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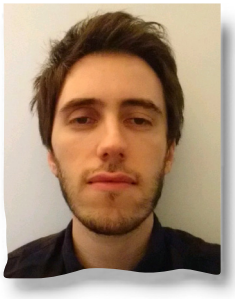
Potential selection of genetically balanced spermatozoa based on the hypo-osmotic swelling test in chromosomal rearrangement carriers

Alexandre Rouen [a,*], ***Léa Carlier*** [a], ***Solveig Heide*** [a], ***Matthieu Egloff*** [a], ***Pauline Marzin*** [a], ***Flavie Ader*** [a], ***Mathias Schwartz*** [a], ***Eli Rogers*** [a], ***Nicole Joyé*** [a], ***Richard Balet*** [b], ***Nathalie Lédée*** [b], ***Laura Prat-Ellenberg*** [b], ***Nino Guy Cassuto*** [c], ***Jean-Pierre Siffroi*** [a]

[a] *Medical Genetics Department, APHP, INSERM UMR_S933, Armand-Trousseau Hospital, 75012 Paris, France*
[b] *Reproductive Medicine Unit, Bluets Hospital, 75012 Paris, France*
[c] *ART unit, Drouot laboratory, 75009 Paris, France*



Alexandre Rouen is a medical geneticist at Trousseau Hospital, Paris, France. He specializes in infertility of genetic origin, and conducts research focusing on improving reproductive care in patients with genetic defects, notably chromosomal rearrangements.

KEY MESSAGE
In male chromosomal rearrangement carriers, sperm selection based on the hypo-osmotic swelling test could potentially be used to select for genetically balanced spermatozoa, prior to pre-implantation genetic diagnosis, in order to improve reproductive prognosis in affected couples.

A B S T R A C T

Chromosomal translocations and other balanced rearrangements, although usually associated with a normal phenotype, can lead to the transmission of an abnormal unbalanced genome to the offspring. Balanced and unbalanced spermatozoa, being indistinguishable, cannot be selected or deselected for prior to IVF and pre-implantation genetic diagnosis. Spermatozoa from 16 chromosomal rearrangement carriers were studied. After incubation in a hypo-osmotic solution (hypo-osmotic swelling test, or HOST), spermatozoa were fixed on microscope slides. The chromosomally balanced or unbalanced status corresponding to each observed class of flagellar conformation was evaluated through fluorescent in-situ hybridization (FISH). We show here a specific type of spermatozoa, with a distinct flagellar conformation that was associated with a balanced genetic content. HOST is a simple, low-cost and time-honoured procedure initially developed to distinguish immotile viable from non-viable spermatozoa. We demonstrate that it can also be used to identify genetically balanced spermatozoa in chromosomal rearrangement carriers, with a 96% decrease in the proportion of unbalanced spermatozoa after selection. This may potentially improve reproductive prognosis in affected couples if used prior to pre-implantation genetic diagnosis (PGD), and clinical utility and efficacy should be evaluated in further studies.

* Corresponding author.
E-mail address: alexandrerouen@gmail.com (A Rouen).
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Introduction

Chromosomal rearrangements, such as translocations and inversions, exist with a prevalence of 1/500 in the general population. In most cases, they are balanced and asymptomatic but are nevertheless associated with a risk of transmitting an unbalanced karyotype to the offspring. Indeed, in these patients, a variable proportion of gametes are genetically unbalanced and are associated with different adverse outcomes such as spontaneous abortions, pregnancy terminations, fetal malformations, or learning disabilities in resulting children (McKinlay Gardner and Sutherland, 2011). Genetically unbalanced spermatozoa are morphologically indistinguishable from their balanced counterparts. Affected couples are therefore advised to either conceive naturally, with planned prenatal diagnosis, or to seek pre-implantation genetic diagnosis (PGD) (Hirshfeld-Cytron et al., 2011). However, the former option implies a pregnancy termination in the case of an adverse result, while the latter is not available in every country and leads to the loss of oocytes fertilized with chromosomally unbalanced spermatozoa.

It has been previously described that discontinuous gradient centrifugation increases the rate of balanced spermatozoa in structural chromosomal rearrangement carriers (Rouen et al., 2013a). However, selection is only partial, with a mean decrease in abnormal spermatozoa of around 30%, which led us to seek a more discriminating procedure, which furthermore needed to be compatible with subsequent assisted reproduction techniques. We postulated that the hypo-osmotic swelling test (HOST) could be used for this purpose. This procedure has been used for several decades to differentiate viable from non-viable spermatozoa, particularly in patients with akinetozoospermia (Jeyendran et al., 1984; World Health Organization, 2010). In the last few years, several authors have shown a correlation between sperm morphology after HOST and several parameters such as DNA fragmentation, membrane integrity, protamine deficiency and aneuploidy (Bassiri et al., 2012; Pang et al., 2010; Stanger et al., 2010; Zeyneloglu et al., 2000).

We therefore postulated that the flagellar conformation after incubation in a hypo-osmotic solution could be correlated with chromosomal content in spermatozoa from structural chromosomal rearrangement carriers.

Materials and methods

Fifteen chromosomal translocation carriers (10 reciprocal translocations and five Robertsonian translocations) as well as one pericentric inversion carrier were studied. Additionally, five normal controls were included for comparison of the proportion of the different HOST classes between them and the rearrangement carriers. These controls took part in IVF cycles for female indication. Most of these patients were initially consulted for infertility, recurrent spontaneous abortions or fetal malformations. Some of them contacted our centre through a chromosomal rearrangement carrier organization (Association Valentin, France). The study was conducted as part of the normal medical care of the patients, and genetic analysis was performed by certified medical geneticists according to French legislation (Agence de la Biomédicine). The Ethical Committee of the Fédération Française des CECOS indicated that the study was exempt from ethical approval on 3 April 2017. Informed consent was obtained from each patient. Semen samples were obtained by masturbation, after a 3–5 day abstinence period. All except three patients had normal semen characteristics (semen volume >1.5 ml, sperm concentration >15 million/ml, progressive motility >32%, normal forms >4%; World Health Organization, 2010); patient 1 (P1) and P4 presented with oligozoospermia (P1: 2 million sperm/ml, P4: 3 million sperm/ml), and patient P6 presented with oligozoospermia and teratozoospermia (0.5 million sperm/ml, 1% normal forms). The semen sample from each patient was then divided into two fractions. One fraction was washed in PBS (Eurobio, France), fixed in a freshly made methanol and acetic acid solution (3:1) at room temperature, and spread onto microscope slides; this was later used to establish the proportion of unbalanced spermatozoa in the native ejaculate for each subject. The second fraction underwent discontinuous gradient centrifugation (DGC) through two layers of 40% and 80% PureSperm solution (Nidacon, Mölndal, Sweden), before being incubated for 10 min at 37°C in a hypo-osmotic 150 mOsm solution (75 mM fructose, 25 mM sodium citrate) prior to spreading on microscope slides and fixation. Fluorescent in situ hybridization (FISH) was conducted for chromosomal segregation evaluation on both slides. Chromosomal probes were chosen for each chromosomal rearrangement as previously described (Rouen et al., 2013a): one telomeric probe for each of the translocated chromosomal ends, as well as one additional centromeric probe, of one of the two rearranged chromosomes in reciprocal translocations, and of another chromosome in Robertsonian translocations. In the inversion, probes were chosen for each of the telomeric ends of the concerned chromosome in order to evaluate the proportion of recombinants. In-house contiguous probes were used for telomere identification, and commercial probes were used for the centromeres (Abbott Molecular, USA). The hybridization efficiency of the probes was evaluated on anaphase chromosomes before the study (pictures not shown). Balanced chromosomal contents gave the signal combination blue–green–red, while all other signal combinations corresponded to unbalanced modes (adjacent 1, adjacent 2, and 3:0 in Robertsonian translocations, 3:1 in reciprocal translocations, and recombinant sperm in inversion translocations).

Analysis was performed on a fluorescence microscope (Olympus BX61). For each sperm cell, flagellar morphology was first analysed under

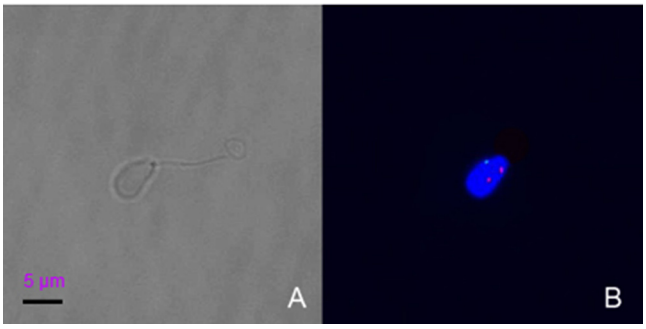

Figure 1 – Spermatozoa were first analysed under white light, in order to evaluate the flagellum and HOST category. (A) A HOST C spermatozoon, with a looped distal flagellum. (B) The same spermatozoon, under fluorescent light, which shows an unbalanced chromosomal combination. In this reciprocal translocation carrier, a blue probe was used for the centromere of one of the centromeres involved, and a red and a green probe were used for the two involved telomeric ends (Olympus BX61 microscope, ×1000). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

white light using a phase contrast device. Immediately after, chromosomal segregation was evaluated under UV light (**Figure 1**). For each patient, approximately 500 spermatozoa were first analysed to evaluate the proportion of unbalanced cells in the native ejaculate. Depending on the relative proportion of the various classes of spermatozoa after HOST, different numbers of spermatozoa were analysed for each HOST class: at least 50 (A), 20 (B), 20 (B+), 20 (C), 25 (D/E), 30 (F) and 70 (G) were analysed. For the B and B + classes, the total of 20 sperm cells was found to be enough for all patients to obtain statistically significant results. Higher numbers of spermatozoa could be analysed for the other classes, because they were found in higher proportions. The proportion of unbalanced cells in each HOST class was compared with that in the native ejaculate using a chi-squared test and results with $P < 0.05$ were considered statistically significant. A Wilcoxon signed rank test was used to evaluate the global efficiency of the procedure to select for genetically balanced sperm (CSBSJ University Statistics tools website, Graphpad website).

Results

When incubated in a hypo-osmotic solution, spermatozoa from translocation carriers underwent morphological modifications of their flagella, just as spermatozoa from patients with a normal karyotype do. As a whole, these modifications were similar to those originally reported by Jeyendran et al. (1984) and included in the WHO document (World Health Organization, 2010), and allowed us to classify sperm cells in one of the HOST A to G classes (**Figure 2**).

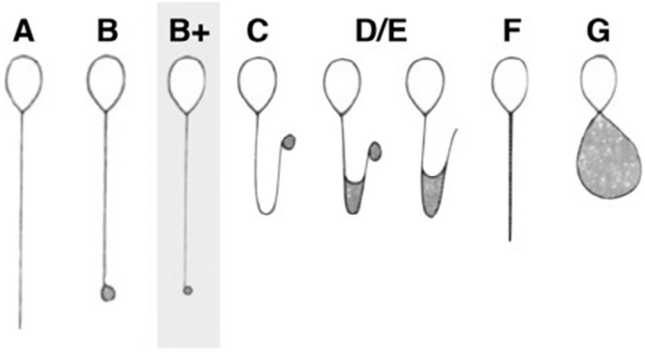

Figure 2 – The different HOST categories, as established in the *WHO laboratory manual for the examination and processing of human semen*, with the addition of the B + type described here (World Health Organization, 2010).

Because the FISH analysis was not conducted on live spermatozoa (in 'real-life' intracytoplasmic sperm injection [ICSI] conditions), but on fixed spermatozoa, we first guaranteed that our fixation protocol did not interfere with HOST morphology by evaluating the proportion of the different HOST classes, in seven subjects, before and after fixation. Indeed, it was important to ascertain that the fixation protocol did not alter the HOST-related flagellar morphology. We did not find any statistical significance (data not shown).

We then evaluated the proportion of the different HOST classes for each patient, as well as for five controls with normal karyotypes (**Figure 3**). For the patients, the majority of spermatozoa belonged to the G class, which is in accordance with the literature (Bassiri et al., 2012; Stanger et al., 2010). The differences observed between the normal subjects and the rearrangement carriers were statistically significant for classes C, D/E, F and G ($P < 0.05$). For the A, B and B + classes, the proportions were not statistically different between the rearrangement carriers and the controls.

By looking at the chromosomal content with respect to the translocation, we found that these different HOST classes comprised chromosomally balanced and unbalanced spermatozoa in variable proportions. We established the proportion of unbalanced spermatozoa in each HOST class; these figures were compared with the corresponding proportion in the native sperm sample. The HOST classes with the highest proportion of unbalanced spermatozoa were, depending on subjects, either F, G or A (the latter indicating non-viable spermatozoa). The classes with significantly reduced proportions ($P < 0.05$) of unbalanced spermatozoa were B (–55%), C (–20%) and D/E (–18%) (**Figure 4**, **Table 1**). Among these, a specific type of spermatozoa, i.e. those with a very small and perfectly round swollen region at the tip of the flagella, showed a drastically lower proportion (–83%) of chromosomally unbalanced cells than that found in unselected spermatozoa (**Table 2**). These spermatozoa resemble the B type in the WHO classification but their looped flagellar segment is smaller and more distal. We suggest naming them 'B + ' spermatozoa (**Figure 5**). In this B + category, the mean decrease in the proportion of spermatozoa carrying an unbalanced chromosomal content was 83% when compared with the ejaculate ($P < 0.001$). Decrease values ranged from 60% (translocation carrier 11) to 100% (translocation carriers 2, 3 and 5). For Robertsonian translocations, the global decrease was 96% (*P*

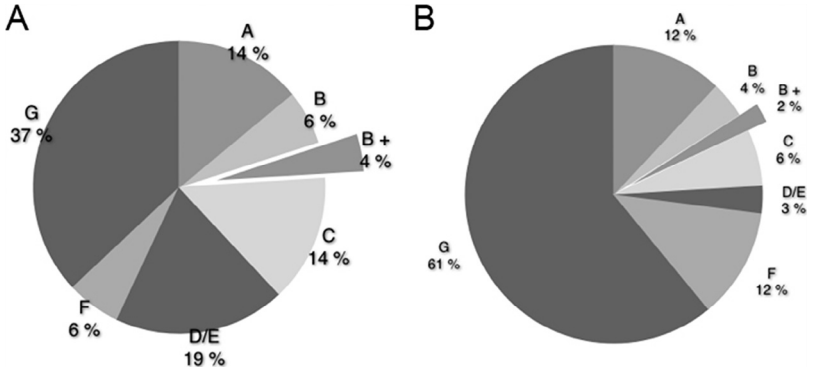

Figure 3 – Respective proportions of the different HOST classes, in five normal controls (A) and in the 16 subjects (B). For controls versus rearrangement carriers $P < 0.05$ for classes C, D/E, F and G.

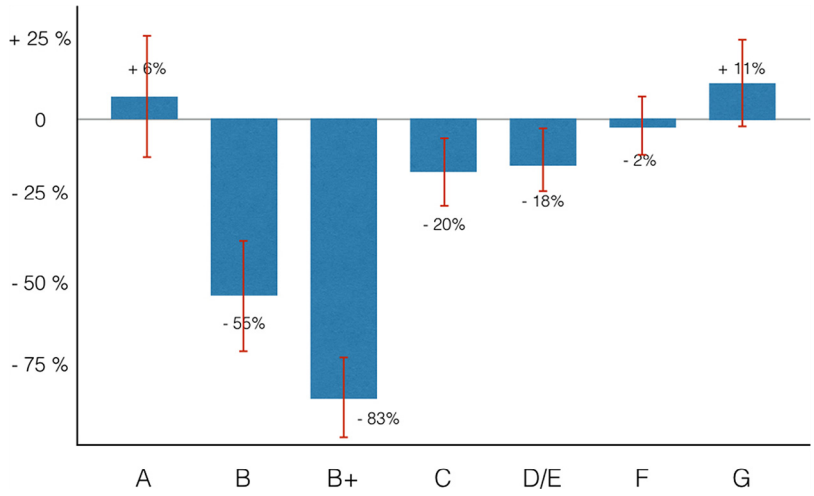

Figure 4 – Average variation of the proportion of unbalanced spermatozoa for each class in HOST-treated ejaculate compared with the proportion in the native ejaculate. The B + category shows the most important decrease (–83%). The red lines show the confidence intervals for each class (95% CI). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

< 0.001) while it was 78% ($P < 0.001$) for reciprocal translocations. In the subject with a pericentric inversion (patient 14), the decrease was 73% ($P = 0.001$). B + spermatozoa can be identified using the following criterion: the ratio L/D (L being the length of the flagellum and D being the diameter of the loop) should be above 20. In other words, the flagellum should be 20 times longer than the diameter of the loop (**Figure 6**), as this variety of spermatozoa presents the highest likelihood of balanced chromosomal content.

To evaluate the ability of the procedure to select balanced spermatozoa, globally and not individually on each patient, we used a non-parametric Wilcoxon signed rank test. We found that our procedure allowed for the selection of chromosomally balanced spermatozoa in a statistically significant manner ($P = 0.0004$).

We then looked for a correlation between the proportion of unbalanced spermatozoa before and after the selection procedure, for all 16 patients (**Figure 7**). We found those two variables to be highly correlated, with a Spearman's rank correlation of 0.875 ($P = 0.0007$). For a given patient, the lower the initial proportion of unbalanced spermatozoa was, the lower it was after the selection procedure.

Discussion

It has been previously demonstrated that in chromosomal rearrangement carriers, unbalanced spermatozoa have a higher level of apoptosis and DNA fragmentation (Brugnon et al., 2010; Rouen et al., 2013a), that their nuclei are less dense (Rouen et al., 2013b) and significantly larger (Rouen et al., 2015). We suggest that the fine spermatic nuclear architecture may be disrupted by chromosomal imbalances,

Table 1 – The proportion of unbalanced spermatozoa in native ejaculate and in the hypo-osmotic swelling test (HOST) treated ejaculate for each HOST category, in all 16 subjects.

Subject	Chromosomal rearrangement	% of unbalanced spermatozoa (native ejaculate)	A (%)	B (%)	B + (%)	C (%)	D/E (%)	F (%)	G (%)
1	rob(13;14)(q10;q10)	33.6	45[a]	20[a]	5.5[a]	30	29	33	40[a]
2	rob(13;14)(q10;q10)	15.8	22[a]	5[a]	0[a]	10	12	18	25[a]
3	rob(13;14)(q10;q10)	47	66[a]	15[a]	0[a]	33[a]	29[a]	59[a]	50
4	rob(13;14)(q10;q10)	36	40[a]	21[a]	2[a]	25[a]	33	41[a]	37
5	rob(14;21)(q10;q10)	45	40[a]	0.9[a]	0[a]	38[a]	27[a]	45	48[a]
6	t(7;20)(p13;q12)	75	76	35[a]	20[a]	39[a]	61[a]	70[a]	78[a]
7	t(11;22)(q24;q11)	72	72	28[a]	14.8[a]	63[a]	63[a]	75	74
8	t(1;18)(p22;q21.1)	73	72	30[a]	15[a]	40[a]	38[a]	77[a]	71
9	t(5;12)(q13;q13)	69	70	25[a]	14.8[a]	59[a]	62[a]	50[a]	67
10	t(4;10)(q31.3;q26.1)	70	50[a]	20[a]	15[a]	56[a]	56[a]	62[a]	87.5[a]
11	t(3;22)(q21;q11.2)	77	66[a]	40[a]	31	76	90[a]	70[a]	78[a]
12	t(5;10)(q34;p12.1)	65	63	28[a]	9[a]	52[a]	40[a]	47[a]	68
13	t(8;11)(q24.2;q12)	59	63	44[a]	1.5[a]	70[a]	62	61	64[a]
14	t(7;20)(q21.1;p12)	49	67	33	13[a]	43	49	49	58
15	t(5;9)(q10;q10)	52	70	40	14[a]	46	42	52	54
16	inv(7)(p12q32)	53	25[a]	19[a]	14.6[a]	35[a]	40[a]	47[a]	61[a]

[a] Statistically significant results compared with the proportion of unbalanced spermatozoa in the native ejaculate ($P < 0.05$).

Table 2 – Proportion of unbalanced spermatozoa in chromosomal rearrangement carriers, before and after HOST-based selection (for B + spermatozoa only). All the results are statistically significant.

Subject	Chromosomal rearrangement	% of unbalanced spermatozoa (native ejaculate)	% of unbalanced spermatozoa (after selection)	% decrease
1	rob(13;14)(q10;q10)	33.6	5.5 ($P< 0.05$)	84
2	rob(13;14)(q10;q10)	15.8	0 ($P< 0.01$)	100
3	rob(13;14)(q10;q10)	47	0 ($P< 0.01$)	100
4	rob(13;14)(q10;q10)	36	2 ($P< 0.05$)	94
5	rob(14;21)(q10;q10)	45	0 ($P< 0.01$)	100
6	t(7;20)(p13;q12)	75	20 ($P< 0.05$)	73
7	t(11;22)(q24;q11)	72	14.8 ($P<0.001$)	79
8	t(1;18)(p22;q21.1)	73	15 ($P< 0.01$)	79
9	t(5;12)(q13;q13)	69	14.8 ($P<0.001$)	79
10	t(4;10)(q31.3;q26.1)	70	15 ($P< 0.01$)	79
11	t(3;22)(q21;q11.2)	77	31 ($P< 0.05$)	60
12	t(5;10)(q34;p12.1)	65	9 ($P< 0.01$)	86
13	t(8;11)(q24.2;q12)	59	1.5 ($P< 0.01$)	97
14	t(7;20)(q21.1;p12)	49	13 ($P< 0.05$)	73
15	t(5;9)(q10;q10)	52	14 ($P< 0.05$)	73
16	inv(7)(p12q32)	53	14.6 ($P=0.001$)	72

and that this may hinder the normal condensation of the nucleus. Indeed, spermatic DNA is characterized by a high degree of condensation, which is made possible by a very specific three-dimensional chromosomal conformation: chromosomes are bent at their centromeres, which are all located in the centre of the nucleus, while the telomeric ends are situated at the periphery (Ioannou and Griffin, 2011; Mudrak et al., 2005). Although chromosomally balanced and unbalanced spermatozoa cannot be distinguished on the basis of their morphology under light microscopy (Cassuto et al., 2011), we postulated that the presence of translocated chromosomes in the spermatic nucleus could affect the nuclear architecture and disrupt the normal nuclear condensation process. We therefore suggested that structural chromosomal abnormalities could have subtle effects on nuclear architecture, which could be used in a way to select genetically balanced spermatozoa prior to IVF.

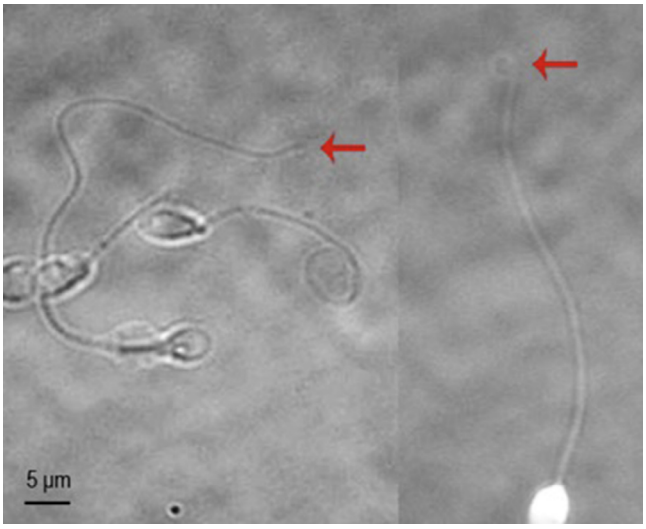

Figure 5 – B + type spermatozoa. These spermatozoa show a perfectly round and small loop (red arrow) at the tip of their flagellum, after HOST treatment. This population shows a very low proportion of chromosomal unbalanced cells, in rearrangement carriers (Olympus BX61 microscope, ×1000). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

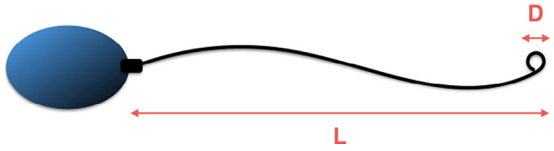

Figure 6 – B + spermatozoa have an *L*/*D* ratio >20, *L* being the length of the flagellum, and *D* the diameter of the loop.

Multiple procedures have been developed to partially eliminate spermatozoa with lower nuclear density levels as well as those with increased apoptosis or DNA fragmentation indices (Sakkas, 2013). These procedures are used prior to IVF in order to increase pregnancy rates. The two most commonly used in IVF laboratories are DGC and the swim-up procedure (Henkel, 2012). HOST has been used for decades in order to differentiate viable from non-viable spermatozoa, especially in patients with total akinetozoospermia (Verheyen et al., 1997; World Health Organization, 2010). Spermatozoa react to the changes in osmolarity in their environment by activating ion channels, which in turn act on the shape of the flagellum (Peris et al., 2000). The unmodified spermatozoa (class A) are the non-viable ones.

It has been postulated that HOST would detect apoptosis particularly early in the process, because membrane alterations (translocation of phosphatidyl serine from the inner to the outer leaflet of the membrane) are one of the most precocious phenomena during apoptosis (Pretorius et al., 2016). This could possibly explain why HOST allows the identification of balanced spermatozoa in these patients.

Furthermore, different classes have been individualized according to the shape of the flagellum. In previous studies, the types that were associated with the lowest degree of DNA fragmentation and the lowest degree of protamine deficiency were D and C in one study (Bassiri et al., 2012) and D, E and F in another (Stanger et al., 2010). In contrast, the groups associated with DNA of lesser quality were A and G in both studies. Concerning chromosomal abnormalities, sperm morphology after HOST was shown to be correlated with aneuploidy (Zeyneloglu et al., 2000). It has been previously reported that DGC could decrease the proportion of unbalanced spermatozoa by 23% (Rouen et al., 2013b). In the present study, we performed DGC prior to HOST in a way to further op-

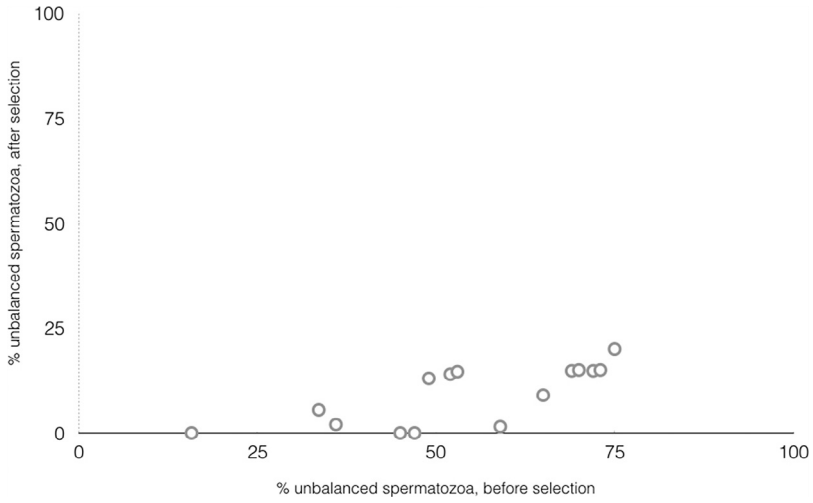

Figure 7 – The proportion of unbalanced spermatozoa after the selection procedure (i.e. among the B + spermatozoa) was found to be highly correlated with that of the initial samples (Spearman's rank correlation: 0.875, *P* = 0.0007).

timize the selection process. HOST alone was not tested, because DGC is routinely used in IVF laboratories before ICSI.

Here, we show that HOST is also related to structural chromosomal aberrations. The HOST category with the highest proportion of unbalanced spermatozoa was the G group, and the one with the lowest was the B group. This led us to further analyse spermatozoa that resembled the B type, only with an even smaller flagellar loop. Spermatozoa of this new category, coined 'B + ', indeed exhibit a very low proportion of unbalanced chromosomal content, when classified by an *L/D* ratio of at least 20. It is therefore likely that the presence of a chromosomal imbalance may hinder the proper nuclear condensation process resulting in nuclear volume increase, DNA fragmentation and apoptosis (De Iuliis et al., 2009). Even though these effects do not impair sperm production, because unbalanced spermatozoa are ultimately found in the ejaculate, they may lead to modifications of the sperm shape after HOST. It is furthermore interesting to note that the proportion of G sperm, which was associated with poor DNA quality in previous studies (Bassiri et al., 2012; Stanger et al., 2010) is almost twice as prevalent among rearrangement carriers compared with normal controls.

Sperm aneuploidy was not evaluated in the present study. Indeed, it has been shown that chromosomal rearrangement carriers present with a slightly elevated proportion of aneuploid spermatozoa (Vozdova et al., 2013). Furthermore, a correlation between aneuploidy and HOST morphology has been shown in patients with a normal karyotype (Zeyneloglu et al., 2000). However, the present study aimed to evaluate the utility of the HOST procedure in selecting spermatozoa originating from the balanced, known as the 'alternate', segregation mode. Evaluating aneuploidy would have been technically difficult, because of the low proportion of aneuploid spermatozoa found for a given chromosome. It would have been mandatory to analyse tens of thousands of spermatozoa, because the B + class only accounts for 2% of spermatozoa.

Conflicting studies exist regarding the duration of incubation for the HOST procedures (Martini et al., 2006; Matson et al., 2009; World Health Organization, 2010). In our study, there were no significant differences in the respective proportions of the different HOST classes, between 5 and 10 min of incubation (data not shown).

The number of spermatozoa studied for each HOST class is arguably low (from 20 to 100). However, the proportions of certain HOST classes are low: 2% for B + , 4% for B, and 3% for D/E spermatozoa (**Figure 3**). Finding 20 B + spermatozoa requires manually analysing 2000 spermatozoa. However, chi-squared calculations showed significant results for all patients due to the significant difference in proportions found in the B + group compared with the native proportions of unbalanced spermatozoa.

These findings should be confirmed on a greater number of patients. However, statistical analyses are not performed here between patients and controls, but rather between the proportion of unbalanced spermatozoa after selection versus before selection. The global number of spermatozoa that were studied was large, thereby allowing statistically significant results. Furthermore, our population included several different types of chromosomal rearrangements (Robertsonian and reciprocal translocations, as well as a pericentric inversion).

The pathophysiological mechanisms of our findings are not entirely uncovered. It was noted that HOST has already been shown to be correlated with other sperm quality parameters. Bassiri et al. (2012) showed that DNA fragmentation levels in B, C and D/E sperm were 2 to 4 times lower compared with that of A, F or G spermatozoa (4% in B, 17% in A). Similar conclusions were reached for aneuploidy (Pang et al., 2010). The prevalence of aneuploid spermatozoa in oligoasthenoteratozoospermic patients in the G class was found to be 1.16%, while it was 0% in B–D spermatozoa ($P < 0.05$). These findings suggest a correlation between sperm tailing patterns and nuclear characteristics, which is in accordance with our findings.

Although the precise mechanism of these modifications remains to be elucidated, these findings have immediate clinical applications. Indeed, there was previously no way to identify and select balanced spermatozoa for IVF in chromosomal rearrangement carriers, leaving patients with the option of prenatal genetic diagnosis of natural pregnancies, or of PGD. The latter option comprises the genetic testing of each embryo in a way to discard the genetically abnormal ones, a process both costly and wasteful of oocytes. We therefore suggest the selection technique we present here be used prior to PGD in order to limit the number of oocytes fertilized with unbalanced spermatozoa, and ultimately maximize the chances of normal pregnancy. HOST is safe to use in IVF: it has been used for several decades in patients with akinetozoospermia to differentiate between dead and living spermatozoa, and is referenced in the WHO

'Examining and processing human semen' guidelines (World Health Organization, 2010). For each new patient, the effect of HOST-based sperm selection should be evaluated through FISH, prior to PGD. In settings and countries where PGD is not available, we suggest that this selection technique could be used prior to conventional ICSI. In both cases, selection of genetically normal spermatozoa in chromosomal rearrangement carriers is thought to improve reproductive outcomes in affected couples. It is important to mention, however, that this selection procedure is not 100% accurate. Indeed, it is likely that selection on living spermatozoa will not be as effective as that on fixed spermatozoa. Consequently, further studies should aim at confirming the effectiveness of this procedure in selecting for genetically balanced spermatozoa not only upon fixed spermatozoa on microscope slides, but upon living spermatozoa in 'real-life' ICSI conditions.

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Chromosome
HOST
Hypo-osmotic swelling test
Rearrangement
Sperm selection
Translocation

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