Long Y chromosome is not a fetal loss risk

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Abstract

Purpose To assess the long Y chromosome genetic effect on human pregnancy outcomes.

Methods We studied all records of pregnancies by human sperm donors after artificial insemination or in vitro fertilization at the Reproductive and Genetic Hospital of Citic-Xiangya. Fetal losses were compared from two groups of sperm donors: the observation group (with long Y chromosome) and the control group (without long Y chromosome).

Results 2885 pregnancies were achieved with donor sperm by artificial insemination and 1746 by in vitro fertilization. The rates of fetal loss, congenital malformation and donor fecundity in the observation group after both assisted reproductive technique were the same as for the control group.

Conclusions A long Y chromosome may therefore be considered as a normal variant.

Keywords Congenital malformation · Donor fecundity rate · Fetal loss · Long Y chromosome · Sperm donors

Introduction

The major Y chromosomal polymorphisms are classified as follows: pericentric inversion of the constitutive heterochromatin of the long arm (inv qh); enlarged heterochromatic region of the long arm (Yqh+); and small or deficient heterochromatic region in the long arm (Yqh-) [1]. Size variation of the long arm of the human Y chromosome differs from person to person and even between ethnic groups, and its inheritance at a constant length has been well documented [2, 3].

Constitutive heterochromatin is formed by tandemly organized highly repeated sequences of satellite DNA that do not encode proteins [4, 5]. Mattei et al concluded that frequently occurring polymorphisms including Yqh+ do not appear to have any functional or phenotypic effect [6], while Ghosh et al found Yqh+ was highly prevalent in two Indian populations (18% in Rajputs, 19% in Punjabis), and without deleterious effect on the phenotype [7]. Similarly, Rodriguez et al suggested that Y chromosome length variability is polymorphism in human males, unassociated with reproductive problems [8], and Verma et al observed that Yqh+ in fathers may be unrelated to fetal loss [9].

In China, Shen Wan Ying reported that Yqh+ carrier percentage in the general population is 13.8% and not associated with spontaneous abortion, stature or abnormal behavior [10]. Since Yqh+ is regarded as polymorphism in China [11–13], potential sperm donors with this trait are accepted at the sperm bank of the Reproductive and Genetic Hospital of Citic-Xiangya. However, a possible association between Yqh+ and fetal loss has still not been definitively ruled out.

Several studies have suggested that Yqh+ may be associated with a clinical phenotype. For example, Nielsen found that mothers of Yqh+ boys had increased spontane-

Capsule A long Y chromosome (Yqh+) did not affect rates of fetal loss, congenital malformation and donor fecundity, and may be considered a normal variant.

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ous abortions (22%) compared to mothers of boys without it (13%) [14]. Additionally, Wang et al reported that 78.5% of Yqh+ carriers (77 out of 98) had spontaneous abortions, still births or infertility [15], while Patil et al investigated 4400 consecutive births and indicated that Yqh+ may be an important cause of fetal loss [16].

The possible association between Yqh+ and risk of fetal loss is clinically very important, in order to clarify this matter, we used the records of the Reproductive and Genetic Hospital of Citic-Xiangya and analyzed the pregnancy outcomes after artificial insemination by donor (AID) and in vitro fertilization by donor (IVF-D), from sperm donors with and without Yqh+.

Materials and methods

A retrospective cohort study was performed on all qualified sperm donors who participated in assisted reproduction during January 2004 to January 2008 in the human sperm bank of the Reproductive and Genetic Hospital of Citic-Xiangya, Changsha, China—approved by the Ethics Committee of the hospital.

Semen samples from potential sperm donors were obtained after 3–5 days of sexual abstinence. Both semen analysis prior to cryopreservation and postthaw cryosurvival tests were performed according to World Health Organization guidelines [17]. Cryoprotectant (glycerol egg yolk citrate medium) was added to the qualified semen samples in the proportions of 1:3 [18], then aliquoted to 1 mL per vial in a laminar flow chamber, and the vials put into Thermo CryoMed Freezers (model 7541, Thermo Electron Corporation, Waltham, MA) for slow programmed freezing. Finally the vials were plunged into liquid nitrogen (model LS4800, Taylor-wharton Cryogenic Refrigerator, Harrisburg, AL) for long term storage.

Complete medical, social, sexual, family, and genetic histories were taken at the first interview of potential sperm donors according to the guidelines of the Ministry of Health of China [19]. They were physically examined for evidence of high-risk behavior, sexually transmitted diseases and drug use [20]. Eligible donors were negative in infectious disease testing for HBV (enzyme-immunoassay kits, Shanghai Kehua Biotech Co., Ltd, China), HCV (ELISA diagnostic kits, Livzon Group Reagent Factory, China), HIV (sandwich ELISA diagnostic kits, Livzon Group Reagent Factory, China), *Treponema pallidum* (TRUST kits, Shanghai Rongsheng Bio-pharm Co., Ltd, China), *Neisseria gonorrhoeae* (Yangzhou Shuangling Medical Company, China), *Chlamydia trachomatis* (Chlamydia trachomatis diagnostic kits, Nanjing Niming Biotech Co., Ltd, China), *Ureaplasma urealyticum* (ureaplasma urealyticum culture kits, Zhuhai Langfeng Biotech Co., Ltd, China), and genetic screening for

glucose-6-phosphate dehydrogenase (G6PDH) deficiency (G6PDH kits, Guangzhou Micky Medical Instrument Co., Ltd). All donors recruited in this study were psychologically normal native Chinese college students from Changsha, Hunan Province with no criminal record, with age at donation of 21.3 ± 1.3 years.

All potential sperm donors underwent double-blind karyotyping analyses in the cytogenetics department of the Reproductive and Genetic Hospital of Citic-Xiangya. Metaphase chromosome preparations were obtained from phytohemagglutinin stimulated lymphocyte cultures according to standard procedures [21]. Chromosome 18 and Y lengths from 30 metaphases were measured, and the ratio of $Y/18 > 1$ was defined as long Y chromosome [22]. In our sperm bank, only two kinds of karyotype were accepted: 46, XY and 46, XYqh+.

Qualified sperm donors could donate their sperm 15–20 times in 6 months, and all donated semen was then quarantined for 6 months. After the quarantine period, all donors were retested for HBV, HCV and HIV prior to release of their semen for clinical use. A maximum of 5 offspring are permitted from one sperm donor in mainland China [23].

The semen samples from 694 and 492 donors were provided to the AID and IVF-D departments, respectively. The primary indication for semen recipients was a diagnosis of azoospermia in their spouse. Inclusion criteria for these women were: under 35 years of age, normal ovarian function, and body mass index (weight in kilograms divided by height in meters squared) between 18–29 kg/m². All recipient women were given physical examination including chest x-rays and electrocardiograms, and were tested for infectious diseases, hormonal levels in follicular phase, and leukorrhea. Only healthy women could continue their medical treatment, and if found to be HBV or HCV seropositive, were required to have normal liver function in order to participate.

All AID recipient women underwent hysterosalpingography (HSG) to exclude bilateral fallopian tube blockage, anatomical abnormalities and cervical incompetence. AID was then carried out according to routine procedures [24, 25]. Similarly, for IVF-D recipient women, the main indication was bilateral fallopian tube blockage confirmed by HSG examination. IVF-D was then performed according to routine procedures as described [26].

Several factors were recorded for each case: sperm donor age, sperm donor fecundity, recipient age, treatment cycles, and follow up of the pregnancy outcome, including any fetal loss or congenital malformation. Clinical pregnancy was confirmed by the detection of fetal heart beats by transvaginal ultrasonography examination at gestational age of 6 weeks, and ongoing pregnancy defined by ultrasonography examination at 12 weeks. Fetal loss included

spontaneous abortion, stillbirth and fetal death. Congenital malformation was diagnosed by physicians (most often pediatricians, obstetricians, and family practitioners) in live born children through 1 year of age.

Donor fecundity rate was defined as the total number of achieved pregnancies divided by the total number of medical treatment cycles.

Fetal loss rate was defined as the total number of fetal losses divided by total pregnancies, and congenital malformation rate as the total number of congenital defects divided by total live born babies.

Results were indicated as percentage. The chi-square test was calculated where appropriate with the use of SPSS 15.0 (Chicago, IL). Statistical significance was set at $p < 0.05$.

Results

A total of 2541 donors were screened in our sperm bank during January 2004 to January 2008, out of which 95 (3.74%) cases had Yqh+. Samples of 1186 donors (694 for AID, 492 for IVF-D) completed sperm recipient cycles. Of the remaining donors, some did not finish donation, and still others did not complete sperm recipient cycles. The donors were divided into two groups: A (46, XYqh+) and B (46, XY), in both the AID and IVF-D departments, and each donor produced 3–5 offspring.

In the AID department, a total of 2885 pregnancies were obtained after 13,089 treatment cycles. In group A (238 pregnancies obtained after 1112 treatment cycles), there are 210 deliveries, 28 fetal losses (11.8%) and 1 (0.47%) with congenital defects. In group B (2647 pregnancies obtained after 11977 treatment cycles), there were 2295 deliveries, 352 (13.3%) fetal losses and 12 (0.51%) with congenital defects. (Table 1)

In group A, 1 child was born with a cleft lip and palate, while in Group B, 12 children born with congenital malformations included 4 cases of congenital heart disease, 3 cases of cleft lip and palate, 1 case of premature birth with chromosomal anomaly, 2 cases of congenital dislocation of the hip, and 2 cases of hypospadias. The congenital defects rate was not high when compared with previous research [27].

There was no significant difference between groups A and B group in rates of donor fecundity, fetal loss, and congenital defects ($p > 0.05$).

In the IVF department, a total of 1746 pregnancies occurred after 2619 treatment cycles. In group A (72 pregnancies occurred after 104 treatment cycles), there were 69 deliveries, 3 (4.2%) fetal losses and 1 (1.11%) congenital defects. In group B (1674 pregnancies occurred after 2515 treatment cycles), there were 1518 deliveries,

Table 1 AID pregnancy outcome

Group	A (observation)	B (control)
Sperm donor karyotype	46,XYqh+	46,XY
Sperm donors number	53	641
Total AID cycles	1112	11977
Total pregnancies	238	2647
• Singleton births	207	2252
• Twin births	3	43
• Triplet births	0	0
• Fetal losses	28	352
○ spontaneous abortion	20	329
○ stillbirth	4	17
○ fetal death	4	6
Total live born babies	213	2338
Congenital malformation	1	12
Congenital malformation rate ^a	0.47%	0.51%
Donor fecundity rate ^b	21.4%	22.1%
Fetal loss rate ^c	11.8%	13.3%

$$^a \chi^2 = 0.007, p = 0.932$$

Congenital malformation rate = (Congenital malformation/Total live born babies)

$$^b \chi^2 = 0.185, p = 0.667$$

Donor fecundity rate = (Total pregnancies/Total AID cycles)

$$^c \chi^2 = 0.449, p = 0.503$$

Fetal loss rate = (Fetal losses/Total pregnancies)

156 (10.3%) fetal losses and 16 (0.82%) congenital defects. (Table 2)

In group A, 1 child was born with ventricular septal defect, while in group B, 16 children were born with congenital malformations, 3 with congenital heart disease, 2 with thalassemia, 2 with cleft lip and palate, 2 with unilateral undescended testis, 2 with hypoplasia of lungs, 1 with hypothyroidism, 1 with hydrocephalus, 1 with abnormal vertebrae, 1 with congenital dislocation of the hip, and 1 with epilepsy (Table 3).

There was no significant difference between groups A and B in rates of donor fecundity, fetal loss and congenital defects ($p > 0.05$).

Discussion

In this study, there was no significant difference between groups A (with Yqh+) and B (without Yqh+) after AID or IVF-D treatment in rates of donor fecundity, fetal loss and congenital defects ($p > 0.05$). Thus, these findings support Yqh+ as being a normal variant in the human population.

Table 2 IVF-D pregnancy outcome

Group	A (observation)	B (control)
Sperm donors karyotype	46,XYqh+	46,XY
Sperm donors number	22	470
Total IVF-D cycles	104	2515
Total pregnancies	72	1674
• Singleton births	48	1093
• Twin births	21	425
• Triplet births	0	0
• Fetal losses	3	156
○ spontaneous abortion	3	137
○ stillbirth	0	15
○ fetal death	0	4
Total live born babies	90	1943
Congenital malformation	1	16
Congenital malformation rate ^a	1.11%	0.82%
Donor fecundity rate ^b	69.2%	66.6%
Fetal loss rate ^c	4.2%	9.3%

^a $P=0.538$ (Fisher's exact test)^b $\chi^2=0.320$, $P=0.571$ ^c $\chi^2=2.214$, $P=0.137$

To the best of our knowledge, this is the first Chinese study to evaluate the relationship between Yqh+ and fetal loss in semen recipients. The main advantages of using sperm donors as research objects were that the men: were 21.3 ± 1.3 years old and in the best fecundity status in life; were strictly screened for physical and reproductive

health; and had 3–5 offspring in a short period via AID or IVF-D.

The distal end of the long arm of the Y chromosome is the heterochromatin region, one of the most variable in length in the human genome [28]. Increased length of the Y chromosome is heritable, and Yqh+ has not been associated with an abnormal phenotype [29]. Interestingly, it has been correlated with racial origin [30].

A number of possible mechanisms influencing the variation in length of the Y chromosome have been reported by different investigators. Bishop et al inferred that increased length of the Y chromosome might be due to a difference in the degree of contraction of chromosomes during cell division [31]. Gripenberg explained Yqh+ as the result of an addition of chromosomal substance because it bears two secondary constrictions [32], while Ghosh et al concluded that the difference in the size of the Y chromosome is a morphological feature without any functional significance [7].

In our study population, 95 (3.74%) of 2541 sperm donors had Yqh+, consistent with results of previous surveys (31, 32, 33). Interestingly, in a very small and early study, Park et al found 28% (8 cases out of 28 total) in overseas Chinese in the USA [33]. The incidence of Yqh+ varies greatly among different geographical areas and ethnic groups [2].

In our research, 75 sperm donors with Yqh+ had normal sperm parameters and fertility. Consistent with our own findings, Kadotani found no difference in the mean length of the Y chromosome between fertile and infertile subjects [34]. In contrast, Sahin et al considered that polymorphic chromosome variants may be related to infertility because they found 1.09% of infertile patients (3 of 276 total) and 0.09% of fetuses (1 of 1130 total) with Yqh+/- [35]. The main reason for the discrepancy between their results and our own may be because Sahin's subjects were all patients.

We found recipients of Yqh+ donors did not have increased fetal loss and congenital malformation rates. Similarly, Hu et al showed Yqh+ had no significant influence on fertilization rate, cleavage rate, embryo quality, clinical pregnancy, miscarriage, or ectopic pregnancy after IVF-embryo transfer [13]. Rodriguez studied 100 couples with recurrent reproductive wastage and 106 control couples with at least 2 healthy children and no spontaneous abortions, and concluded that Y chromosome length variability was not related to reproductive wastage [8]. Furthermore, the average dimensions of the Y chromosome were the same for fathers of 27 normal babies and 18 babies with birth defects [36].

Hou found Yqh+ was inherited from the biological fathers in more than 99% of 288 boys, with the incidence of the Y polymorphisms being similar in 3 groups: children with mental retardation, other chromosomal aberrations or

Table 3 Congenital defects (AID vs. IVF-D)

Congenital defect	AID Department		IVF Department	
	Group		Group	
	A	B	A	B
abnormal vertebrae				1
cleft lip & palate	1	3		2
dislocation of the hip		2		1
epilepsy				1
heart disease		4		3
hydrocephalus				1
hypospadias		2	1	
hypothyroidism				1
lung hypoplasia				2
premature birth ^a		1		
thalassemia				2
undescended testis				2
Total	1	12	1	16

^a with chromosomal anomaly

congenital anomalies, and normal controls [37], and they are not associated with phenotypic abnormalities. However, previous reports have indicated an association between Yqh+ and increased risk of abortion [38].

Nielsen et al found 58 boys with Yqh+ (0.52%) among 11,148 newborn children, and the mothers of these boys had 22% abortions, compared to 13% among 4,895 mothers of children without Yqh+ [14]. They theorized that Yqh+ heteromorphisms may be related to clinical abnormalities, because large Y chromosome heterochromatin represents excessive duplication of DNA that may in turn be related to errors in mitosis, gene regulation or cell differentiation, ultimately leading to spontaneous abortion. Madon et al karyotyped 842 individuals attending an IVF clinic due to primary infertility (Group A) or repeated miscarriages (Group B), found polymorphic variants in 28.8% of the males and 17.2% of the females, and suggested gamete donors with chromosome variants should be screened out to enhance take-home baby rate [39]. Interestingly results extracted from this same article to compare Yqh+ incidence in two groups, revealed values of 7.7% (20/260) for Group A, and 8.1% (16/198) for Group B. Statistical analysis demonstrated Yqh+ incidence in these two groups was the same ($\chi^2=0.023$, $p=0.878$), thus lending further support to our current study.

Verma et al had an interesting rationale for this issue: if Yqh+ plays a significant role in fetal loss, then through natural selection, the East Indian race should have almost vanished by now because 96.7% of male Indians have a long Y chromosome [9]. In reality, Indians are the second most populous race in the world. Hou [37] found only 2 (0.75%) cases with Yqh+ occurred *de novo*, while 263 cases all were inherited from their biological fathers, and there were no history of reproductive problems in their fathers. Therefore, Yqh+ carriers appear to have normal fertility.

One of the strengths of this study was that our research objective differed from those of previous investigations. The qualified sperm donors used in the current study were healthy and each had 3–5 offspring, permitting easy observation of the clinical effects of Yqh+. In previous studies, research objects were clinical phenotype-positive, so their conclusions were based on patients who had a variety of health problems. It is very difficult to gather information about Yqh+ in the general population, because of the problems with recruiting healthy volunteers to participate in research projects.

Our research study covered a span of 4 years, and included 95 long Y chromosome sperm donors (3.74%) out of the total 2541. Of these, only 75 with Yqh+ were involved in this study. The 20 donors not included were omitted for 3 reasons: failure to complete the sperm donation procedure; failure to finish clinical usage, or insufficient quantity of stored semen for clinical use.

The current study is limited by its retrospective design and potential for multiple confounders from sperm donors or recipient women. In this retrospective research, we did not karyotype all recipient women until January 2007, and the overwhelming majority had normal karyotypes. Women with abnormal karyotypes were given genetic counseling about preimplantation genetic diagnosis or oocyte donation. Further medical and psychological follow-up of the offspring from Yqh+ sperm donors are also required. Finally, it would be desirable to increase both the sample size and duration of this study.

Our study, consistent with and supported by previous reports, suggests that Yqh+ does not carry an increased risk of fetal loss and may be considered a normal variant. This information is useful in screening sperm donors and offering genetic counseling to patients with Yqh+.

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