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No beneficial effect of preimplantation genetic screening in women of advanced maternal age with a high risk for embryonic aneuploidy

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BACKGROUND: Human preimplantation embryos generated through *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatments show a variable rate of numerical chromosome abnormalities or aneuploidies. Preimplantation genetic screening (PGS) has been designed to screen for aneuploidies in high risk patients, with the aim of improving live birth rates in IVF/ICSI. We assessed whether the effect of PGS on live births rates differs in women of advanced maternal age with variable risks for embryonic aneuploidy, and weighed these effects against the results obtained after IVF/ICSI without PGS. METHODS: The effect of PGS on live birth rates was compared between groups defined by maternal age, number of previous miscarriages, semen quality, total amount of recombinant FSH (rFSH) administered during ovarian stimulation and total number of top-quality embryos, using data from a randomized controlled trial among women of advanced maternal age (35–41 years). RESULTS: There was no significant differential effect of PGS in groups based on maternal age (*P*-value of interaction 0.16), the number of previous miscarriages (*P*-value of interaction 0.93), semen quality (*P*-value of interaction 0.26), rFSH dose (*P*-value of interaction 0.15) or the number of top-quality embryos (*P*-value of interaction 0.59). Live birth rates after IVF/ICSI with PGS were lower in all groups when compared with live birth rates after IVF/ICSI without PGS. CONCLUSIONS: The paradigm that the effect of PGS is determined by a woman's risk for embryonic aneuploidy seems incorrect. In fact, PGS has no clinical benefit over standard IVF/ICSI in women of advanced maternal age regardless of their risk for embryonic aneuploidy.

Keywords: preimplantation genetic screening; *in vitro* fertilization; aneuploidy

Introduction

Human preimplantation embryos generated through *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatments show a high rate of numerical chromosome abnormalities or aneuploidies (Wilton, 2002). It is thought that most of these aneuploid embryos do not develop to term, but either fail to implant or abort spontaneously (Munne, 2003).

To detect such embryos, preimplantation genetic screening (PGS) was developed with the aim to improve live birth rates after IVF/ICSI (Munne *et al*., 1993a, b). In PGS, a single blastomere is aspirated from a cleavage stage embryo and the copy number of a given set of chromosomes is determined. Subsequently, embryos shown to be euploid for the chromosomes tested are transferred and aneuploid embryos are discarded.

The extent to which human embryos display aneuploidies varies strongly (Wilton, 2002; Rubio *et al*., 2005). Aneuploidy rates are reported to be especially high in women of advanced maternal age, and in women with a history of recurrent miscarriage (Munne *et al*., 1995; Marquez *et al*., 2000; Werlin *et al*., 2003; Kahraman *et al*., 2004; Wilding *et al*., 2004; Platteau *et al*., 2005; Rubio *et al*., 2005). In addition, semen quality, ovarian stimulation and morphological quality of the embryo also appear to correlate with embryonic aneuploidy rates (Munne *et al*., 1995, 1997; Almeida and Bolton, 1996; Gianaroli *et al*., 2000; Marquez *et al*., 2000; Rubio *et al*., 2005; Baltaci *et al*., 2006; Baart *et al*., 2007).

A recent randomized controlled trial (RCT) that included unselected women of advanced maternal age regardless of their risk for embryonic aneuploidies showed a negative

effect of PGS on live birth rates (Mastenbroek *et al*., 2007). However, given the variable expression of embryonic aneuploidy, several investigators hypothesized that the effect of PGS on live birth rates might be different for various groups of women (Handyside and Thornhill, 2007; Munne *et al*., 2007). More precisely, PGS would most likely have a beneficial effect on live birth rates in groups of women with a high risk for generating aneuploid embryos.

To test this hypothesis, we assessed whether the effect of PGS on live birth rates differs between groups with different a priori risks of embryonic aneuploidy, and weighed these effects against the results obtained after standard IVF/ICSI, using data from the above mentioned RCT (Mastenbroek *et al*., 2007).

Materials and Methods

Participants and study protocol

Data from a recent large double-blind RCT were used for this study. Details of this trial have been described elsewhere (Mastenbroek *et al*., 2007). Briefly, women aged 35–41 years starting with their first IVF/ICSI treatment, who had signed informed consent were randomly allocated to three IVF/ICSI cycles with embryo selection based on PGS (PGS group) or to three standard IVF/ICSI cycles with embryo selection based on morphology (control group).

In the PGS group, a single blastomere was aspirated from each embryo and analysed for aneuploidies using probes for chromosomes 1, 13, 16, 17, 18, 21, X and Y.

In both groups, two embryos, when available, were transferred on Day 4 after follicular aspiration. In the PGS group, two chromosomally normal embryos with the best morphological features were selected for transfer. If no chromosomally normal embryos were available, undetermined embryos were transferred if available. The reason for transferring undetermined embryos (when no chromosomally normal embryos were available) was to give the woman a change to achieve a live birth. The other option would be to do no transfer, but we considered this to be unethical because in that scenario a woman would certainly not get pregnant. For women allocated to the control group, the selection of embryos for transfer was based solely on the morphology of the embryos.

The primary outcome for the current study was live birth. Groups were divided based on factors known to affect the risk for embryonic aneuploidy, i.e. maternal age ($<$38 years, $\geq$38 years), previous miscarriages (none, one, and two or more), semen quality (total motile count during fertility work-up $<1 \times 10^6$ or $\geq 1 \times 10^6$), total amount of recombinant FSH (rFSH) administered during ovarian stimulation (subdivision based on quartiles) and total number of top-quality embryos (none, one or two, three or more). A top-quality embryo was defined as an embryo having a combined embryo score of 24 or more, calculated by multiplying the number of cells on Day 3 after follicular aspiration by the morphological score of the embryo (Steer *et al*., 1992).

In the analysis of total amount of rFSH administered during ovarian stimulation and total number of (top-quality) embryos, only the first treatment cycle with follicular aspiration of each patient was included since these variables differed for each cycle.

Statistical analysis

Relative risks (RR) for live birth rates were calculated for the separate subgroups. To compare directly the effect of PGS within the various groups, in order to determine whether a subgroup of women might benefit from PGS, logistic regression analysis was performed with live birth as the dependent variable, and treatment (IVF/ICSI with or without PGS), the variable on which the group was based, and the interaction between treatment and group as independent variables. In case the interaction is statistically significant (*P*-value of interaction <0.05), this is a clear indication that the effect of treatment, i.e. IVF/ICSI with or without PGS in a specific group is significantly different from the effect in the other group based on the same variable, or in case of several groups, from the overall study population (Matthews and Altman, 1996; Assmann *et al*., 2000). All primary analyses were performed on an intention-to-treat basis. The analyses were also repeated on a per protocol basis.

Results

A total of 408 women were included in the RCT. Two hundred and six of them underwent IVF/ICSI with PGS and 202 underwent IVF/ICSI without PGS. A total of 836 cycles of IVF were performed (434 cycles with PGS and 402 cycles without PGS). Baseline characteristics of the participating women are presented in Table I.

As described previously, overall live birth rates were significantly lower in the PGS group compared with the control group (RR 0.68, 95% CI 0.50–0.92) (Mastenbroek *et al*., 2007). The results of the analyses in the groups based on embryonic aneuploidy risk are presented in Table II.

There was no significant differential effect of PGS in groups based on maternal age (*P*-value of interaction 0.16). The RR to achieve a live birth after IVF/ICSI with PGS was higher for women between 35 and 38 years of age when compared with the women aged 38 years or older (0.83 and 0.47, respectively).

Similarly, there was no significant differential effect of PGS in groups based on the number of previous miscarriages (*P*-value of interaction 0.93). The RR to achieve a live birth after IVF/ICSI with PGS was lower for women with no previous miscarriages compared with the women with one, or two or more previous miscarriages (0.63, 0.76 and 0.82, respectively).

No significant differential effect of PGS was found in groups based on semen quality (*P*-value of interaction 0.26). The RR to achieve a live birth after IVF/ICSI with PGS was higher when the total motile count was below 1×10^6 than when the total motile count was 1×10^6 or higher (0.99 versus 0.62).

There was no significant interaction between treatment effect and rFSH groups (*P*-value of interaction 0.15). In women who were administered a total rFSH dose between 2401 and 3300 IU live birth rate was higher in the PGS group when compared with the control group (RR 1.26; 95% CI 0.49–3.29). In the groups of women who were administered a total rFSH dose between 900 and 1800, 1801 and 2400 or 3301 and 8100 live birth rates were lower in the PGS group when compared with the control group (RR 0.78, 0.61 and 0.10, respectively).

There was no significant interaction between treatment effect and number of top-quality embryos (*P*-value of interaction 0.59). The RR to achieve a live birth after IVF/ICSI with PGS was highest in the group of women with one or two top-quality embryos (RR 0.99) compared with the group women with no, or three or more top-quality embryos

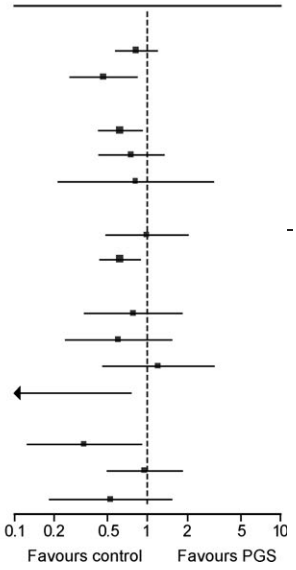
Table I. Patient characteristics.

	PGS group ($n = 206$)	Control group ($n = 202$)
Age (years), mean (SD)	38.0 (1.7)	37.9 (1.6)
Nulliparous, *n* (%)	139 (67)	123 (61)
Women without previous miscarriages, *n* (%)	162 (79)	166 (82)
Body mass index (kg/m^2), mean (SD)[a]	24.6 (4.4)	24.0 (3.7)
Smoking, *n* (%)[b]	38 (19)	37 (19)
Cause of infertility, *n* (%)[c]		
Poor semen quality	78 (38)	78 (39)
Unexplained	77 (37)	74 (37)
Tubal	48 (23)	44 (22)
Anovulation	14 (7)	11 (5)
Endometriosis	12 (6)	7 (3)
Cervical	8 (4)	9 (4)
Ovarian failure	2 (1)	1 (<1)
Total motile count (×10^6), mean (SD)	57 (78)	63 (91)
Total rFSH used (IU), mean (SD)[d]	2736 (1318)	2801 (1321)
Fertilization procedure, *n* (%)[d]		
IVF	144 (72)	138 (71)
ICSI	56 (28)	57 (29)
Number of embryos, mean (SD)[d]	5.0 (4.5)	4.8 (3.7)
Number of top embryos, mean (SD)[d]	1.2 (1.7)	0.9 (1.3)

[a]Data available for 348/408 women (85%); [b]data available for 397/408 women (97%);[c]more than one diagnosis per couple was possible; 353 couples had one diagnosis, and 55 couples had two diagnoses; [d]Only cycles with follicular aspiration are included [395/408 women (97%)].

Table II. Effect of adding preimplantation genetic screening to IVF/ICSI on live birth rates in different groups based on the risk for embryonic aneuploidies.

	Group	*n* PGS/*n* control	PGS live birth *n* (%)	Control live birth *n* (%)	Relative risk (95% CI)	*P* value of interaction[a]	Forrest plot
Baseline variables							
Age	<38 years	103/102	35 (34)	42 (41)	0.83 (0.58–1.18)	0.16	
	≥38 years	103/100	14 (14)	29 (29)	0.47 (0.26–0.83)		
Previous miscarriages	0	162/166	33 (20)	54 (33)	0.63 (0.43–0.91)	0.93	
	1	33/27	13 (39)	14 (52)	0.76 (0.44–1.33)		
	≥2	11/9	3 (27)	3 (33)	0.82 (0.22–3.11)		
Total motile count	<1 x 10e6	32/35	10 (31)	11 (31)	0.99 (0.49–2.02)	0.26	
	≥1 x 10e6	174/167	39 (22)	60 (36)	0.62 (0.44–0.88)		
First cycle variables*[b]							
Total rFSH	900–1800	50/49	8 (16)	10(20)	0.78 (0.34–1.82)	0.15	
	1801–2400	64/50	7 (11)	9 (18)	0.61 (0.24–1.52)		
	2401–3300	39/47	7 (18)	7 (15)	1.21 (0.46–3.14)		
	3301–8100	48/48	1 (2)	10 (21)	0.10 (0.01–0.75)		
Top-quality embryos	0	106/100	5 (5)	14 (14)	0.34 (0.13–0.90)	0.59	
	1–2	62/73	13 (21)	16 (22)	0.96 (0.50–1.83)		
	≥3	33/21	5 (15)	6 (29)	0.53 (0.19–1.52)		



[a]Interaction between treatment (IVF/ICSI with or without PGS) and the variable on which the group was based; [b]only cycles with follicular aspiration are included (395/408 women).

(RR 0.34 and 0.53, respectively). The analyses on a per protocol basis gave similar results (data not shown).

Discussion

In contrast to the hypothesis underlying the rationale behind PGS, we found that the effect of PGS in women of advanced maternal age did not differ in women with a high embryonic aneuploidy risk when compared with the women with a low embryonic aneuploidy risk. Moreover, live birth rates were lower after IVF/ICSI with PGS when compared with IVF/ICSI without PGS in all groups.

As it is generally accepted that embryonic aneuploidy rates increase significantly with increasing maternal age, we were surprised to find that PGS worked relatively better in the younger age group. Apart from having an effect on embryonic aneuploidy rate, it is also known that embryo morphology and developmental competence are related to maternal age, i.e. embryos from younger women are in general of better morphology and competence than embryos from older women (Roseboom *et al*., 1995; Hsu *et al*., 1999; Shapiro *et al*., 2002). It may

therefore be that embryos from younger women are less vulnerable to the biopsy procedure than embryos from older women and that this underlies the observed difference in relative chance to achieve a live birth after IVF/ICSI with PGS between the two age groups. These results are in line with two RCTs that showed an RR of 1.0 in an RCT in women below 36 years of age and an RR of 0.69 in an RCT in women 36 years of age or older (Staessen *et al*., 2004, 2007, Abstract).

PGS success rate for live birth was higher after PGS in couples with a low total motile count compared with couples with a high total motile count (RR: 0.99 versus 0.62), although this interaction was not statistically significant (*P*-value of interaction 0.26). Despite the fact that in the current study live birth rates after PGS were still lower than after standard IVF treatment in both these groups, we cannot completely rule out a possible effect of PGS in couples of advanced maternal age with very poor semen quality.

The success rate for live birth in women with one or more previous miscarriages was higher compared with the women without previous miscarriages [RR 0.63 (95% CI 0.43–0.91), 0.76 (95% CI 0.44–1.33) and 0.83 (95% CI 0.22–3.11) for no, one and two or more miscarriages, respectively], suggesting a possible differential effect of PGS in these groups. However, the *P*-value of interaction was very high ($P = 0.93$), which counteracts this possible suggestion.

We did not find a differential effect of PGS in women using a high rFSH dose, or in women with many top-quality embryos.

A well-known shortcoming of subgroup analyses is that it can produce false-positive findings in case every subgroup is tested separately or false-negative findings due to a lack of power. We handled these issues by using interaction tests. As these tests include all available data in one test, they have emerged as the most effective statistical method to restrain inappropriate subgroup findings, while still having the ability to detect interactive effects, if present (Schulz and Grimes, 2005).

In nearly all aneuploidy risk categories IVF/ICSI with PGS leads to lower live birth rates than standard IVF/ICSI treatment and we could not detect any significant interaction for the proposed risk categories. In our view, this suggests that the theoretical concept of improving IVF/ICSI success rates by screening embryos for aneuploidies using PGS is wrong. Several mechanisms might be responsible for the failure of PGS to improve IVF/ICSI success rates. First, the technique itself, more precisely the biopsy of a blastomere from a cleavage stage embryo, could hamper the potential of the embryo to successfully develop and implant. Second, PGS may not be sufficiently able to detect all aneuploid embryos, due to the limited number of chromosomes tested. Third, embryonic chromosomal mosaicism, i.e. the phenomenon that the chromosomal constitution of one blastomere is not representative for the entire embryo, could lead to the transfer of embryos that are abnormal as well as to the discarding of potentially viable embryos.

Whatever the exact biological mechanism behind the failure of PGS, from a clinical point of view, the results of our current study show there is no clinical benefit of PGS in women of advanced maternal age, regardless of their risk for embryonic aneuploidies.

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