, PreQC No. ANO-23-25537 (PQ); **Reviewed:** 28-Jul-2023, QC No. ANO-23-25537; **Revised:** 04-Aug-2023, Manuscript No. ANO-23-25537 (R); **Published:** 11-Aug-2023, DOI:10.35248/2167-0250.23.12.294

Citation: Jeyendran RS, Fishel L (2023) Inequality of X- and Y-Sperm in Human Ejaculates. Andrology. 12:294.

in Table 1, the variation was significant. More critical: the possibility that any ejaculate may be more likely to deviate from an expected 1:1 ratio than conform to it. The significant difference in the mean between the two populations is probably due to the small numbers of ejaculates evaluated (Table 1).

Sample	%X sperm	%Y sperm
1	51	49
2	32	68
3	48	52
4	51	49
5	94	6
6	52	48
7	57	43
8	56	44
9	57	43
10	56	44
11	52	48
12	48	52
13	45	55
14	53	47
Mean*	53.55	46.45
S.D.	12.84	12.84

Note: *Means were significantly different ($p<0.05$) from each other.

Table 1: Percentages of X and Y-Sperm in ejaculates from fourteen randomly selected men.

Six of the fourteen samples had more than 10% difference between the X or Y-sperm from the respective ejaculates.

CONCLUSION

The disparity in percent X-sperm and Y-sperm in individual ejaculates is supported by studies such as the following that showed ligand activation of Toll-Like Receptors 7/8 (TLR7/8) selectively encoded by the X-chromosome produced over 90% male embryos in mice following *in vitro* fertilization increased proportion of X sperm in mice and humans with increased Y aneuploidy in spermatozoa, that selectively eliminated the Y sperm.

Therefore, genetic factors may differentially affect the sperm sex ratio by making one type more sensitive to loss than the other, confirming the possibility of disproportion between the percentage of X and Y-sperm in an ejaculate as found in our studies.

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