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3 **PREIMPLANTATION GENETIC DIAGNOSIS OF GENDER SELECTION IN THE**
4 **UNITED STATES**

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31 ABSTRACT

32 Preimplantation genetic diagnosis (PGD) of gender selection for non medical reasons has been
33 considered an unethical procedure by several authors and agencies in the Western society on the
34 basis of disrupting the sex ratio, being discriminatory againsts women and disposal of normal
35 embryos of the non desired gender. In this study, the analysis of a large series of PGD
36 procedures for gender selection from a wide geographical area in the United States, shows that in
37 general there is no deviation in preference towards any specific gender except for a preference of
38 males in some ethnic populations of Chinese, Indian and Middle Eastern origin that represent a
39 small percentage of the US population. In cases where only normal embryos of the non-desired
40 gender are available, 45.5% of the couples elect to cancel the transfer, while 54.5% of them are
41 open to have transferred embryos of the non-desired gender, this fact being strongly linked to
42 cultural and ethnical background of the parents. In addition this study adds some evidence to the
43 proposition that in couples with previous children of a given gender there is no biological
44 predisposition towards producing embryos of that same gender. Based on these facts, it seems
45 that objections to gender selection formulated by ethics committees and scientific societies are
46 not well-founded.

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50 SUMMARY

51 Preimplantation genetic diagnosis of gender selection for non medical reason is considered
52 unethical by scientific societies and ethics committees claiming that the sex ratio of the
53 population can be disrupted, that it is discriminatory against women and regarding the fate of

54 normal embryos of the undesired gender. Some studies show there is a bias towards males in the
55 general population suggesting that may be originated by some biological processes. This study of
56 a large series of PGD cases for gender selection shows that there is no deviation towards male or
57 females in the gender preferences of patients from an ethnically diverse western society like the
58 United States, with the exception of some ethnic populations of Chinese, Indian and Middle
59 Eastern origin where a bias towards males has been observed. Since these populations represent
60 an extremely small percentage of the population in the United States, the general sex ratio
61 cannot be disrupted. This study also shows that in couples with previous children of a given
62 gender there is no predisposition to conceive embryos of that gender and that in general there is
63 no bias towards males in the gender of embryos, suggesting that the bias towards males in the
64 general population is not reflected in the early stages of development. Based on these facts, it
65 seems that objections to gender selection formulated by ethics committees and scientific societies
66 are not well-founded.

67

68 **KEY WORDS**

69 Preimplantation Genetic Diagnosis, gender selection, FISH, ethics committees

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73 **INTRODUCTION**

74 Preimplantation genetic diagnosis (PGD) analysis is being used to improve ART outcome
75 (Gianaroli *et al.*, 1999; Munné *et al.*, 1999; Munné *et al.*, 2003), for couples with idiopathic
76 recurrent pregnancy loss (Munné *et al.*, 2005; Garrisi *et al.*, 2008), carriers of structural

77 chromosome abnormalities (Otani *et al.*, 2006; Escudero *et al.*, 2008), and gene defects (Harper
78 *et al.*, 2002; Fiorentino *et al.*, 2003).

79 Since aneuploidy of sex chromosomes in human embryos can lead to offspring with Turner's
80 Syndrome, Klinefelter's Syndrome, and other abnormalities compatible with post-natal viability,
81 probes for chromosomes X and Y have been included in most PGD protocols using FISH, with
82 occasional exceptions for indications of structural chromosome abnormalities. Currently, most
83 X-linked genetic defects are diagnosed by PGD using molecular methods that allow specific
84 identification of the mutation (Amor and Cameron, 2008). However, in the recent past,
85 karyotype-based gender determination was used to prevent X-linked disorders like hemophilia.
86 For those syndromes with no clear genetic association and an increased male incidence like
87 autism, FISH is still used for gender determination.

88 While sex selection of embryos for medical indications is well accepted (Ethics Committee of
89 the American Society for Reproductive Medicine, ASRM, 2001), controversy arises regarding
90 sex selection for family balance or gender preference purposes, which many people believe to be
91 unethical. Two main reasons are cited: one is the risk of biasing sex ratios in the population at-
92 large and/or gender discrimination, and the other that chromosomally normal embryos are being
93 discarded. Considering gender bias, many believe gender selection is discriminatory and sexist;
94 they argue that it would lead to a severe distortion of the sex ratio based on the assumption that a
95 large proportion of couples would select male offspring (American College of Obstetricians and
96 Gynecologists, ACOG, 2007; Robertson 2002; Human Fertilization and Embryology Authority,
97 2003; International Federation of Gynecology and Obstetrics, FIGO, 2006).

98 However, several studies have demonstrated that although in some countries like China and India
99 the sex ratio can be distorted in favor of males, in Western societies there is no evidence of such

100 effect and that gender preferences are usually the result of a desire to have a family with children
101 of both genders (family balancing) (Dahl *et al.*, 2003; Heyd 2003; Jain *et al.*, 2005; Dahl *et al.*,
102 2006a; Dahl *et al.*, 2006b; Fejes *et al.*, 2006). All but one of these studies have been carried out
103 through opinion surveys, questionnaires, or analysis of newborn data. The one exception is based
104 on results from a series of 92 PGD assays for gender selection in the New York area (Gleicher
105 and Barad, 2007). Overall, the data suggest a strong sex selection towards males remains
106 confined in that area to some minority ethnic groups of Chinese, Middle Eastern/Muslim and
107 Indian origin and that no bias or a slight preference for females is observed among couples of
108 Western origin.

109 The second controversial issue regarding gender determination is the disposal of chromosomally
110 normal embryos because they are the unwanted gender. As a result of this concern, pre-
111 fertilization techniques like sperm sorting are favored over post-fertilization techniques like PGD
112 (Robertson 2002; ACOG 2007). No studies have been done to determine the proportion of
113 couples seeking PGD for gender determination who are willing (non-absolute preference) or not
114 willing (absolute preference) to transfer embryos from the unwanted gender when these are the
115 only ones available .

116 The policy of our laboratory has been that we offer PGD for all indications but not specifically
117 for gender determination. However, since FISH procedures usually involve the analysis of X and
118 Y chromosomes, and regulating agencies (i.e. New York State Department of Health) request the
119 disclosure of all genetic information obtained, we know the test is being used by some doctors
120 for gender determination as well as aneuploidy.

121 Before deciding whether to modify our policy or not, we chose to request further information
122 from those IVF centers sending us PGD samples so we could uncover any bias in gender
123 prediction.

124 A second purpose of this study was to evaluate the popular belief that families with all same-
125 gender children are predisposed to produce either more girls or more boys than the population at-
126 large. Previous studies of very large birth cohorts have not supported this belief (Maconochie
127 and Roman, 1997). But no published reports have looked directly for gender bias at the stage of
128 fertilization and embryo production within the two subpopulations of couples who have children
129 all of one gender or the other. Here we present the confirmed data of 276 PGD cycles involving
130 gender selection from 53 different IVF centers throughout the United States.

131

132 **MATERIALS AND METHODS**

133 **Sample population ascertainment**

134 The couples included in this study were selected from PGD cycles performed in our referring
135 facilities from January 2007 to August 2008. Centers referring these cycles were asked to
136 provide information on the first (AMA, RPL, etc.) and second indication (gender selection) for
137 PGD, and if gender selection was mentioned, race and gender desired was recorded.

138 Those confirmed to have requested gender determination were classified according to reason for
139 the request, including X-linked diseases, family balance, gender bias, or unknown; and classified
140 by ethnicity into Chinese, Indian, Middle Eastern and Western (Caucasian, Hispanic and
141 African-American).

142 Statistical comparisons between groups were made using Chi-square and Fisher's exact test, with
143 a level of significance of $P < 0.05$ (GraphPad InStat 3).

144

145 Biopsy, fixation and FISH

146 Embryos were biopsied on day 3 of development by removing a single blastomere, followed by
147 nuclear fixation using the slightly modified Carnoy method (Velilla *et al.*, 2002). The fixed cells
148 were sent to Reprogenetics laboratories either at Livingston, NJ or South San Francisco, CA, for
149 FISH analysis, and results were provided on days 3 to 5 of embryo development.

150 PGD was performed by FISH as part of the analysis of 5 (X, Y, 13, 18, 21), 9 (X, Y, 13, 15, 16,
151 17, 18, 21, 22) or 12 chromosomes (X, Y, 8, 13, 14, 15, 16, 17, 18, 20, 21, 22) as previously
152 reported (Munné *et al.*, 1998a; Colls *et al.*, 2007). In cycles analyzing 9 and 12 chromosomes,
153 chromosomes X and Y were analyzed by using probes [CEP X, DXZ1 within Xp11.1-q11.1] and
154 [CEP Y, DYZ1 Yq12 For cases where five chromosomes were analyzed, the FISH analysis was
155 performed by using the MultiVysion PGT panel (Abbot, Downers Grove, IL), which includes
156 the same probe for chromosome X used in the 9- or 12-chromosomes test and probe [CEP Y,
157 DYZ3 within Yp11.1-q11.1] for chromosome Y.

158 Our “No Result Rescue” (NRR) approach was applied after the regular FISH panels, in cases
159 where doubtful results for one or more of the analyzed chromosomes were obtained (Colls *et al.*,
160 2007). NRR for chromosomes X and Y was performed by using one of these probes: [Telomeric
161 Xp22.3/Yp11.3, DXYS129], [Telomeric Xq28/Yq12, EST Cdy 16c07] or [LSI Xq12, Androgen
162 Receptor] (Abbott, Downers Grove, IL).

163 FISH signals were scored applying criteria previously described (Munné *et al.*, 1998b)

164

165 RESULTS

166 A total of 3,339 PGD cycles using a 5-, 9- or 12-chromosome test were reviewed. Of these, 381
167 (11.4%) were ascertained to be for gender selection from 53 different US-based IVF centers
168 among more than 150 centers referring case material to us. Of them, 276 (72.4%), stated the
169 preferred gender, while in the remaining 105 (27.6%) there was no disclosure, so they could not
170 be used here.

171 Of the 276 cases included in the study, 145 (52.5%) were referred from IVF centers located in
172 the Western half of the USA, vs. 131 (47.5%) referred from the Eastern half of the country. The
173 reasons provided were as follows: 21/276 (7.6%) were selecting females due to X-linked
174 diseases, 9 (3.3%) were selecting females to potentially reduce the chances of autism, 36 (13%)
175 were selecting females or males due to family balance, 97 (35.2%) were selecting females or
176 males due to primary gender selection and 113 (40.9%) were selecting females or males without
177 disclosure of the reason.

178 When we excluded the 30 cases where selection of female embryos was requested for therapeutic
179 reasons we determined that 119/246 (48.4%) of the non-therapeutic requests were for female
180 embryos while 127/246 (51.6%) were selecting for male embryos. However, when gender
181 preference was analyzed taking into account the reason for gender selection and ethnicity, a
182 significant bias toward male selection was seen in couples of Chinese, Indian and Arab/Muslim
183 origin compared with patients of Western origin. Results are summarized in Table 1.

184 Information regarding the gender of previous offspring was obtained in 30 cases requesting
185 gender selection for family balance, showing that 8 (26.6%) requested family balance after
186 having one child of the opposite requested gender, 15 (50%) after having two, 6 (20%) after
187 having three and 1 (3.3%) after having four.

188 A total of 1,647 embryos were analyzed from the 246 cases of gender selection for non-
189 therapeutic reasons. The gender outcome of these embryos showed that there was no difference
190 in the embryo sex ratio of couples wanting either males or females. Limiting the analysis to only
191 Western couples, who are less likely to have a bias towards females, and more likely to want
192 family balance, the results showed no difference in the sex ratios regardless of the desired
193 gender. Likewise, no difference in the sex ratio was observed for normal embryos or for embryos
194 abnormal for other chromosomes. Results are summarized in Table 2.

195 In 33 cases of gender selection for non-therapeutic reasons, none of the normal embryos obtained
196 were of the desired gender. Of these, 18/33 (54.5%) elected to have a transfer of embryos of the
197 initially undesired gender while 15 (45.5%) decided to cancel the transfer. Regarding ethnicity,
198 in the first group, 2/18 (11.1%) were of Indian, Chinese or Middle Eastern origin versus 6/15 (40
199 %) in the second group.

200 Table 3 compares a series of 6977 PGD cycles for aneuploidy testing (PGD-A), with known
201 number of embryos replaced, with the cycles of PGD for gender selection (PGD-G) identified in
202 this study. Although IVF centers referring PGD cycles do not always specify the indication for
203 the test, it is obvious from this table that the vast majority of cycles in which 5 chromosomes are
204 tested or less is for the indication of gender selection.

205 It is also interesting to observe that in both groups of PGD cycles the same number of embryos
206 was replaced (1.5) (Table 3), although the number of chromosomally normal and not replaced
207 was higher in the PGD-G (2.6 embryos) than in the PGD-A group (0.8 embryos). Because the
208 average of embryos tested was actually less in the PGD-G (6.7) than in the PGD-A (8.7), the
209 difference in non-transferred normal embryos is due to the fact that more chromosome
210 abnormalities are detected with 9-12 probes tested, than with 5, and that only 5% of PGD-A

211 tested for 5 chromosomes compared to 58% of PGD-G (Table 3). Indeed, on average 25%
212 (2.2/8.7) embryos were classified as normal by PGD-A and 63% (4.2/6.7) by PGD-G. Assuming
213 a similar rate of chromosome abnormalities in the PGD-G group, 25% of 6.7 embryos or 1.7
214 would be normal, barely enough to replace 1.5 of them. This also means that by only testing 5
215 chromosomes in 58% of cycles, those cycles are replacing less than 1.5 normal embryos and
216 leaving behind potential normal embryos for transfer.

217 Indeed, when only PGD-G cycles with 9-12 chromosomes tested are taken into account (Table 3)
218 the average number of normal embryos not replaced in the PGD-G group decreases from 2.6 to
219 1.6, much closer to the 0.8 embryos in PGD-A.

220

221 **DISCUSSION**

222 In contrast to poll studies that survey opinions regarding gender selection in the general
223 population, our study was designed to evaluate the choices actually made by couples who have
224 decided to gender-select through the use of PGD. Overall, the results obtained in this study
225 are in agreement with previous findings that suggest that in an ethnically diverse Western
226 society, like the United States, sex ratio cannot be disrupted by sex selection. Indeed, our data
227 showed no significant differences regarding gender preference. In the group patients of Western
228 origin, there is actually a slight but not significant preference for females. This finding
229 invalidates the unsubstantiated ethical claim, suggested by some, that sex selection is always a
230 sexist procedure favoring males (United Nations 1995; ACOG 2007; Hanson *et al.*, 2002;
231 Shenfield 2005).

232 However, a significant deviation towards preference for males was observed in patients of
233 Chinese, Indian and Middle Eastern/Muslim ethnicity. Similar results were obtained by Gleicher

234 and Barad (2007) also in the analysis of a series of PGD cases for sex selection in the New York
235 area, showing a strong preference for males in the same ethnic subpopulations, but not a
236 significant difference when the overall population is taken into account, although a slight
237 deviation towards males is described. This bias towards males in Gleicher and Barad (2007) and
238 perhaps toward females in our study may be a reflection of the ethnic composition of the group
239 of patients included in the study and thus a reflection of the geographical origin of the samples.
240 In the Gleicher and Barad study, the percentage of Chinese, Indian and Muslim patients is
241 higher, and therefore, the overall results show a slight deviation towards male. However, since
242 the patients included in our study come from 53 different IVF centers throughout the United
243 States, the ethnic composition of the patients included in this study can be considered a more
244 accurate representation of the population composition of the whole country, and therefore the
245 present results are a more accurate representation of the lack of effect of sex selection on the sex
246 ratio.

247 Dahl *et al.* (2006b) say that for a severe disruption in the sex ratio of a population, there must be
248 a strong preference for a specific gender and at the same time there must be a high demand of
249 assisted reproductive technology with PGD for sex selection. We can agree with the former but
250 simple observation shows the latter need not be present. In China, where the population control
251 laws of the late 1970s require not more than one child per family, a strong preference for males
252 has led to abortion and infanticide of female newborns, creating an excess of males. Chinese
253 society has long fiercely discriminated against females, just as is often the case in Hindu and
254 Muslim societies. The results of male bias mean many males will not find wives, and -- in theory
255 -- that females at last have a choice of suitors. Few women in this situation will accept forcibly
256 arranged marriages or discriminatory mistreatment when multiple choices of husband are

257 available. There are many examples in the natural world, particularly among herbivores, (Fisher
258 1930; Maynard Smith 1980) and in the human history (Trivers 1985; Sureau 1999) that echo this
259 situation. It is our opinion, therefore, that in the longer term, an excess of males in a society will
260 have two obvious effects: one, that discriminatory behavior against females will diminish and
261 eventually disappear; and two, any continued activities for direct sex selection will change to
262 return the sex ratio to equilibrium. Thus an unbalanced sex ratio can only be self-correcting in
263 the longer term.

264 However, in the ethnically diverse United States there is no overall preference for any particular
265 gender when PGD for sex selection is requested, with the exception of some ethnic populations,
266 which represent an extremely small percentage of the US population (Chinese 0.9%, Indian
267 0.6%) (U.S. Census Bureau 2000). Neither does the United States have a high demand for
268 assisted reproductive technology with PGD for sex selection, since only a small fraction of the
269 total population does request PGD, and of those, only 11.4% also request gender selection.

270 In our study only 10.9% of the cases referred for sex selection were for a medical reason, usually
271 for female embryos because of X-linked diseases or to decrease the chance of autism which
272 primarily affects males (Yeargin-Allsopp *et al.*, 2003; Chakrabarti and Fombonne, 2005).

273 Then there is the popular belief that some couples with multiple children of the same gender
274 must have been predisposed in this direction. To assess the validity of this belief, a 1997 study
275 of all 549,048 births in Scotland over a 14 year period, looked at the gender of fourth and fifth
276 newborns from families in which all previous children were of one gender or the other
277 (Maconochie and Roman, 1997). If gender predisposition is a real phenomenon, the gender of
278 the later-born children should be skewed toward the gender of their older siblings. But the data
279 failed to support this hypothesis.

280 Our study extended these findings by considering the gender ratio of embryos produced by
281 couples who were proactive in their desire to gender-balance an unbalanced family. If there was
282 a predisposition to conceive embryos of one sex, we would expect it to show up within these
283 particular patient groups. But we found no difference in the sex ratio of their embryos, either as a
284 total or taking into account only the embryos diagnosed as 'normal', suggesting that such
285 predisposition does not exist; or, that if there is any biological selection against one gender, it did
286 not occur at this stage of development. However, since 76.6 % of couples in the family balance
287 group requested gender selection after having only one or two previous children, they may not be
288 realistically considered to have a predisposition to produce embryos of a given gender, and a
289 much larger population of couples with 3 or more babies of the same sex would need to be
290 screened.

291 Based on large-scale cross-cultural statistics of newborns, it can be seen that there is a slight but
292 uniform skewing of sex ratio worldwide in favor of males with an average of 1.05 (Central
293 Intelligence Agency 2004). Different biological factors have been proposed to explain this shift
294 towards males, such as different survival rates between male and female embryos during early
295 embryo development (Crawford *et al.*, 1987; Boklage 2005), nutritional factors (Rosenfeld and
296 Roberts, 2004; Jimenez *et al.*, 2003; Ménézo 2006), evolutionary degeneration of the Y
297 chromosome and differential fertilization potential of X-bearing and Y-bearing sperm (Cheng *et*
298 *al.*, 2007). However, the present results demonstrate that if there is any factor leading to a bias
299 towards male at the newborn stage, it does not affect the early stages of embryo development or
300 that assisted reproductive technologies neutralize that effect. It certainly does not support a better
301 fertilization potential of Y-bearing sperm (Cheng *et al.*, 2007) at least for babies conceived
302 through ART.

303 The fate of normal embryos that are not transferred is a difficult moral aspect of this procedure
304 for some persons. Our study showed that 54.5% of couples undergoing gender selection PGD for
305 non-therapeutic reasons elected to have any-sex embryos available transferred when there were
306 no embryos of the desired gender; meaning that 45.5% chose to discard. Taken into account that
307 only 13.4 % of the PGD-G failed to produce normal embryos of the desired gender, 6.1% of all
308 cycles had no transfer due to lack of normal embryos of the preferred gender.

309 In those cases where no embryos were replaced there was a significant deviation towards Indian,
310 Chinese and Middle Eastern origin (40%) versus the 11.1% found in the group that elected to
311 have embryos transferred even when not the desired gender, which is a natural reflection of those
312 cultural backgrounds.

313 One can argue that in addition to this 6.1% of cycles with no transfer, many other normal
314 embryos are not replaced because of gender in cycles with transfer. However Table 3 shows that
315 the same average number of embryos is replaced (1.5 embryos) in PGD-A and PGD-G cycles.
316 Extrapolating the number of abnormalities seen in PGD-A (75%) to PGD-G, the number of
317 normal embryos not replaced would be actually less than in PGD-A. In PGD-A the residual
318 number of normal embryos not replaced are usually those of poor morphology since on average
319 <2% of cycles with PGD-A produce frozen embryos. Thus, apparently PGD-G does not increase
320 significantly the number of non-replaced embryos, but this is misleading. For those PGD-G
321 cycles in which only 5 chromosomes were tested, less detection of chromosome abnormalities
322 means that less normal embryos were replaced in total. Thus, to have a less controversial PGD-G
323 program one should test for as many chromosome abnormalities as possible, furthermore when
324 now it is known that embryo biopsy produces a slight to significant detrimental impact on
325 implantation, depending on the biopsier and cells biopsied (Cohen et al. 2007, Goessens et al.

326 2008, Munne et al. 2007), thus, if the biopsy is to be done, it should confer the maximum
327 selection and thus maximum advantage to that cycle. Testing more chromosomes in PGD-G
328 would prevent that less normal embryos are left non-replaced, since couples may decide to
329 replace those of another gender if non of the desired gender are found, and this possibility will
330 increase with more chromosomes tested. In addition, by testing more chromosomes after the
331 same biopsy, more normal embryos in general will be replaced, and thus the pregnancy outcome
332 should increase. This should be further analyzed with a large dataset.

333

334 To conclude, this study demonstrates that sex selection by PGD in an ethnically diverse Western
335 society, like the United States, does not have any significant effect on population sex ratio, does
336 not discriminate against female embryos, and seldom results (6%) in the non replacement of any
337 normal embryos because such embryos were not of the desired gender, provided that the PGD-G
338 test analyzes as many chromosomes as possible. Since these are the main concerns that ethics
339 committees and scientific societies from Western countries raise in support of their opposition to
340 gender selection by PGD, it seems clear that Western objections to gender selection are not well-
341 founded. Furthermore, the alternative to PGD or sperm selection for gender in some Asian
342 countries is infanticide, which is universally repugnant. Thus, for those couples who desire
343 gender selection, earlier methods are clearly preferable. We believe that it is incumbent upon
344 committees and scientific societies who have formulated policy statements on gender selection to
345 start anew with the actual facts in the pursuit of rational policymaking that protects private
346 interests when those interests bear no negative consequences for society at-large.

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348

349 **REFERENCES**

350

351 American College of Obstetricians and Gynecologists, Committee of Ethics 2007 ACOG

352 Committee Opinion (Number 360). *Obstetrics Gynecology* **109**, 475-478.

353

354 Amor DJ, Cameron C 2008 PGD gender selection for non-Mendelian disorders with unequal sex

355 incidence. *Human Reproduction* **23**, 729-734.

356

357 Boklage CE 2005 The epigenetic environment: secondary sex ratio depends on differential

358 survival in embryogenesis. *Human Reproduction* **20**, 583-587.

359

360 Chakrabarti S, Fombonne E 2005 Pervasive developmental disorders in preschool children:

361 confirmation of high prevalence. *American Journal of Psychiatry* **162**, 1133-1141.

362

363 Cheng H, Shang X, He Y, Zhang T, Zhang Y, Zhou R 2007 Insight into human sex ratio

364 imbalance: the more boys born, the more infertile men. *Reproductive Biomedicine Online* **15**,

365 487-494.

366

367 Central Intelligence Agency 2004 *The World Factbook 2004*368 www.cia.gov/cia/publications/factbook

369

370 Cohen J, Wells D, Munné S (2007) Removal of two cells from cleavage stage embryos is likely
371 to reduce the efficacy of chromosomal tests employed to enhance implantation rates. *Fertil Steril*,
372 **87**:496-503

373

374 Colls P, Escudero T, Cekleniak N, Sadowy S, Cohen J, Munné S 2007 Increased efficiency of
375 preimplantation genetic diagnosis for infertility using “no result rescue”. *Fertility and Sterility*
376 **88**, 53-61.

377

378 Crawford MA, Doyle W, Meadows N 1987 Gender differences at birth and differences in fetal
379 growth. *Human Reproduction* **2**, 517-520.

380

381 Dahl E, Beutel M, Brosig B, Hirsch KD 2003 Preconception sex selection for non-medical
382 reasons: a representative survey from Germany. *Human Reproduction* **18**, 2231-2234.

383

384 Dahl E, Beutel M, Brosig B, Grussner S, Stobe-Richter Y, Tinneberg HR, Braehler E 2006a
385 Social sex selection and the balance of the sexes: empirical evidence from Germany, the UK and
386 the US. *Journal Assisted Reproduction Genetics* **23**, 311-318.

387

388 Dahl E, Gupta RS, Beutel M, Stobel-Richter Y, Brosig B, Tinneberg HR, Jain T 2006b
389 Preconception sex selection demands and preferences in the United States. *Fertility and Sterility*
390 **85**, 468-473.

391

- 392 Escudero T, Estop A, Fischer J, Munné S 2008 Preimplantation genetic diagnosis for complex
393 chromosome rearrangements. *American Journal of Medical Genetics* **146**, 1662-1669.
394
- 395 Ethics Committee of the American Society for Reproductive Medicine 2001 Preconception
396 gender selection for non medical reasons. *Fertility and Sterility* **75**, 861-864.
397
- 398 Fejes I, Szollosi J, Zavačzki Z, Koloszar S, Pal A 2006 A boy or a girl? A Hungarian survey
399 regarding gender selection. *Acta Obstetrica Gynecologica Scandinavia* **85**, 993-996.
400
- 401 Fiorentino F, Magli MC, Podini D, Ferraretti AP, Nuccitelli A, Vitale N, Baldi M, Gianaroli L
402 2003 The minisequencing method: an alternative strategy for preimplantation genetic diagnosis
403 of single gene disorders. *Molecular Human Reproduction* **9**, 399-410.
404
- 405 Fisher RA 1930 *The genetic Theory of Natural Selection*. Clarendon Press, Oxford.
406
- 407 Garrisi JG, Colls P, Ferry KM, Zheng X, Garrisi MG, Munné S 2008 Effect of infertility,
408 maternal age, and number of previous miscarriages on the outcome of preimplantation genetic
409 diagnosis for idiopathic recurrent pregnancy loss. *Fertility and Sterility* **Epub ahead of print**.
410
- 411 Gianaroli L, Magli MC, Ferraretti AP, Munné S 1999 Preimplantation diagnosis for aneuploidies
412 in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories
413 for which it should be proposed. *Fertility and Sterility* **72**, 837-844.
414

415 Gleicher N, Barad DH 2007 The choice of gender: is elective gender selection, indeed, sexist?

416 *Human Reproduction* **22**, 3038-3041.

417

418 Goossens V, De Rycke M, De Vos A, Staessen C, Michiels A, Verpoest W, Van Steirteghem A,

419 Bertrand C, Liebaers I, Devroey P, Sermon K (2008) Diagnostic efficiency, embryonic

420 development and clinical outcome after the biopsy of one or two blastomeres for Preimplantation

421 genetic diagnosis. *Human Reprod*, 23:481-492

422

423 Hanson C, Hamburger L, Janson PO 2002 Is any form of gender selection ethical? *Journal*

424 *Assisted Reproduction Genetics* **19**, 431-432.

425

426 Harper JC, Wells D, Piyamongkol W, Abou-Sleiman P, Apeessos A, Ioulianos A, Davis M, Doshi

427 A, Serhal P, Ranieri M, Rodeck C, Delhanty JD 2002 Preimplantation genetic diagnosis for

428 single gene disorders: experience with five single gene disorders. *Prenatal Diagnosis* **22**, 525-

429 533.

430

431 Heyd D 2003 Male or female, we will create them: the ethics of sex selection for non-medical

432 reasons. *Ethical Perspectives* **10**, 204-214.

433

434 Human Fertilization and Embryology Authority 2003 Code of Practice, 6th edition. London:

435 *HEFA*.

436

- 437 International Federation of Gynecology and Obstetrics (FIGO). Committee for the Ethical
438 Aspects of Human Reproduction and Women's Health 2006 Ethical guidelines on sex selection
439 for non-medical purposes. *International Journal Gynaecology Obstetrics* **92**, 329-330.
440
- 441 Jain T, Missmer SA, Gupta RS, Hornstein MD 2005 Preimplantation sex selection demand and
442 preference in an infertile population. *Fertility and Sterility* **83**, 649-658.
443
- 444 Jimenez A, Madrid-Bury N, Fernandez R, Perez-Garnelo S, Moreira P, Pintado B, De la Fuente
445 J, Gutierrez-Adan A 2003 Hyperglycemia-induced apoptosis affects sex ratio of bovine and
446 murine preimplantation embryos. *Molecular Reproduction and Development* **65**, 180-187.
447
- 448 Maconochie N, Roman E 1997 Sex ratios: are there natural variations within the human
449 population?. *British Journal of Obstetrics and Gynecology* **104**, 1050-1053.
450
- 451 Maynard Smith J 1980 A new theory of sexual investment. *Behaviour Ecology Sociobiology* **7**,
452 247-251.
453
- 454 Ménézo YJ 2006 Paternal and maternal factors in preimplantation embryogenesis: interaction
455 with the biochemical environment. *Reproductive Biomedicine Online* **12**, 616-621.
456
- 457 Munné S, Magli C, Bahce M, Fung J, Legator M, Morrison L, Cohen J, Gianaroli L 1998a
458 Preimplantation diagnosis of the aneuploidies most commonly found in spontaneous abortions
459 and live births: XY, 13, 14, 15, 16, 18, 21, 22. *Prenatal Diagnosis* **18**, 1459-1466.

460
461 Munné S, Marquez C, Magli C, Morton P, Morrison L 1998b Scoring criteria for preimplantation
462 genetic diagnosis of numerical abnormalities for chromosomes X, Y, 13, 16, 18 and 21.
463 *Molecular Human Reproduction* **4**, 863-70.

464
465 Munné S, Magli MC, Cohen J, Morton P, Sadowy S, Gianaroli L, Tucker M, Márquez C, Sable
466 D, Ferraretti AP, Massey JB, Scott R 1999 Positive outcome after preimplantation diagnosis of
467 aneuploidy in human embryos. *Human Reproduction* **14**, 2191-2199.

468
469 Munné S, Sandalinas M, Escudero T, Velilla E, Walmsley R, Sadowy S, Cohen J, Sable D 2003
470 Improved implantation after preimplantation genetic diagnosis of aneuploidy. *Reproductive*
471 *Biomedicine Online* **7**, 91-97.

472
473 Munné S, Chen S, Fischer J, Colls P, Zheng X, Stevens J, Escudero T, Oter M, Schoolcraft B,
474 Simpson JL, Cohen J 2005 Preimplantation genetic diagnosis reduces pregnancy loss in women
475 aged 35 years and older with a history of recurrent miscarriages. *Fertility and Sterility* **84**, 331-
476 335.

477
478 Munné S, Gianaroli L, Tur-Kaspa I, Magli C, Sandalinas M, Grifo J, Cram D, Kahraman S,
479 Verlinsky Y, Simpson JL 2007. Sub-standard application of PGS may interfere with its clinical
480 success. *Fertil Steril*, **88**:781-784

481

- 482 Otani T, Roche M, Mizuike M, Colls P, Escudero T, Munné S 2006 Preimplantation genetic
483 diagnosis significantly improves the pregnancy outcome of translocation carriers with a history
484 of recurrent miscarriage and unsuccessful pregnancies. *Reproductive Biomedicine Online* **13**,
485 869-874.
- 486
- 487 Robertson JA 2002 Sex selection for gender variety by preimplantation genetic diagnosis.
488 *Fertility and Sterility* **78**, 463.
- 489
- 490 Rosenfeld CS, Roberts RM 2004 Maternal diet and other factors affecting offspring sex ratio: a
491 review. *Biology of Reproduction* **71**, 1063-1070.
- 492
- 493 Shenfield F 2005 Procreative liberty, or collective responsibility? Comment on the House of
494 Commons report Human Reproductive Technology and the Law, and on Dahl's response.
495 *Reproductive Biomedicine Online* **11**, 155-157.
- 496
- 497 Sureau C 1999 Gender selection: a crime against humanity or the exercise of a fundamental
498 right? *Human Reproduction* **14**, 867-868.
- 499
- 500 Trivers R 1985 *Social Evolution*. Benjamin-Cummings, Menlo Park, California
- 501 United Nations 1995 Gender equality, equity and empowerment of women. *Population and*
502 *Development: Programme of Action Adopted at the International Conference on Population and*
503 *Development, 5-13 September 1994. Cairo, New York. UN*, 17-21.
- 504

505 United States Census Bureau 2000 Profiles of General Demographic Characteristics. 2000
 506 Census of Population and Housing. <http://www.census.gov/prod/cen2000/dp1/2kh00.pdf>

507

508 Velilla E, Escudero T, Munné S 2002 Blastomere fixation techniques and risk of misdiagnosis
 509 for preimplantation genetic diagnosis of aneuploidy. *Reproductive BioMedicine Online* **4**, 210-
 510 217.

511

512 Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C, Murphy C 2003 Prevalence
 513 of autism in a US metropolitan area. *The Journal of the American Medical Association* **289**, 49-
 514 55.

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524 **Table 1. Gender preference by reason and ethnicity.**

525

526 Reason	Ethnicity	Cases	Male	Female	significance
527 Family balance	Middle East	1	1 (100%)	0 (0%)	

528		Chinese	2	1 (50%)	1(50%)	
529		Indian	10	10 (100%)	0 (0%)	
530		Total	13	12 (92.3%)	1 (7.7%)	P<0.05
531		Western	23	12 (52.2%)	11 (47.8%)	P=1.0000
532						
533	Primary selection	Middle East	8	6 (75%)	2 (25%)	
534		Chinese	6	4 (66.6%)	2 (33.3%)	
535		Indian	12	9 (75%)	3 (25%)	
536		Total	26	19 (73.1%)	7 (26.9%)	P=0.1534
537		Western	71	21 (29.6%)	50 (70.4%)	P=0.0252
538						
539	Unknown reason	Middle East	8	8 (100%)	0 (0%)	
540		Chinese	7	7 (100%)	0 (0%)	
541		Indian	8	7 (87.5%)	1 (12.5%)	
542		Total	23	22 (95.7%)	1 (4.3%)	P=0.0017
543		Western	90	44 (48.9%)	46 (51.1%)	P=1.0000
544						
545	Total	Middle East	17	15 (88.2%)	2 (11.8%)	P=0.0255
546		Chinese	15	12 (80.0%)	3 (20.0%)	P=0.1281
547		Indian	30	26 (86.7%)	4 (13.3%)	P=0.0048
548		Total	62	53 (85.5%)	9 (14.5%)	P<0.0001
549		Western	184	74 (40.2%)	110 (59.8%)	P=0.0748
550						

552 **Table 2. Gender outcome of analyzed embryos from different groups of patients.**

553

554 Patient	Total	Total	Normal	Normal
555 Group	Male	Female	Male	Female
557 Total patients	784 (47.6%)	863 (52.4%)	507 (49.6%)	515 (50.4%)
558 Select Male	418 (49.2%)	432 (50.8%)	278 (50.9%)	268 (49.1%)
559 Select Female	366 (45.9%)	431 (54.1%)	229 (48.1%)	247 (51.9%)
561 Western patients	581 (47.1%)	653 (52.9%)	361 (48.6%)	381 (51.4%)
562 Select Male	251 (49.4%)	257 (50.6%)	158 (50.6%)	154 (49.4%)
563 Select Female	330 (45.4%)	396 (54.6%)	203 (47.2%)	227 (52.8%)
565 Family balancing	89 (50.6%)	87 (49.4%)	60 (52.6%)	54 (47.4%)
566 Select Male	58 (49.6%)	59 (50.4%)	36 (52.2%)	33 (47.8%)
567 Select Female	31 (52.5%)	28 (47.5%)	24 (53.3%)	21 (46.7%)

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574 **Table 3: Comparison of PGD cycles of aneuploidy and gender selection**

575		PGD for aneuploidy		PGD for gender selection	
576		#	Av.	#	Av.
578	Age <35				
579	cycles	1867		85	
580	av. Age	31.1		31.1	
581	# embryos tested	19548	10.5	610	7.2
582	# embryos normal	6497	3.5	423	5.0
583	# embryos replaced	3456	1.9	119	1.4
584	Av. Normal non replaced		1.6	3.6	
585	cycles with 5 chromosomes tested*	150.0	8.0%	61	71.8%
586					
587	age 35-39				
588	cycles	2321		75	
589	av. Age	37.2		36.9	
590	# embryos tested	20314	8.8	467	6.2
591	# embryos normal	5452	2.3	245	3.3
592	# embryos replaced	3783	1.6	113	1.5
593	Av. Normal non replaced		0.7	1.8	
594	cycles with 5 chromosomes tested*	108	4.7%	41	54.7%
595					
596	age >39				

597	cycles	2789		86	
598	av. Age	43.5		44.1	
599	# embryos tested	20947	7.5	570	6.6
600	# embryos normal	3589	1.3	354	4.1
601	# embryos replaced	2941	1.1	139	1.6
602	Av. Normal non replaced		0.2	2.5	
603	cycles with 5 chromosomes tested*	102	3.7%	40	46.5%
604					
605					
606	Total cycles with 9-12 chromosomes tested				
607	cycles	6617		104	
608	av. Age		38		38.5
609	# embryos tested	58167	8.8	703	6.8
610	# embryos normal	14477	2.2	306	2.9
611	# embryos replaced	9600	1.4	138	1.3
612	Av. Normal non replaced		0.8		1.6
613					
614	Total				
615	cycles	6977		246	
616	av. Age	38.1		37.4	
617	# embryos tested	60809	8.7	1647	6.7
618	# embryos normal	15538	2.2	1022	4.2
619	# embryos replaced	10180	1.5	371	1.5

620	Av. Normal non replaced		0.8		2.6
621	cycles with 5 chromosomes tested*	360	5.2%	142	57.7%

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624 * The rest of cycles had 9 to 12 chromosomes tested