



HAL
open science

Does the prognosis after PGT for structural rearrangement differ between female and male translocation carriers?

Anne Mayeur, Naouel Ahdad, Laetitia Hesters, Michael Grynberg, Serge Romana, Charlotte Sonigo, Nelly Frydman

► To cite this version:

Anne Mayeur, Naouel Ahdad, Laetitia Hesters, Michael Grynberg, Serge Romana, et al.. Does the prognosis after PGT for structural rearrangement differ between female and male translocation carriers?. *Reproductive BioMedicine Online*, 2020, 40, pp.684 - 692. 10.1016/j.rbmo.2020.01.025 . hal-03490933

HAL Id: hal-03490933

<https://hal.science/hal-03490933>

Submitted on 22 Aug 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Does the prognosis of PGT-SR differ in female translocation carriers compare to male**
2 **carriers? A lesson learned from 331 couples**

3

4 **Running Title:** PGT for chromosomal translocations

5 Naouel Ahdad^{1*}, Anne Mayeur², Laetitia Hesters², Michael Grynberg^{1,3,4}, Serge Romana⁵,

6 Charlotte Sonigo^{1,6}, Nelly Frydman^{2,3}

7 ¹ Department of Reproductive Medicine and Fertility Preservation, Hôpital Antoine Bécclère,
8 Hôpitaux Universitaires Paris Sud, Assistance Publique - Hôpitaux de Paris, Clamart 92140,
9 France

10 ² Reproductive Biology Unit, Paris-Sud University, Paris-Saclay University, Antoine Bécclère
11 Hospital, Clamart 92140, France

12 ³ Université Paris-Sud, Université Paris Saclay, Le Kremlin Bicêtre 94276, France

13 ⁴ Inserm U1133 Université Paris Diderot, Paris 75013, France

14 ⁵ Necker Enfants Malades Hospital, Genetic Unit, AP-HP, Paris, France

15 ⁶ Inserm U1185 Université Paris-Sud, Université Paris Saclay, Le Kremlin Bicêtre 94276,
16 France

17 *Correspondence address: Service de médecine de la reproduction et de préservation de la
18 fertilité, Naouel Ahdad, 157 rue de la Porte de Trivaux 92140 Clamart, France. Tel: +33-14-
19 53-74-979. E-mail: naouel.yata@aphp.fr

20

21 **ABSTRACT**

22 **Research question:** Chromosomal translocations are known genetic causes of premature
23 ovarian insufficiency syndrome. Are certain translocations associated with decreased capacity
24 of small antral follicles to respond to exogenous FSH? Does the prognostic of PGT-SR differ
25 in female translocation carriers?

26 **Design:** This is a monocentric retrospective observational study covering a 10-year period.
27 One hundred and thirty-nine females carrying a translocation were compared with 192
28 partners of male translocation carriers. To evaluate ovarian response to FSH, we used the
29 follicular output rate (FORT), defined by ratio between the pre ovulatory follicle count (PFC)
30 on day of hCG x 100 / antral follicle count (AFC). In an effort to determine a cut-off of
31 metaphase II oocytes and biopsied embryos as predictor of obtaining a balanced embryo
32 transfer, we performed ROC curves.

33 **Result:** We found a decreased capacity of small antral follicles to respond to exogenous FSH
34 in female translocation carriers. The number of metaphase II oocytes in both groups was
35 weakly informative as a predictor of obtaining an ET. On the contrary the number of biopsied
36 embryos had some clinical value and allowed to determine a cut-off of 6.5 for female
37 translocation carriers versus 5.5 for the partners of male translocation carriers.

38 **Conclusions:** Female translocation carriers may respond poorly to COH and present a higher
39 rate of unbalanced embryos, which means that a higher gonadotropins dose may be used
40 safely in an attempt to increase the number of biopsied embryos.

41 **Keys words:** chromosomal translocation, controlled ovarian stimulation, FORT, PGT-
42 outcomes.

43 **STUDY FUNDING/COMPETING INTEREST(S):** None

44 INTRODUCTION

45 Patients bearing a balanced translocation present an increased risk of recurrent
46 implantation failure and obstetrical complications, such as recurrent abortion, stillborn or live
47 born fetuses affected by a chromosomal syndrome (Jacobs *et al.*, 1974; Simopoulou *et al.*,
48 2003). Preimplantation Genetic Testing for structural rearrangement (PGT-SR) provides the
49 opportunity to distinguish between normal/balanced and unbalanced embryos. In this
50 procedure, *in vitro* fertilization (IVF) with intra-cytoplasmic sperm injection (ICSI) is
51 performed and the embryos obtained are biopsied to determine their chromosomal status. On
52 the one hand, PGT-SR represents a real chance to minimize the risk of recurrent implantation
53 failure and miscarriage due to abnormal chromosomal segregation and offers couples the
54 chance to have an unaffected child, without facing prenatal diagnosis and termination of
55 pregnancy (Munné *et al.*, 1998, 2000; Otani *et al.*, 2006; Scriven *et al.*, 2013). On the other
56 hand, PGT-SR is a complex procedure with several critical steps. A major issue is the need
57 for a large number of embryos to increase the chance to get a balanced embryo since the rate
58 of embryonic chromosomal unbalance varies from 67% to over 80% (Sermon *et al.*, 2004; Ko
59 *et al.*, 2010; Harper *et al.*, 2012; Scriven *et al.*, 2013; Moutou *et al.*, 2014; Kato *et al.*, 2016).

60 It is well known that autosomal translocations, particularly Robertsonian
61 translocations (ROBT) and some reciprocal translocations (RCT) are associated with an
62 increased risk of sperm quality alteration (Sasagawa *et al.*, 1993; Penna Videau *et al.*, 2001;
63 Rao *et al.*, 2005; Mayeur *et al.*, 2019). For women, cytogenetic studies are more often focused
64 on premature ovarian insufficiency (POI) with identifiable causes mainly related to X
65 chromosome disorders and more rarely to autosome-autosome translocations (Therman and
66 Susman, 1990; Schlessinger *et al.*, 2002). However, autosomal translocations may also affect
67 the ovarian function less drastically. Some investigators addressed this issue by merely

68 analyzing the strength of ovarian response to controlled ovarian stimulation (COS) in female
69 translocation carriers compared to controls and reported contradictory results (Chen *et al.*,
70 2005; Dechanet *et al.*, 2011). To objectively evaluate antral follicle responsiveness to
71 exogenous FSH, the follicular output rate (FORT) may be helpful (Genro *et al.*, 2011; Gallot
72 *et al.*, 2012). The objectives of the present study, based on a 10-year period in practising
73 PGT-SR were: (i) to evaluate the possible relationship between the FORT and the female
74 chromosomal status, (ii) to evaluate if the number of metaphase II oocytes or biopsied
75 embryos could be predictive of the chance to reach an embryo transfer.

76

77 **MATERIALS AND METHODS**

78 The present study is a monocentric retrospective observational study carried out from January
79 2006 through April 2017. Consent from all patients-couples was obtained at the time of PGT-
80 SR attempt for the use of their medical data for research. Our database was approved by the
81 National Data Protection Authority (Commission Nationale de l'Informatique et des Libertés,
82 CNIL no. 1217921) on 21 February 2007. According to the "Jardé Law" (decree n° 2016-
83 1537, 16 November 2016), Institutional Review Board approval was not required for this
84 retrospective study.

85

86 **Subjects**

87 All women referred to our PGT centre (PGT Centre of Antoine Bécclère - Necker
88 hospitals), were tested for ovarian reserve with a blood sample for anti-Müllerian hormone
89 (AMH) level and Antral Follicles Count (AFC) determined by ultrasonography before

90 inclusion in our PGT-SR program. Only patients aged ≤ 38 years with AMH > 1 ng/mL or AFC
91 > 10 follicles were eligible for PGT-SR procedure.

92 As shown in Figure 1, three hundred and forty-three couples underwent PGT-SR to avoid
93 transmission of an unbalanced karyotype. Patients with a translocation involving sex
94 chromosomes have been excluded of the study (12 patients with 31 cycles). Three hundred
95 and thirty-one couples were finally included in this study in 760 COS cycles.

96 For the FORT calculation, we have selected the first cycle of a sub population of women (89
97 female translocation carriers and 145 partners of male translocation carriers) that met the
98 following inclusion criteria: (i) ovaries without morphological abnormalities (such as cysts,
99 endometriomas, etc.); (ii) regular menstrual cycles; (iii) total number of antral follicles
100 measuring 3–8 mm in diameter after pituitary desensitization (baseline) < 25 ; (iv) BMI
101 ranging from 17 to 29 kg/m².

102 For our second objective we studied the overall population constituted from 139 females
103 bearing a translocation (102 RCT and 37 ROBT) and 192 partners of male translocation
104 carriers (119 RCT and 73 ROBT).

105 **Standard Karyotyping and FISH**

106 Standard chromosomal analyses were performed on cultured peripheral lymphocytes
107 from the patient using standard procedures [G-banding with Trypsin using Giemsa (GTG); R-
108 banding after Heat denaturation and Giemsa (RHG)]. Fluorescence *in situ* hybridization
109 (FISH) analyses were performed on metaphase spreads of lymphocytes from the patient. In
110 accordance with breakpoints visualized on a standard Karyotype, specific DNA probes were
111 used, with the manufacturers' recommendations, to identify chromosome segments involved
112 in translocation.

113 **Controlled ovarian hyperstimulation protocols**

114 Controlled ovarian hyperstimulation (COH) was achieved using long agonist or
115 antagonist protocol. For long agonist protocol, patients received a single dose of GnRH
116 agonist, triptorelin (3mg, I.M, Decapeptyl®, Ipsen, France) on the first or 20th day of
117 menstrual cycle. Three weeks later, pituitary desensitization was confirmed by the detection
118 of low serum level of progesterone (P4), estradiol (E2), and LH; and follicle size <10mm.
119 Thereafter, recombinant FSH (recFSH) (Gonal-F®, Merck-Serono; Puregon ®, MSD, France)
120 was initiated for at least 6 days. Patients undergoing GnRH antagonist protocols received pre-
121 treatment with oral contraceptive pill or estradiol valerate (provames ®). Then, recFSH was
122 administered from day 3 for at least 5 days at initial dosage and 0,25 mg GnRH antagonist
123 (Ganirelix, Orgalutran ®, MSD France or Cetorelix, Cetrotide ®, Merck-Serono, France)
124 was systematically started on day 6. From the sixth day of recFSH therapy onwards, daily
125 FSH doses were adjusted according to E2 levels and/or the number of growing follicles.
126 During the last days of COH, patients had daily visits at our institution for ultrasonographic
127 and hormonal examinations to define the proper timing for hCG administration.
128 Administration of hCG to trigger ovulation was performed as soon as ≥ 4 pre-ovulatory
129 follicles (16-22mm in diameter) were observed and E2 levels per pre-ovulatory follicle were >
130 200 pg/mL. Oocytes were retrieved 36 hours after hCG administration by transvaginal
131 ultrasound-guided aspiration.

132

133 **Gametes preparation, Embryo culture, biopsy procedure and genetic analysis**

134 Retrieved Cumulus Oocyte Complexes (COCs) were preserved in 3 mL of IVF culture
135 medium (Origio, Lyon, France) until being denudated. Liquefied ejaculate was drop into a

136 discontinuous Pure Sperm (Nidacom, JCD, France) preparation (45% and 90%) and the sperm
137 pellet was washed in Ferticult medium (FertiPro NV, JCD, France) by centrifugation at 600 g
138 for 10 min. All matured oocytes obtained were inseminated by ICSI. Fertilization was
139 checked 18h after sperm injection and zygotes were cultured in micro-drops of 40 μ L of
140 SAGE 1-Step™ (Origio, Lyon, France) at 37°C under an ambient atmosphere enriched to 5%
141 CO₂.

142 Embryo biopsy was scheduled on day-3 using laser procedure (Fertilase; MTGMedical
143 Technology Vertriebs-GmbH, Altdorf, Germany). Two blastomeres were removed from
144 embryo with a minimum of 6 cells and < 30% fragmentation and spread on slides (Superfrost
145 Plus) according to Coonen *et al.* (1994). FISH was used with fluorescent DNA probes
146 specifically designed for the studied translocation. The slides were analysed by two operators
147 and the diagnosis was based on concordant results from two mononucleate cells. Only
148 blastomeres showing two signals per probe were defined as normal or balanced.
149 One or two balanced embryos were transferred at blastocyst stage and supernumerary
150 blastocysts were cryopreserved on day 5 or day 6 according embryo development.

151 **Embryo transfer and luteal phase support**

152
153 Embryo transfers were performed under ultrasound guidance (Voluson 730 Expert;
154 General Electric Medical Systems, Paris, France) using a classic Frydman catheter ® (CCD
155 Laboratories, Paris, France).

156
157 For fresh embryo transfers, luteal phase was supported with natural micronized
158 progesterone 600mg/day and oral oestrogen supplementation (4mg/day), starting the evening
159 of embryo transfer (ET) (Utrogestan® 200mg, Provames® 2mg, France) until the pregnancy
160 test. Treatment was continued up to 8 weeks of amenorrhea in case of pregnancy.

161 For frozen embryo transfers, endometrium was prepared with a programmed cycle
162 regimen using oestrogen priming at a dose of 4 mg daily (Provames® 2mg, France) on day 1-
163 3 of menstrual cycle. Progesterone treatment (micronized progesterone vaginally Progestan®
164 800mg, Besins, France) was added when endometrial thickness reached 7 mm or more.
165 Clinical pregnancy was defined as the presence of gestational sac observed at ultrasound
166 scans around 7 weeks of amenorrhea.

167

168 **Follicular Output Rate calculation**

169 The FORT was used to assess antral follicle responsiveness to recFSH independently
170 of the follicular cohort size before treatment, as previously described (Genro *et al.*, 2011).
171 Ovarian ultrasound scans were performed using a 5.0-9.0 MHz multi-frequency transvaginal
172 probe (Voluson 730 Expert®, General Electric Medical Systems, and Paris, France). The
173 objective of ultrasound examination was to evaluate the number and sizes of small antral
174 follicles. All follicles measuring 3 to 22 mm in mean diameter (mean of two orthogonal
175 diameters) in each ovary were counted. Only antral follicles < 8 mm were considered for
176 antral follicle count (AFC) at baseline (d0) and follicles between 16 and 22 mm on dhCG
177 were considered for pre-ovulatory follicles count (PFC). To optimize the reliability of ovarian
178 follicular assessment, the ultrasound scanner was equipped with a tissue harmonic imaging
179 system, which allowed improved image resolution and adequate recognition of follicular
180 borders. The FORT was calculated by the ratio between the PFC on dhCG x 100/AFC at d0.
181 The choice of considering only 16-22 mm follicles for the calculation of FORT was validated
182 in previous investigation (Genro *et al.*, 2011; Gallot *et al.*, 2012).

183

184

185 **Statistical analysis**

186 Statistical analyses were performed using Graph Pad Prism (ver. 5.02, Graphpad SoftwazaInc,
187 San Diego, California, USA).

188 The measure of central tendency used was the mean and the measure of variability was the
189 standard error (SE). Differences between two groups were evaluated with Student's t-test or
190 Mann-Whitney-Wilcoxon, when appropriate. A P value <0.05 was considered statistically
191 significant.

192 By the use of a univariate linear regression, we confirmed that the number of metaphase II
193 oocytes and the number of biopsied embryos were two factors significantly correlated with
194 the number of transferred embryos ($P<0.001$ and $P<0.0001$; respectively). We thus performed
195 ROC curves to determine a cut-off that would maximize the chance to reach an embryo
196 transfer according to the number of metaphase II oocytes and the number of biopsied
197 embryos. ROC curves were performed to determine a cut-off that would maximize the chance
198 to reach an embryo transfer according to the number of metaphase II oocytes and the number
199 of biopsied embryos. The area under the curve (AUC) was numerically calculated with the
200 use of trapezoids, and its 95% confidence interval (CI) was calculated based on the Wilcoxon
201 statistic (Hanley and McNeil, 1982). As usual, a calculated AUC equal to 0.5 was considered
202 as not informative and between 0.5 and 0.7 as weakly informative. When it was comprised
203 between 0.7 and 0.9, AUC was considered as moderately informative and between 0.9 and 1
204 as accurately informative. When AUC was > 0.7 an optimal cut off level was determined with
205 the corresponding sensitivity and specificity.

206

207 RESULTS

208 Response to exogenous FSH assessed by FORT

209 Out of 139 female translocation carriers and 192 partners of male translocation carriers, 89
210 (25 ROBT and 64 RCT) and 145 met the inclusion criteria retained for the FORT calculation.

211 Population and COS characteristics in both groups are summarized in Table 1. As females
212 bearing a Robertsonian or reciprocal translocation were similar, they were combined together.

213 As shown, the two female groups were comparable in terms of women's ages, BMIs, serum

214 AMH, AFC at baseline and total recFSH dose used for COS, duration of recFSH therapy and

215 serum E2 levels on dhCG. On the contrary, PFC values and FORT were significantly

216 decreased in female translocation carriers suggesting diminished antral follicle sensitivity to

217 FSH. As shown in figure 2, we sorted the population of female carriers in five FORT

218 categories according to whether FORT values were under the 20th percentile (<27.0%),

219 between the 20th and the 40th percentile [27.0%-35.3%], between the 40th and the 60th

220 percentile [35.4%-52.6%], between the 60th and the 80th percentile [52.7%-70.3%] and

221 above the 80th percentile [70.4%-100%]. Then, we compared the proportion of female

222 carriers and partners of male carriers in each defined FORT category. A significantly higher

223 proportion of women carrying balanced translocations had a FORT value under 35.3%

224 compared to women whose partner had a balanced translocation. Furthermore, a significantly

225 lower proportion of women carrying balanced translocations had a FORT value comprised

226 between 52.3% and 70.6% compared to partners of male translocation carriers.

227 The distribution of chromosomes involved in translocations in the female carriers for which

228 FORT value was under the 20th percentile (<27%) is summarized in the Table 2. Most of

229 them carried a reciprocal translocation (12 out of 15). Although, relatively short or short

230 metacentric (chromosomes 16-18) and submetacentric (chromosomes 19-20) were involved in
231 6 translocations, no redundant breakpoint region was associated with a very low FORT.

232

233 **PGT-SR outcomes between female and male according the type of translocation**

234 Table 3 summarizes PGT-SR outcomes between females and males according the type of
235 translocation. The number of retrieved oocytes, metaphase II oocytes, obtained and biopsied
236 embryos were similar between females and males regardless the type of translocation.
237 Conversely, whether in the case of reciprocal or Robertsonian translocation the number of
238 balanced embryos and transferred embryos were significantly lower in female carriers.
239 However and interestingly, the clinical pregnancy and live birth rates per oocyte retrieval
240 (OR) and ET as such as the cumulative live birth rates per OR and ET were similar between
241 females and males in each type of translocation.

242

243 **Parameters influencing the chance to reach an embryo transfer**

244 Figure 3 and Table 4 present AUCs for the numbers of metaphase II oocytes or biopsied
245 embryos as predictor of obtaining an ET in female translocation carriers and partners of male
246 translocation carriers with the yielded estimated sensitivity and specificity for the used cut-
247 off. The AUCs calculated for the number of metaphase II oocytes in both groups were < 0.7
248 while for the number of biopsied embryos, AUCs were > 0.7 . A cut-off of 6.5 biopsied
249 embryos in female translocation carriers yielded sensitivity estimates of 87% and specificity
250 of 37%. For partners of male translocation carriers, this cut-off was 5.5 led to sensitivity
251 estimates of 82% and specificity of 45%.

252

253 DISCUSSION

254 To the best of our knowledge, this is the largest study investigating ovarian
255 responsiveness to COH as also PGT-SR outcomes in female translocation carriers compared
256 to partners of male translocation carriers. Its major advantage is the large number of PGT-SR
257 cycles analysed. Our investigation presents also some limitations. Some confounding factors
258 such as smoking, drinking and environmental toxic exposure are missing. We can probably
259 expect that due to the relative large size of the two groups some factors will be levelled out
260 but we cannot exclude this may be a potential bias. Due to French legal issue, blastomere
261 chromosomal analysis was performed by the use of fluorescent in situ hybridization with
262 DNA probes targeting the sole chromosomes involved in the translocation. Therefore, inter
263 chromosomal effect could not be assessed (Gianaroli et al., 2002; Pujol et al., 2006; Xie et al.,
264 2018).

265 One of the central findings of the present investigation is the diminished capacity of
266 small antral follicles to respond to exogenous FSH, assessed by the FORT index, in female
267 translocation carriers. The influence of chromosomal abnormalities on ovarian response to
268 stimulation was not yet well established. So far, only two contradictory studies evaluating
269 ovarian response in female translocation carriers have been published (Chen *et al.*, 2005;
270 Dechanet *et al.*, 2011). The first one compared 61 cycles in 46 women with balanced
271 translocations to 42 cycles in 32 women whose male partner had a balanced translocation. A
272 significantly higher proportion of women carrying balanced translocations responded very
273 poorly (E2 on the day of hCG <1,000 pg/mL) to ovarian stimulation compared to women
274 whose partner had a balanced translocation. Conversely, the second study reported that the
275 ovarian response to COS was not impaired by balanced translocation status, considering 79
276 cycles in 33 female translocation carriers and 116 cycles in 55 partners of male translocation

277 carriers. To better quantify follicle responsiveness to exogenous FSH, we used the FORT
278 known yet to be a qualitative indicator of ovarian follicular competence (Genro *et al.*, 2011)
279 in infertile patients. FORT has the advantage of being independent of the AFC before FSH
280 administration allowing a better understanding of the capacity of these follicles to respond to
281 ovarian stimulation. As FORT was found to be positively related to IVF-ET outcome in
282 normo-cycling women devoid of polycystic ovaries (Gallot *et al.*, 2012), we excluded women
283 endowed with AFC>25 from both studied groups. We found that female translocation carriers
284 had a significant lower FORT than partners of male translocation carriers. Furthermore, by
285 sorting both groups according FORT categories, we show that the proportion of female
286 translocation carriers with a low FORT value (<35.3%) was higher than in the partners of
287 male carriers. This suggests that in addition to cases of obvious ovarian insufficiency; female
288 translocation carriers may present a functional ovarian impairment. Although, no redundant
289 breakpoint region was evidenced in female translocation carriers with a very low FORT, 2
290 female carriers share the same reciprocal translocation [46,XX,t(6;20) and 46,XX,t(9;11)] as
291 already described in Chen *et al.* (2005). We hope that the present study will allow information
292 to be shared with other researchers. Indeed, if we could obtain several patients with this
293 phenotype and with the same break points, we could hypothesize that a gene of interest for
294 follicular function has been disrupted.

295 In line with Tur Kaspas *et al.*, (2012), we show that despite the mild functional ovarian
296 impairment in female translocation carriers once the patients had an ET, PGT-SR outcomes
297 were similar. Albeit, in the population of female translocation carriers with a FORT value
298 fewer than 27%, none pregnancy was obtained. Since aging greatly influences the number of
299 small antral follicles available before treatment, female aging could progressively be an
300 additional negative factor.

301 Our second finding was that the embryo-unbalanced rate was significantly higher in
302 female translocation carriers as compared to male translocation carriers. Several hypotheses
303 may be raised to explain this result. First, is the progressive removal of abnormal spermatozoa
304 during spermatogenesis, which reduces sperm counts but allows the selection of balanced
305 spermatozoa. The defect of spermatogenesis originated from a mechanical mechanism during
306 meiosis (reviewed by Van Assche et al., 1996). Meiotic arrest may occur by a gradual
307 association of the asynaptic region of trivalent or tetravalent with the transcriptional inactive
308 XY body, thus disturbing the meiotic sex chromosomes inactivation (Sciurano *et al.*, 2007).
309 Or only for the fact that asynaptic regions of trivalent or tetravalent might result in autosome
310 meiotic silencing (Naumova *et al.*, 2013).

311 Second, sperm preparation was performed by the use of a discontinuous gradient
312 centrifugation. This technic could improve the selection of spermatozoa with a greater nuclear
313 integrity (Centola *et al.*, 1998; Larson et al., 1999) and decrease the proportion of
314 chromosomally unbalanced spermatozoa used during ICSI procedure (Perrin *et al.*, 2011;
315 Rouen *et al.*, 2013). Third, the higher unbalanced embryo rate observed in female compared
316 to male carriers may be explained by meiotic segregation pattern. Indeed, some studies
317 reported that male meiosis was different from female with an increased incidence of 3:1 and
318 4:0 segregation in females carriers compared to males carriers (Mackie Ogilvie and Scriven,
319 2002; Tease *et al.*, 2002; Ko *et al.*, 2010).

320 Finally, we tried to determine the minimal number of metaphase II oocytes or embryos
321 biopsied to maximize the chance of achieving an embryo transfer. We analysed ROC curves
322 widely used to predict an outcome according to a specific indicator. The AUCs level for the
323 number of metaphase II oocytes was <0.7 in both groups suggesting that this indicator is
324 weakly informative for evaluating the chance to reach embryo transfer. Some confounding
325 factors such as sperm/egg genetic integrity, *in vitro* procedures and environment should be

326 more deeply analyzed to understand this result. Interestingly, the number of biopsied embryo
327 seems to be more informative. In consistency with the increased unbalanced embryos rate
328 observed in female translocation carriers, the cut-off value for biopsied embryos was higher in
329 this group. Despite a low specificity calculated with these cut-offs, the sensitivity was more
330 than 0.8 meaning that if we succeed to biopsy a sufficient number of embryos, the chance to
331 reach ET is fairly good. In practice this suggests that if the number of embryos suitable for
332 biopsy does not reach the calculated cut-off, an accumulation of vitrified embryos may be
333 recommended and should increase patient's chances to obtain normal/balanced embryo
334 (Chatziparasidou *et al.*, 2013; Van Landuyt *et al.*, 2013; Chi *et al.*, 2015). This also suggests
335 that we will need more oocytes in female translocation carriers than in partners of male
336 translocation carriers.

337 In conclusion, female translocation carriers may respond poorly to COH and present a
338 higher rate of unbalanced embryos, which means that a higher gonadotropins dose may be
339 used safely in an attempt to increase the number of biopsied embryos. At the same time, while
340 the ovarian response to COH may be decreased, the outcome of PGT-SR is not affected.

341 **ACKNOWLEDGEMENTS**

342 The authors thank the staff members at the Reproductive Biology and Clinical Units of the
343 Antoine Bécère Hospital (Clamart, France). We are grateful to couples-patients who consent
344 to the use of their medical data.

345 **Authors' roles**

346 N.A, A.M., L.H, C.S. and N.F. were involved in the study conception, design, assembly of
347 data, data analysis and interpretation and manuscript writing. S.R. was involved in the genetic
348 analysis of embryonic cells. MG was involved in reviewing the paper. N.F was also involved

349 in the revision and final approval of the manuscript. All authors have approved the final
350 version and submission of this article.

351 **FUNDING**

352 No external funding was either sought or obtained for this study.

353 **CONFLICT OF INTEREST**

354 The authors have nothing to disclose.

355

356

357 **REFERENCES**

- 358 Centola, G.M., Herko, R., Andolina, E., Weisensel, S., 1998. Comparison of sperm separation
359 methods: effect on recovery, motility, motion parameters, and hyperactivation. *Fertil. Steril.* 70, 1173–
360 1175.
- 361 Chatziparasidou, A., Nijs, M., Moisdou, M., Chara, O., Ioakeimidou, C., Pappas, C., Christoforidis,
362 N., 2013. Accumulation of oocytes and/or embryos by vitrification: a new strategy for managing poor
363 responder patients undergoing pre implantation diagnosis. *F1000Research.* 2, 240.
- 364 Chen, S.H., Escudero, T., Cekleniak, N.A., Sable, D.B., Garrisi, M.G., Munne, S., 2005. Patterns of
365 ovarian response to gonadotropin stimulation in female carriers of balanced translocation. *Fertil.*
366 *Steril.* 83, 1504–1509.
- 367 Chi, F., Luo, C., Yin, P., Hong, L., Ruan, J., Huang, M., Duan, T., Tong, G., 2015. Vitrification of day
368 3 cleavage-stage embryos yields better clinical outcome in comparison with vitrification of day 2
369 cleavage-stage embryos. *Zygote Camb. Engl.* 23, 169–176.
- 370 Coonen, E., Dumoulin, J.C., Ramaekers, F.C., Hopman, A.H., 1994. Optimal preparation of
371 preimplantation embryo interphase nuclei for analysis by fluorescence in-situ hybridization. *Hum.*
372 *Reprod. Oxf. Engl.* 9, 533–537.
- 373 Dechanet, C., Castelli, C., Reyftmann, L., Hamamah, S., Hedon, B., Dechaud, H., Anahory, T., 2011.
374 Do female translocations influence the ovarian response pattern to controlled ovarian stimulation in
375 preimplantation genetic diagnosis? *Hum. Reprod. Oxf. Engl.* 26, 1232–1240.
- 376 Gallot, V., Berwanger Da Silva, A.L., Genro, V., Grynberg, M., Frydman, N., Fanchin, R., 2012.
377 Antral follicle responsiveness to follicle-stimulating hormone administration assessed by the Follicular
378 Output RaTe (FORT) may predict in vitro fertilization-embryo transfer outcome. *Hum. Reprod. Oxf.*
379 *Engl.* 27, 1066–1072.
- 380 Genro, V.K., Grynberg, M., Scheffer, J.B., Roux, I., Frydman, R., Fanchin, R., 2011. Serum anti-
381 Müllerian hormone levels are negatively related to Follicular Output RaTe (FORT) in normo-cycling
382 women undergoing controlled ovarian hyperstimulation. *Hum. Reprod. Oxf. Engl.* 26, 671–677.
- 383 Hanley, J.A., McNeil, B.J., 1982. The meaning and use of the area under a receiver operating
384 characteristic (ROC) curve. *Radiology* 143, 29–36.
- 385 Harper, J.C., Wilton, L., Traeger-Synodinos, J., Goossens, V., Moutou, C., SenGupta, S.B., Pehlivan
386 Budak, T., Renwick, P., De Rycke, M., Geraedts, J.P.M., Harton, G., 2012. The ESHRE PGD
387 Consortium: 10 years of data collection. *Hum. Reprod. Update* 18, 234–247.
- 388 Jacobs, P.A., Melville, M., Ratcliffe, S., Keay, A.J., Syme, J., 1974. A cytogenetic survey of 11,680
389 newborn infants. *Ann. Hum. Genet.* 37, 359–376.
- 390 Kato, K., Aoyama, N., Kawasaki, N., Hayashi, H., Xiaohui, T., Abe, T., Kuroda, T., 2016.
391 Reproductive outcomes following preimplantation genetic diagnosis using fluorescence in situ
392 hybridization for 52 translocation carrier couples with a history of recurrent pregnancy loss. *J. Hum.*
393 *Genet.* 61, 687–692.

- 394 Ko, D.S., Cho, J.W., Park, S.Y., Kim, J.Y., Koong, M.K., Song, I.O., Kang, I.S., Lim, C.K., 2010.
395 Clinical outcomes of preimplantation genetic diagnosis (PGD) and analysis of meiotic segregation
396 modes in reciprocal translocation carriers. *Am. J. Med. Genet.* 152A, 1428–1433.
- 397 Mackie Ogilvie, C., Scriven, P.N., 2002. Meiotic outcomes in reciprocal translocation carriers
398 ascertained in 3-day human embryos. *Eur. J. Hum. Genet.* 10, 801–806.
- 399 Mayeur, A., Ahdad, N., Hesters, L., Brisset, S., Romana, S., Tosca, L., Tachdjian, G., Frydman, N.,
400 2019. Chromosomal translocations and semen quality: A study on 144 male translocation carriers.
401 *Reprod. Biomed. Online* 38, 46–55.
- 402 Moutou, C., Goossens, V., Coonen, E., De Rycke, M., Kokkali, G., Renwick, P., SenGupta, S.B.,
403 Vesela, K., Traeger-Synodinos, J., 2014. ESHRE PGD Consortium data collection XII: cycles from
404 January to December 2009 with pregnancy follow-up to October 2010. *Hum. Reprod. Oxf. Engl.* 29,
405 880–903.
- 406 Munné, S., Morrison, L., Fung, J., Márquez, C., Weier, U., Bahçe, M., Sable, D., Grundfeld, L.,
407 Schoolcraft, B., Scott, R., Cohen, J., 1998. Spontaneous abortions are reduced after preconception
408 diagnosis of translocations. *J. Assist. Reprod. Genet.* 15, 290–296.
- 409 Munné, S., Sandalinas, M., Escudero, T., Fung, J., Gianaroli, L., Cohen, J., 2000. Outcome of
410 preimplantation genetic diagnosis of translocations. *Fertil. Steril.* 73, 1209–1218.
- 411 Naumova, A.K., Fayer, S., Leung, J., Boateng, K.A., Camerini-Otero, R.D., Taketo, T., 2013.
412 Dynamics of response to asynapsis and meiotic silencing in spermatocytes from Robertsonian
413 translocation carriers. *PloS One* 8, e75970.
- 414 Otani, T., Roche, M., Mizuike, M., Colls, P., Escudero, T., Munné, S., 2006. Preimplantation genetic
415 diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of
416 recurrent miscarriage and unsuccessful pregnancies. *Reprod. Biomed. Online* 13, 869–874.
- 417 Penna Videau, S., Araujo, H., Ballesta, F., Ballescá, J.L., Vanrell, J.A., 2001. Chromosomal
418 abnormalities and polymorphisms in infertile men. *Arch. Androl.* 46, 205–210.
- 419 Perrin, A., Basinko, A., Douet-Guilbert, N., Gueganic, N., Le Bris, M-J., Amice, V., De Braekeleer,
420 M., Morel, F., 2011. Aneuploidy and DNA fragmentation in sperm of carriers of a constitutional
421 chromosomal abnormality. *Cytogenet. Genome Res.* 133, 100–106.
- 422 Rao, K.L., Babu, K.A., Kanakavalli, M.K., Padmalatha, V.V., Deenadayal, M., Singh, L., 2005.
423 Prevalence of chromosome defects in azoospermic and oligoastheno-teratozoospermic South Indian
424 infertile men attending an infertility clinic. *Reprod. Biomed. Online* 10, 467–472.
- 425 Rouen, A., Balet, R., Dorna, M., Hyon, C., Pollet-Villard, X., Chantot-Bastarud, S., Joyé, N., Portnoï,
426 M-F., Cassuto, N.G., Siffroi, J-P., 2013. Discontinuous gradient centrifugation (DGC) decreases the
427 proportion of chromosomally unbalanced spermatozoa in chromosomal rearrangement carriers. *Hum.*
428 *Reprod. Oxf. Engl.* 28, 2003–2009.
- 429 Sasagawa, I., Nakada, T., Adachi, Y., Kato, T., Sawamura, T., Ishigooka, M., Hashimoto, T., 1993. Y-
430 autosome translocation associated with azoospermia. *Scand. J. Urol. Nephrol.* 27, 285–288.

- 431 Schlessinger, D., Herrera, L., Crisponi, L., Mumm, S., Percesepe, A., Pellegrini, M., Pilia, G.,
432 Forabosco, A., 2002. Genes and translocations involved in POF. *Am. J. Med. Genet.* 111, 328–333.
- 433 Scieurano, R., Rahn, M., Rey-Valzacchi, G., Solari, A.J., 2007. The asynaptic chromatin in
434 spermatocytes of translocation carriers contains the histone variant γ -H2AX and associates with the
435 XY body. *Hum. Reprod.* 22, 142–150.
- 436 Scriven, P.N., Flinter, F.A., Khalaf, Y., Lashwood, A., Mackie Ogilvie, C., 2013. Benefits and
437 drawbacks of preimplantation genetic diagnosis (PGD) for reciprocal translocations: lessons from a
438 prospective cohort study. *Eur. J. Hum. Genet.* 21, 1035–1041.
- 439 Sermon, K., Van Steirteghem, A., Liebaers, I., 2004. Preimplantation genetic diagnosis. *Lancet Lond.*
440 *Engl.* 363, 1633–1641.
- 441 Simopoulou, M., Harper, J.C., Fragouli, E., Mantzouratou, A., Speyer, B.E., Serhal, P., Ranieri, D.M.,
442 Doshi, A., Henderson, J., Rodeck, C.H., Delhanty, J.D.A., 2003. Preimplantation genetic diagnosis of
443 chromosome abnormalities: implications from the outcome for couples with chromosomal
444 rearrangements. *Prenat. Diagn.* 23, 652–662.
- 445 Tease, C., Hartshorne, G.M., Hultén, M.A., 2002. Patterns of meiotic recombination in human fetal
446 oocytes. *Am. J. Hum. Genet.* 70, 1469–1479.
- 447 Therman, E., Susman, B., 1990. The similarity of phenotypic effects caused by Xp and Xq deletions in
448 the human female: a hypothesis. *Hum. Genet.* 85, 175–183.
- 449 Tur-Kaspa, I., 2012. Clinical management of in vitro fertilization with preimplantation genetic
450 diagnosis. *Semin. Reprod. Med.* 30, 309–322.
- 451 Van Assche, E., Bonduelle, M., Tournaye, H., Joris, H., Verheyen, G., Devroey, P., Van Steirteghem
452 A., Liebaers, I., 1996. Cytogenetics of infertile men. *Hum. Reprod.* 11, 1–26.
- 453 Van Landuyt, L., Van de Velde, H., De Vos, A., Haentjens, P., Blockeel, C., Tournaye, H., Verheyen,
454 G., 2013. Influence of cell loss after vitrification or slow-freezing on further in vitro development and
455 implantation of human Day 3 embryos. *Hum. Reprod. Oxf. Engl.* 28, 2943–2949.
- 456
- 457

458 **Legends**

459 Figure 1: Study design

460 Three hundred and forty-three couples were referred to our PGT center for either male or
461 female chromosomal translocation. For the FORT calculation, we select the first cycle of a
462 sub population of women that met inclusion criteria described in materials and methods. To
463 answer to our second aim, we studied the PGT-SR outcomes of 456 OR from reciprocal
464 translocations (102 females and 119 males) and of 225 OR from Robertsonian translocations
465 (37 females and 73 males).

466 OR: oocyte retrieval. RCT: Reciprocal translocation. ROBT: Robertsonian translocation.

467

468 Figure 2: Proportion of female carriers and partners of male carriers in each FORT category

469 We sort the population of female carriers in five FORT categories according to whether
470 FORT values were under the 20th percentile (<27.0%), between the 20th and the 40th
471 percentile [27.0%-35.3%], between the 40th and the 60th percentile [35.4%-52.6%], between
472 the 60th and the 80th percentile [52.7%-70.3%] and above the 80th percentile [70.4%-100%].
473 Hence we compare the proportion of female carriers and partners of male carriers in each
474 defined FORT value (**P<0.01).

475

476 Figure 3: Receiver operating characteristic curves for the relationship between the number of
477 biopsied embryos and the outcome of obtaining a PGT-ET in female translocation carriers
478 (dashed line) and partners of male translocation carriers (solid line).

Figure 1

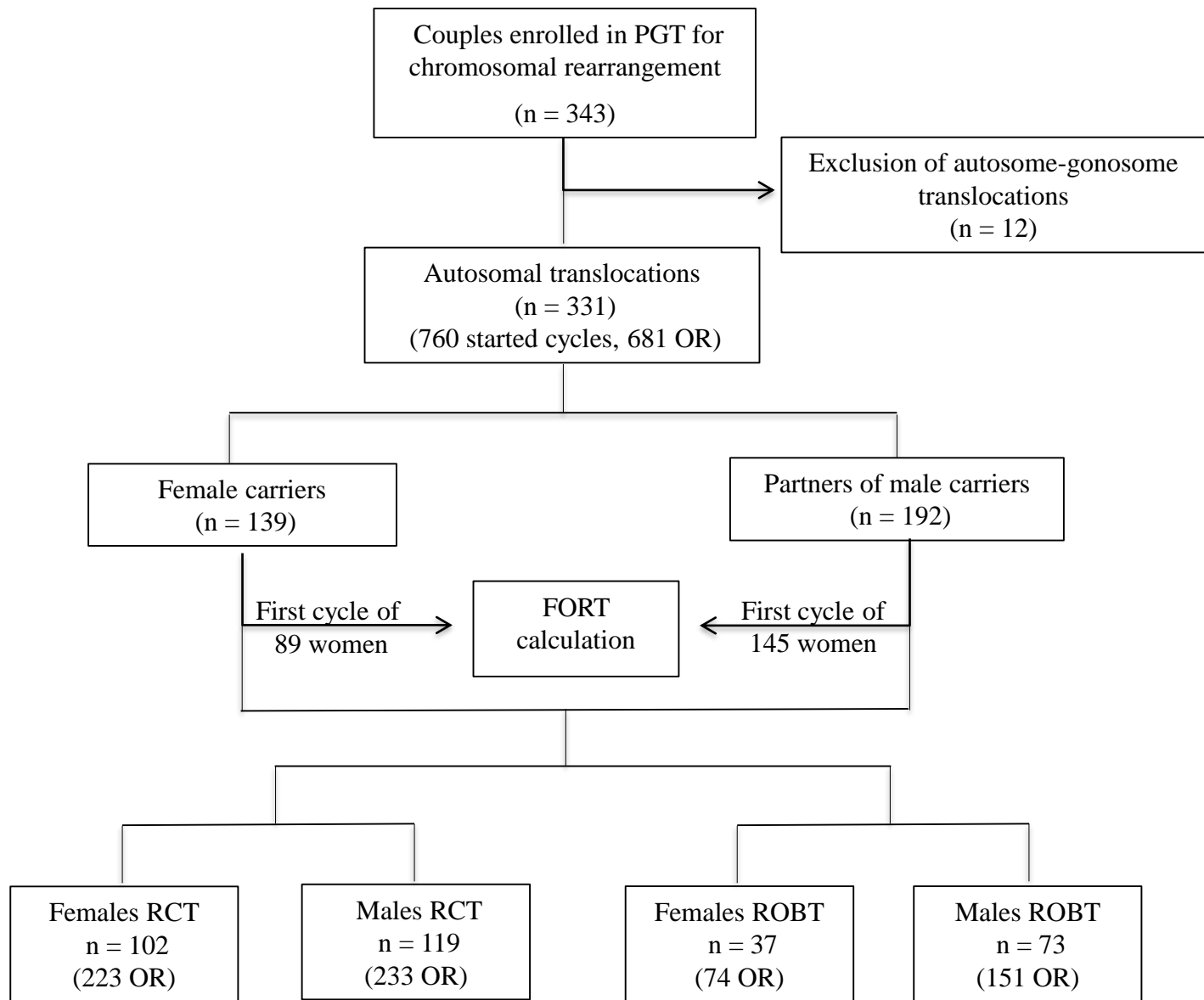


Figure 2

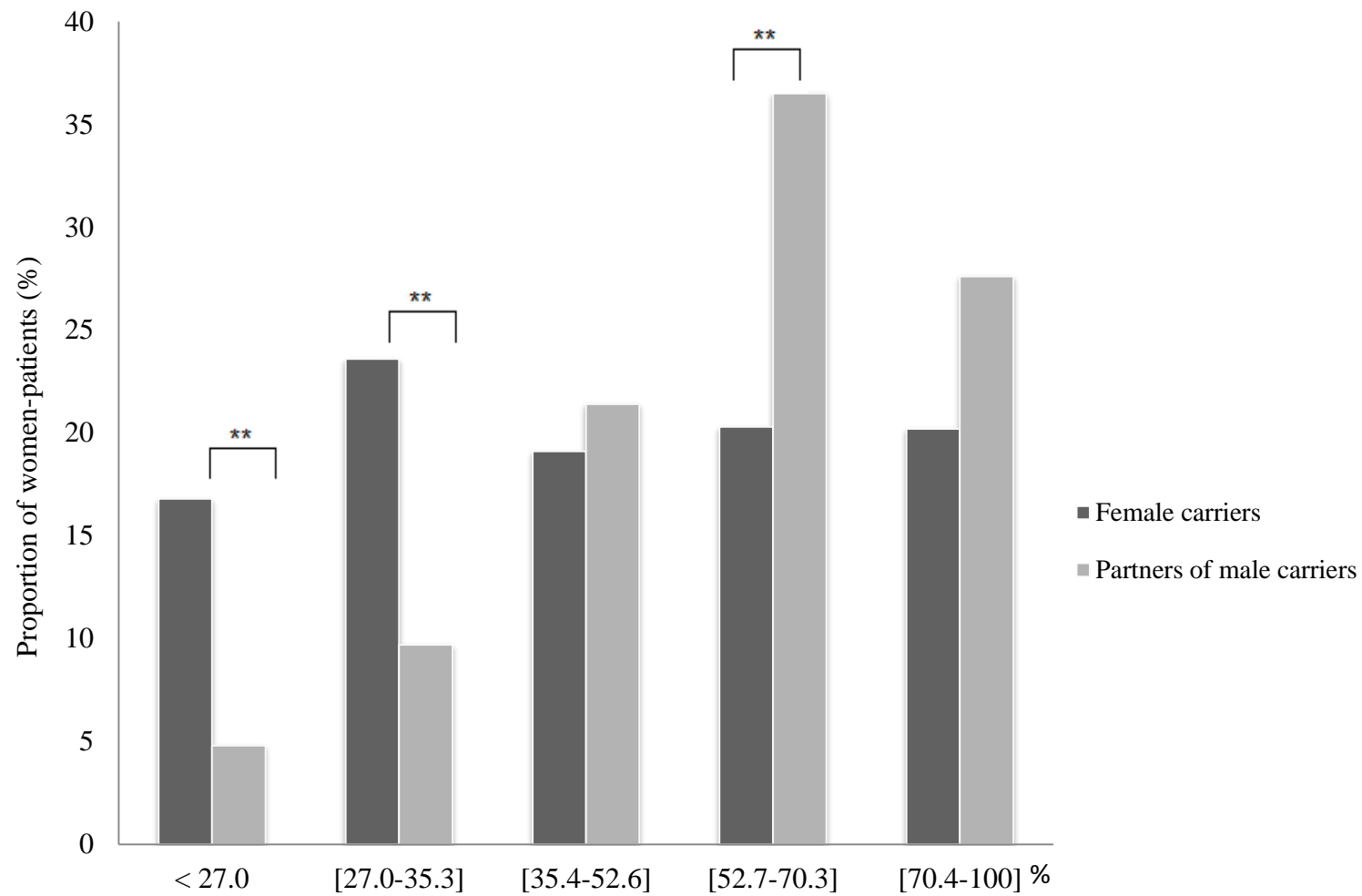


Figure 3

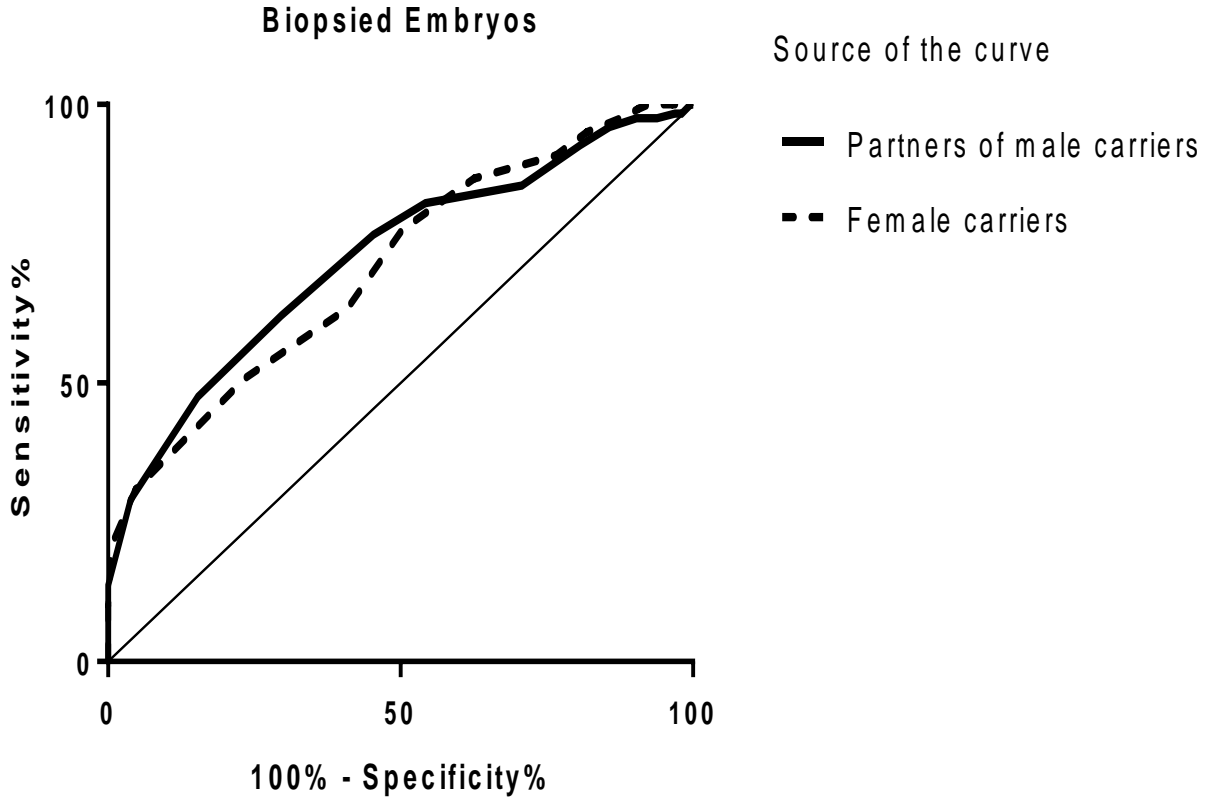


Table 1: Female characteristics and COS outcomes in female carriers or partners of male carriers.

	Females carriers (n=89)	Male carriers (n=145)	<i>P</i> value
Age (years) ^a	32.6±0.4	33.3±0.3	NS
BMI (kg/m ²) ^a	23.5±0.6	23.5±0.4	NS
AMH (ng/mL) ^a	3.1±0.2	3.7±0.2	NS
AFC (3-8 mm) ^a	17.5±0.5	15.2±0.4	NS
Agonist protocol (%)	50 (56.2)	68 (46.9)	NS
Antagonist protocol (%)	39 (43.8)	77 (53.1)	NS
Duration of COS (days) ^a	10.9±0.2	10.9±0.1	NS
Total dose of FSH (IU) ^a	2883.0±110.2	2754.0±68.3	NS
Estradiol level on dhCG (pg/mL) ^a	2501±102.1	2956.0±297.0	NS
PFC (16-22 mm) ^a	9.9±0.4	11.8±0.3	<0.0001
FORT (%)	48.1±2.6	59.4±1.	<0.001

^aData are expressed as mean±SEM. COS: Controlled Ovarian Stimulation; BMI: Body Mass Index; AMH: Antimullerian Hormone, AFC: Antral Follicle Count at baseline (just before the start of FSH treatment); PFC: Preovulatory Follicles Count on dhCG, FORT: Follicular Output RaTe.

Table 2: Listing of karyotypes for female translocation carriers according FORT under the 20th percentile (<27.0 %)

Translocation	AFC	FORT (%)	Retrieved oocytes	Metaphase II oocytes	Biopsied embryos	Outcome
46,XX,t(17;18)(p13;p31)	23	13.0	8	5	2	No ET
46,XX,t(6;20)(p21;q13)	21	14.2	17	13	3	No PR
46,XX,t(1;17)(q42;q25)	21	14.2	21	13	4	No ET
46,XX,t(8;16)(q11;q12)	13	15.3	2	1	1	No ET
46,XX,t(6;10)(q15;p10)	19	15.7	15	10	6	No PR
46,XX,t(3;1)(q21;q32)	24	16.6	11	8	5	No ET
45,XX,der(13;14)(q10;q10)	22	18.1	4	3	1	No ET
46,XX,t(2;16)(q14;q24)	20	20.0	7	7	0	No ET
45,XX,der(13;14)(q10;q10)	24	20.8	7	7	4	No PR
45,XX,der(14;21)(q10;q10)	9	22.2	8	7	2	No PR
46,XX,t(4;21)(q32;q21)	22	22.7	9	8	3	No ET
46,XX,t(11;22)(q23;q11)	21	23.8	2	2	1	No ET
46,XX,t(6;20;13)(p12;p12;q33)	24	25.0	6	6	5	No ET
46,XX,t(4;8)(p16;p23)	23	26.0	16	10	4	No ET
46,XX,t(9;11)(q22;q22)	23	26.1	12	11	7	No ET
<i>Mean±SEM</i>	<i>20.6±1.1</i>	<i>19.6±1.2</i>	<i>9.7±1.4</i>	<i>7.4±0.9</i>	<i>3.2±0.5</i>	<i>NA</i>

ET: embryo transfer; PR: pregnancy

Table 3: PGT-SR outcomes according the type of translocation in female or male carriers

	Female carriers RCT n=102	Male carriers RCT n=119	<i>P</i>	Female carriers ROBT n=37	Male carriers ROBT n=73	<i>P</i>
Cycles (n)	254	257	/	82	167	/
OR (n)	223	233	/	74	151	/
Oocytes retrieved^a	11.5±0.3	11.9±0.3	NS	12.2±0.6	12.5±0.4	NS
Metaphase II oocytes^a	9.6±0.3	9.8±0.3	NS	9.9±0.5	10.2±0.3	NS
Embryos obtained (n)	1481	1492	/	496	957	/
Good embryos obtained (%)	354 (23.9)	313 (21.0)	NS	96 (19.3)	193 (20.2)	NS
Embryos biopsied^a	5.2±0.2	4.7±0.2	NS	4.8±0.3	4.7±0.2	NS
Balanced embryos^a	1.0±0.1	1.2±0.1	<0.05	1.7±0.2	2.1±0.1	<0.05
Unbalanced embryo rate (%)	79.8	71.9	<0.01	63.2	51.8	<0.001
Embryo transfers (% per OR)	122 (54.7)	134 (57.5)	NS	54 (73.0)	126 (83.4)	NS
Embryos transferred^a	0.78±0.05	1.02±0.05	<0.05	1.03±0.11	1.36±0.06	<0.05
Embryos cryopreserved	9	23		13	12	
Pregnancies (n)	43	34	/	14	41	/
Implantation rate (%)	26.3	18.8	NS	17.7	19.9	NS
Clinical pregnancy per OR (%)	19.3	14.6	NS	18.9	27.1	NS
Clinical pregnancy per ET (%)	35.2	25.4	NS	25.9	32.5	NS
Live Births (n)	37	27	NS	13	30	NS
Live Birth Rate per OR (%)	16.6	12.0	NS	17.6	19.9	NS
Live Birth Rate per ET (%)	30.3	20.1	NS	24.1	23.8	NS
Frozen embryo transfer (n)	6	16		10	7	
Frozen live births (n)	3	5		1	0	
Cumulative LB rate per OR (%)	17.9	13.7	NS	18.9	19.9	NS
Cumulative LB rate per ET (%)	31.0	21.3	NS	21.9	22.5	NS

^a Data are expressed as mean±SEM. Unbalanced embryo rate (number of unbalanced embryos/number of diagnosed embryos) x 100. **OR:** oocyte retrieval; **ET:** embryo transfer; **LB:** live birth

Table 4: Receiver Operating Curves results

		AUC	CI	Sensitivity (%)	Specificity (%)	Cutoff level
Number of biopsied embryos	Males	0.724	0.668-0.708	82	45	5.5
	Females	0.707	0.646-0.767	87	37	6.5
Number of metaphase II oocytes	Males	0.595	0.535-0.655	NA	NA	NA
	Females	0.627	0.563-0.691	NA	NA	NA

AUC: Area Under the Curve; **CI:** 95% Confidence Interval; **NA:** Not Applicable