Cytogenetic Analysis of Spermatozoa in a Patient with Chromosome Constitution 45,X/46,X,r(Y) by Intracytoplasmic Injection into Mouse Oocytes

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Abstract—The chromosome set of human spermatozoa was studied by intracytoplasmic injection into mouse oocytes. A total of 85 metaphase plates of male pronuclei of a patient with chromosome constitution 45,X/46,X,r(Y) and 108 metaphase plates of patients with normal sperm parameters (control group) were examined. The ratio between X- and Y-bearing chromosomes in the 45,X/46,X,r(Y) patient and in the control group did not differ from 1 : 1. A significant increase in the rates of diploidy, hypoploidy, hyperploidy of sex chromosomes, and chromosome structure rearrangements in spermatozoa of the patient in comparison with spermatozoa in the control group was recorded.

INTRODUCTION

About 10% of spermatogenesis dysfunctions in men correlate with abnormal chromosome constitution, often involving sex chromosomes [1, 2]. The most frequent abnormalities of sex chromosomes causing infertility include Klinefelter’s syndrome (47,XXY) [3] and structural aberrations of the Y chromosome. The latter often give rise to cell clones devoid of the Y chromosome (45,X) and cause notable phenotypic changes. Phenotypes determined by chromosome constitution 45,X/46,X,r(Y) can vary from female with Turner’s syndrome to male with spermatogenesis dysfunction [4]. Progress in assisted reproductive technologies (ART), in particular, intracytoplasmic injection of a spermatozoon into an egg cell allows coping with many forms of male infertility. However, application of ART to patients with chromosome aberrations increases the risk of birth of children with chromosome abnormalities.

Fluorescence hybridization in situ (FISH) is a common method for investigation of the proportion of imbalanced spermatozoa in patients with chromosome aberrations. However, this method involves all, including unviable, spermatozoa, thus not reflecting the actual risk of inheritance of chromosome aberrations. Intracytoplasmic spermatozoon injection (ICSI) into mouse oocytes, which allows analysis of the chromosome sets of spermatozoa, is free of this drawback.

The goal of the present study was to determine the rates of structural and numerical chromosome aberrations in motile spermatozoa of a patient with chromosome constitution 45,X/46,X,r(Y) by ICSI into mouse oocytes and to estimate the effect of the Y chromosome aberration on the emergence of numerical and structural chromosome aberrations.

MATERIALS AND METHODS

Case record. An ejaculate sample from a 32-year-old patient with infertility and data of chromosome set, microdeletion, hormonal, and sperm analyses were provided by personnel of the Infertility Center, University of Ghent. Standard chromosome set analysis of peripheral blood lymphocytes stimulated with phytohemagglutinin revealed mosaic chromosome constitution 45,X/46,X,r(Y). There were no data on the proportions of cell clones. No deletions in the AZFa, AZFb, or AZFc loci were detected by microdeletion analysis with primers flanking loci sY84, sY86, sY127, sY134, sY254, and sY255. Normal levels of the luteinizing and follicle-stimulating hormones were revealed. Sperm analysis performed twice according to WHO criteria [5] and to the Kruger strict criterion [6] revealed oligoasthenoterozoospermia (OAT) (concentration, 2–4 mln/ml; motility, 6–10%; spermatozoa of normal morphology, 0%).

Ejaculate samples from eight patients with normal sperm parameters were used as the control group [5, 6].

Preparation of ejaculate for injection. Ejaculates were washed from seminal plasma by centrifugation in
were examined with a LUMAM RPO 11 luminescence
used the Hoechst 33258 fluorochrome [9]. Preparations
of preparations.
2.5 mM KCl, 0.35 mM KH$_2$PO$_4$, 0.2 mM MgSO$_4$,
10 mM sodium lactate, 0.2 mM glucose, 0.2 mM
sodium pyruvate, 25 mM NaHCO$_3$, 1.71 mM CaCl$_2$,
1 mM glutamine, 0.01 mM EDTA, 1.0 mg/ml BSA,
1% HEPES; osmolality 265–280 mosm/ml). To
remove cumulus cells, oocytes were incubated in a drop
of KSOM-H medium with 80–100 U/ml hyaluronidase
(Sigma, Germany) for 5 min, washed with KSOM-H,
transferred to KSOM, and stored at 37°C and 5% CO$_2$
for 2–5 h.

**Spermatozoan injections.** Micromanipulations
were performed with a Nicon Diaphot microscope
(Nicon, Japan) equipped with a 40× objective lens, 10×
eyepieces, and Narishige micromanipulators (Narish-
ge, Japan). Injections of motile spermatozoa to oocytes
were performed within 17–23 h after injection of
human chorionic gonadotropin to the mouse. The
spermatozoa were placed into a drop of KSOM-H with 50%
polyvinyl pyrrolidone (Vitrolife, Germany). Oocytes
were transferred to a drop of KSOM-H with 20% fetal
serum (Gibco BRL, Scotland) and incubated at 17°C
for 15 min. One spermatozoa was injected into each
oocyte with a micromanipulator needle [8].

After injection, oocytes were incubated at 17°C for
15 min and at 25°C for 20 min, transferred to a drop
of KSOM, and incubated at 37°C at 5% CO$_2$ for 5–12 h.
After formation of pronuclei, the zygote was trans-
ferred to a drop of KSOM with 2 µg/ml nocodazole
(Sigma, Germany) and incubated until the disappear-
ance of the pronuclei.

**Oocyte fixation.** One hour after pronucleus disappear-
ance, oocytes were incubated in a drop of KSOM-H
with 0.5% pronase E (Sigma, Germany) for 5 min to
remove the bright oocyte envelope and washed in
KSOM-H. Hypotonic treatment was performed in
75 mM KCl with KSOM-H for 8–12 min. Oocytes
were fixed in methanol–glacial acetic acid (3 : 1) at
−25°C for 20–25 s and transferred to a microscopic slide.

**Chromosome staining and microscopic examination of preparations.** For QFH-banding analysis, we
used the Hoechst 33258 fluorochrome [9]. Preparations
were examined with a LUMAM RPO 11 luminescence
microscope equipped with a set of light filters for Hoe-
chst 33258 and a computer image processing system
(VideoTest-Kario 2.0).

Statistical analysis of the results was performed using
$\chi^2$ test and Fisher’s angular transformation ($P < 0.05$).

### RESULTS

A total of 193 metaphase plates of male pronuclei
were examined: 108 plates of spermatozoa with normal
head morphology from 8 patients with normal sperm
parameters (control group) and 85 plates of spermato-
zoa with abnormal heads from the patient with chromo-
osome constitution 45,X/46,X,r(Y).

The ratio between X- and Y-bearing spermatozoa
with normal head morphology and motility in the control
group ($n = 108$) did not differ from 1:1 (45.71 and
52.38%, respectively; $P > 0.05$). The overall rate of
chromosome aberrations was 25.91%. Hyperploidy
(7.4%) was related to additional gonosomes: 24,XY
(two cases; 1.85%) and autosomes: 24,X,+C (two
cases); 24,Y,+C; 24,Y,+D; 24,Y,+E; and 24,Y,+G (one
case each; 5.55%). Hypoploidy (12.03%) was related to
nullisomy for autosomes of groups A, C, D, E, and F
(9.25%) and gonosomes (2.77%). Structural rearrange-
ments (6.48%) involved marker chromosomes, a de
novo translocation 24,X;3,+4,t(5;6),–17,+mar de novo,
and a deletion 23,Y,del(18).

The ratio between X- and Y-bearing spermatozoa in the
patient with chromosome constitution 45,X/46,X,r(Y)
($n = 85$) did not differ significantly from 1 : 1 (37.64 and
27.05%, respectively; $P > 0.05$) (Fig. 1). The overall rate
of de novo chromosome aberrations was 68.25%
(table). Hyperploidy constituted 17.64% (11.76% for
gonosomes and 5.88% for autosomes; Fig. 1). Hypoplo-
dy constituted 48.23% (18.82% for gonosomes and
30.59% for autosomes). Diploidy and tetraploidy con-
stituted 4.70% altogether; structural rearrangements,
29.41% (table). Comparison of chromosome aberrations
in spermatozoa of the patient with the control
group showed elevated rates of gonosome hyperploidy
(11.76 and 1.85%, respectively; $P < 0.05$), polyplody
(4.70 and 0%, respectively; $P < 0.05$); and hypoploidy
(48.23 and 12.03%, respectively, $P < 0.05$). The rate
of spermatozoa with structural rearrangements was
also elevated: 29.41 and 6.48%, respectively, $P < 0.05$
(Fig. 2).

### DISCUSSION

Numerous studies of infertile patients have shown
that 5 to 10% of azoospermy and OAT cases are accom-
panied by microdeletions in the Y chromosome [10]. In
our case, we found no deletions in the AZF loci; i.e., the
rearrangement of the Y chromosome had not involved
these loci. The ratio between X- and Y-bearing sperma-
tozoia did not differ from 1 : 1 (45.71 and 52.38%,
respectively). The rates of spermatozoa devoid of one
of the gonosomes or having an extra gonosome did not