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The relationship between the percent of euploid embryo and the tolerance of embryo biopsy in preimplantation genetic screening

A systematic review and meta-analysis of randomized controlled trials

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Abstract

The aim of this study was to analyze the relationship between the percent of euploid embryo and the tolerance of embryo biopsy in preimplantation genetic screening (PGS).

PubMed and trial registers were searched for clinical studies that patients were randomized to the PGS group or the control group from 1995 to October 2017. The patients of advanced maternal age, repeated implantation failure, and good prognosis with or without PGS in randomized controlled trials (RCTs) were collected.

Original data from 9 RCT studies comparing in-vitro fertilization with and without PGS including 1642 patients were obtained and they were divided into 3 subgroups according to the percent of euploid embryo. PGS significantly increased live birth babies per embryo transferred (risk ratio: 2.98, 95% confidence interval: 1.54–5.75) in ≤30% of euploid embryo subgroups and but in other 2 groups, PGS has no effect. Significant negative correlation was found between the percent of euploid embryo and the tolerance of embryo biopsy in PGS ($r = 0.80$, $P = 0.010$)

The tolerance of embryo biopsy in PGS was associated negatively with the percent of euploid embryo. There was a beneficial effect when PGS was used in the patients with the lowest percent of euploid embryo.

Abbreviations: AMA = advanced maternal age, BE = biopsy embryo, CI = confidence intervals, EE% = percent of euploid embryo, ET = embryo tolerance after PGS, FISH = fluorescence in situ hybridization, LBE = live birth embryo, PGS = preimplantation genetic screening, RCTs = randomized controlled trials, RIF = repeated implantation failure, RR = risk ratios.

Keywords: embryo biopsy, euploid embryo, meta-analysis, PGS, systematic review

1. Introduction

There are higher probability that aneuploidies in pregnancies occur in the women of advanced maternal age (AMA), with a

history of recurrent miscarriage or repeated implantation failure (RIF), and with a partner with low sperm quality.[1–5] The aneuploidy may result in miscarriage, stillbirth, or the birth of a child with chromosomal disorder such as Down syndrome.[6,7] Preimplantation genetic screening (PGS) evaluates 1 or 2 cells from day 3 embryos created through IVF and discovered the chromosome abnormality embryos, which can screen for euploid embryos and remove aneuploid ones and in theory can increase live births rate.[8] However, some articles reported PGS was not helpful for live birth rate or disadvantageous and the biopsy and mosaicism of embryo are the major reasons.[9,10]

In biopsy, an embryo is taken out of the incubator for a couple of minutes, made a hole in the zona pellucida via mechanical dissection, acidic Tyrode solution or a laser and ≥1 cells aspirated from this embryo. Those additional operations could be harmful for an embryo.[11] The chromosomal mosaicism is the phenomenon that not all cells in an embryo have the same chromosomal content, with mosaicism rates varying from 15% to >90%, which is highly relevant for the efficacy of PGS.[12–14] With female age increasing, the frequency of abnormal chromosome and mosaicism becomes higher, as well as the embryo quality becomes poorer. Similarly, their tolerance of additional operations perhaps decreases. The damage of embryos after additional operations perhaps surpass the beneficial effect of PGS with the decreasing percent of euploid embryo, which leads to no effect of PGS in the total process. AMA and RIF of

aneuploidy rates were 20% to 50% in these articles[14–22] and there was not a reference value. However, there has not been some related data to analyze the relationships between the percent of euploid embryo and the tolerance of embryo biopsy in PGS.

2. Method

2.1. Search strategy and data extraction

We performed a literature search in PubMed and databases for registration of randomized controlled trials (RCTs) from 1995 to October 2015 with the following terms: (preimplantation genetic screening OR aneuploidy screening OR preimplantation testing OR embryo screening) and Randomized Controlled Trial. An RCT about PGS used to screen the euploid embryo and not for AMA, RIF, or the general population will be selected.

2.2. Eligibility criteria

We collected randomized clinical trials of women undergoing in vitro fertilization, receiving embryo transfers with previous PGS compared with women without PGS. Main outcomes of interest for the review were those related with the percent of euploid embryo, live birth rates per transfer, and live birth babies per embryo; live birth babies per embryo and embryo tolerance after PGS in theory were assessed as the theoretical calculation of data (Table 1).[14–22] We assessed trials' methodological quality paying special attention to the generation of the randomization sequence, the allocation concealment adequacy, the blinding of investigators, patients and outcome assessment, and the reporting of follow-up.

2.3. Data acquisition

Two authors (XJ and CZ) screened the electronic searches for eligible articles by reading the title and abstract. As the controversy occurred, the third author (RZ) must check the articles. The ineligible articles were confirmed and the reasons were signed in it. Two authors (XJ and RZ) read the eligible articles in detail and find the outcomes. If necessary, we contacted the corresponding author of a report in attempt to retrieve missing data.

2.4. Outcome measures

The primary aim of this study was to analyze the relationships between the percent of euploid embryo and the tolerance of embryo biopsy in PGS. The percent of euploid embryo were firstly obtained. Live birth rate per transfer is as the effect of PGS. As seen in Figure 1, there are 2 ideal situations and we can calculate live birth rate per embryo and embryo tolerance after PGS in theory. The details are shown as follows:

2 ideal situations of live birth rate per embryo is $\frac{LBE}{BE \times EE\%}$ and $\frac{LBE}{BE \times EE\%} \times ET$, respectively, where LBE indicates live birth embryo; BE indicates biopsy embryo; EE% indicates percent of euploid embryo; and ET indicates embryo tolerance after PGS.

2.5. Statistical analysis

We calculated risk ratios (RRs) with 95% confidence intervals (CIs) for each individual trial. Eight RCTs were divided into 3 subgroups according to different percent of euploid embryo. The fixed-effect model was used to combine data for each

indication separately and to combine all included studies. Statistical heterogeneity between results of studies was examined by inspecting the scatter in the data points on the graphs and the overlap of CIs, and by checking the I^2 statistic. A value of ≥50% was considered to indicate substantial heterogeneity. In case of substantial heterogeneity, the random-effects model was used instead of the fixed-effect model. Data were analyzed according to the intention-to-treat principle. Revman Software (Version 5, The Cochrane Collaboration) was used to combine data for meta-analysis. The correlation analyses between sex ratios and implantation rate and between sex ratios and some clinical characteristics were performed by Pearson correlation coefficient.

3. Result

3.1. Study exclusions and inclusions

The literature search produced a list of 534 reports and after reading the title and abstracts, 495 studies were excluded. We obtained 39 full articles that screened further and 15 RCT studies for PGS were potentially eligible (Fig. 1). All trials used fluorescence in situ hybridization (FISH) to classify embryo as euploid or aneuploidy, and CCS was excluded. After detailed analyzing and assessing the above RCTs, 9 studies were selected in the systematic review. Two reports were excluded because they were quasirandomized and the treatment options were on the basis of the couples decision.[23,24] Two studies provided insufficient data to assess the methodological quality, for example, the percent of euploid embryo.[25,26] There were overlapping data between 2 trails[20,27]; the early publication date was excluded. We hypothesize the percent of euploid embryo was the same between the PGS and control group because there was no statistical difference in age, the number of embryo transfers, oocytes retrieved, fertilization rate, and some baseline characteristics. One trial was excluded because the criterion of RIF and AMA was different with other trails.[28] The characteristics and quality features of the selected studies are shown in the Tables 1 and 2. There are 9 trials about PGS that are divided into 3 main groups, such as 4 studies to women of AMA, 3 studies to good prognosis patients, and 1 study to RIF.

3.2. The different percent of euploid embryo

In the PGS groups of 9 RCTs, total 4478 embryos were successfully to biopsy on the cleavage stage and FISH was used to analyze the chromosome of 1–2 blastomeres; 41.56% (1861/4478) of embryos were chromosomally normal (euploid). The percent of euploid embryo were 35.77% (1163/3251, 28.13% to 38.41%), 50.25% (198/394), and 59.80% (500/836, 47.85% to 67.82%), respectively in AMA, RIF, and good prognosis. In this article, we divided the 9 trails into 3 subgroups via the percent of euploid embryos instead of the indication of PGS (Fig. 2).

3.3. The ideal effect of PGS

PGS can detect abnormal copy numbers of chromosomes, or aneuploidies and select euploid embryos to transfer, so in theory it can improve the clinic outcomes. The ideal effect of PGS is shown in Figure 1. In fact, the damage of biopsy can cause the loss of the viable embryo, which is shown in Figure 2. In Table 2, we can calculate live birth rate per embryo and embryo tolerance after PGS in theory

Table 1

Characteristics of studies included in a systematic review and meta-analysis of RCTs.

| First author/ publication year	Indication and criteria	Biopsy	FISH	The select of embryo transferred		Statistical significance between the clinical characteristics
				PGS	control	
Staessen et al (2004)[14]	AMA, ≥37 y, normal karyotype of both partners, need for ICSI with motile sperm	3 Days, blastomeres	X,Y, 13, 16, 18, 21, 22	1. Genetically normal embryos 2. At least one compacting embryo or early blastocyst 3. on day 5	1. At least 1 compacting embryo or early blastocyst 2. On day 5	No
Mastenbroek et al, 2007ba[15]	AMA, 35–41 y, no previous failed IVF cycles, no objection to DET	3 Days, blastomeres	X,Y, 13, 16, 18, 21, 22	1. Chromosomally normal embryos 2. The best morphologic features 3. On day 4	1. The best morphologic features 2. On day 4	No
Staessen et al, (2008)[16]	Good prognosis, <36 y, need for ICSI with motile sperm, both partners normal karyotype	3 Days, blastomeres	X,Y, 13, 16, 18, 21, 22	1. Chromosomally normal embryos 2. At least 1 compacting embryo or early blastocys 3. on day 5	1. At least one compacting embryo or early blastocyst 2. On day 5	No
Mersereau et al, (2008)[17]	Good prognosis, <38 y undergoing a fresh IVF cycle without limiting to poor prognosis	3 Days, blastomeres	X,Y, 13, 16, 18, 21,	1. Chromosomally normal embryos 2. Routine blastocyst transfe 3. On day 5 or 7	1. Routine blastocyst transfer 2. On day 5 or 6	No
Blockeel et al, (2008)[18]	RIF, ≥3 failed IVF/ICSI attempts with embryos of good morphological quality, maternal age, 37 y, normal karyotype of both partners, motile sperm	3 Days, blastomeres	X, Y, 13, 16, 18, 21, 22	1. Chromosomally normal embryos 2. At least 1 compacting embryo or early blastocys 3. on day 5	1. At least 1 compacting embryo or early blastocyst 2. On day 5	No
Hardarson et al, (2008)[19]	AMA, ≥38 y, at least 3 embryos of good morphological quality if DET, at least 2 embryos of good morphological quality if SET	3 Days, blastomeres	X,Y, 13, 16, 18, 21, 22	1. Chromosomally normal embryos 2. The best morphologic features 3. On day 4	1. The best morphologic features 2. On day 3	No
Schoolcraft et al, 2009a[20]	AMA, ≥35 y, presence of at least five embryos with ≥6 cells and ≤15% fragmentation on Day 3	3 Days, blastomeres	X, Y,13, 15, 16, 17, 18, 21, 22	1. Chromosomally normal embryos 2. Routine blastocyst transfer 3. On day 5 or 6	1. Routine blastocyst transfer 2. On day 5 or 6	No
Debrock et al (2009)[21]	AMA, ≥35 y with at least 2 fertilized oocytes available on Day 1 after oocyte retrieval, and with at least 2 embryos consisting of ≥6 cells at day 3 after oocyte retrieva	3 Days, blastomeres	X, Y, 13, 16, 18, 21, 22	1. Chromosomally normal embryos 2. Routine blastocyst transfer 3. On day 5 or 6	1. Routine blastocyst transfer 2. On day 5 or 6	No
Meyer et al, (2009)[22]	Good prognosis, <39 y, normal ovarian reserve, body mass index 30 kg/m², no smoking history, no hydrosalpinx, presence of ejaculated sperm, normal uterus, ≥2 previous failed IVF cycles, ≥4 embryos containing at least 5 cells with, 40% fragmentation	4 Days, blastomeres	X,Y, 13, 16, 17, 18, 21, 22	1. Chromosomally normal embryos 2. Routine blastocyst transfer 3. ON day 5 or 6	1. Routine blastocyst transfer 2. On day 5 or 6	No

AMA = advanced maternal age, DET = double embryo transplantation, FISH = fluorescence in situ hybridization, ICSI = intracytoplasmic sperm injection, IVF = in-vitro fertilization, PGS = preimplantation Genetic Screening, RIF = repeated implantation failure, SET = single embryo transplantation.
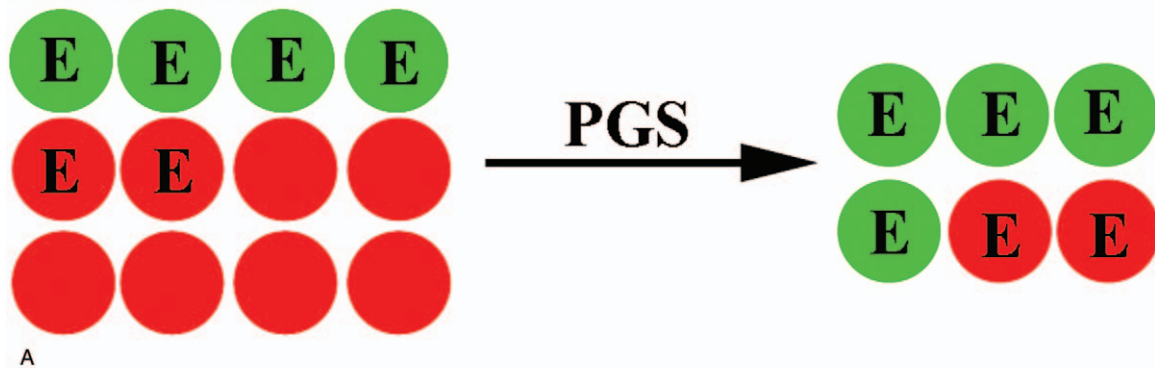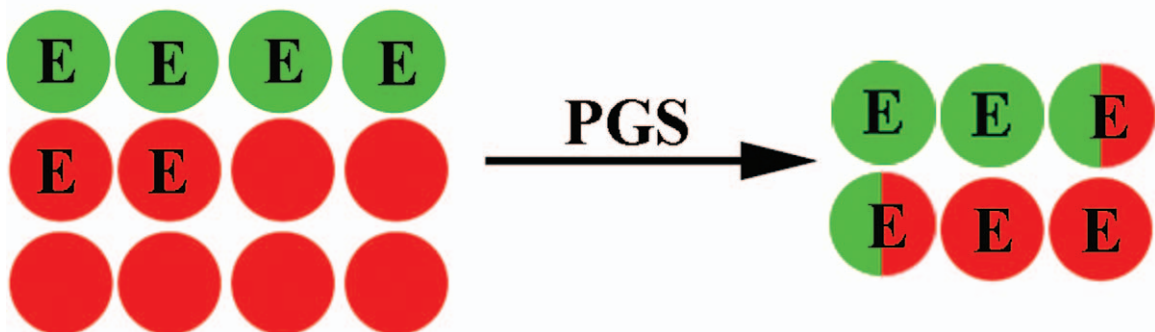
Figure 1. Two situations of PGS, live birth embryo marked by green rounds and other marked by red one. E is the abbreviation for euploid embryo. (A) Ideal situation: all euploid embryos can be successfully screened out and the embryos are not damaged by addition operations and misdiagnosis does not occur. (B) Factor situation: the embryo is damaged by additional operations, which leads the live birth embryos to destroy and not to be selected. PGS = preimplantation genetic screening.
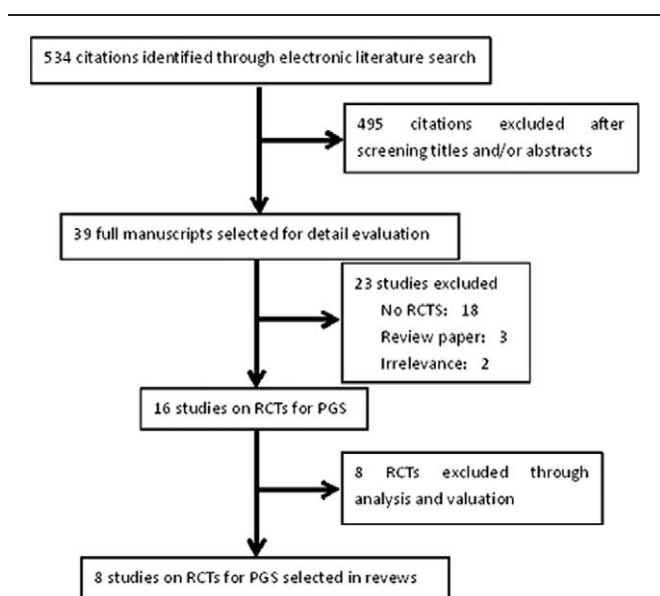
Figure 2. Flow chart of search for RCTs on PGS. PGS = preimplantation genetic screening, RCT = randomized controlled trial.

3.4. The live birth rate per embryo

There were 9 RCTs (2731 participants) which reported the live birth, multiple pregnancies, and the number of transfer embryos, so we can calculate live birth rates per embryo We observed a trend toward increase in the live embryos rate via the pooled data analysis (n = 2844, RR = 0.97; 95% CI, 0.82–1.15; P = .76), whereas there was no significant change and there was substantial heterogeneity between studies (I^2 = 69%).

Two trails (286 participants) were included in "the percent of euploid embryo >60%" subgroup as shown in Figure 2. The live birth rate per embryo was 36.1% (52 of 144) and 31.6% (45 of 142), respectively, in the control and PGS group and there was no significant change (P = .33). It is the meta-analysis (n = 286, RR = 1.17, 95% CI: 0.85–1.60) and there was no significant heterogeneity (I^2 = 0%, P = .38).

Six trials (1498 participants) were included in the "30%<the percent of euploid embryo<60%" subgroup. The live birth rate per embryo was 11.25% (118/1049) and 14.26% (194/1360), respectively in the control and PGS group. PGS significantly decreased live birth rate per embryo (n = 1498; RR = 0.80, 95%, CI: 0.80–0.98).

Single trial (149 participants) was included when evaluating the subgroup "the percent of euploid embryo<30%", which showed a

Table 2

Clinic outcomes of 8 RCTs.

First author/Publication year	Number of patients		Indication for PGS	The percent of euploid embryo (%)	Embryos per transfer		Transferred embryos		Live birth rates per transfer (%)		Live birth babies per embryo (%)		Live birth babies per embryo with PGS in theory (%)	Embry tolerance of PGS
	Ctrl	PGS			Ctrl	PGS	Ctrl	PGS	Ctrl	PGS	Ctrl	PGS		
The percent of euploid embryo >60%														
Staessen et al, (2008)[16]	120	120	Good prognosis	304/499 (60.92)	1	1	89	85	37 (30.83)	37 (30.83)	37 (41.57)	38 (44.71)	68.24	65.52
Mersereau et al, (2008)[17]	25	28	Good prognosis	118/174 (67.82)	2.1	2.1	53	59	8 (32.00)	14 (50.00)	8 (15.09)	14 (23.73)	22.25	100
30%<The percent of euploid embryo <60%														
Staessen et al, (2004)[14]	190	199	AMA	240/653 (36.75)	2.8	2.0	338	164	29 (23.97)	22 (27.16)	35 (10.36)	23 (14.02)	28.18	42.64
Mastenbroek et al, 2007ba[15]	202	206	AMA	653/1700 (38.41)	1.9	1.8	673	642	71 (35.15)	49 (23.79)	85 (12.63)	59 (9.19)	32.88	27.95
Blockeel et al. (2008)[18]	67	72	RIF	198/394 (50.20)	2.1	1.4	132	77	26 (38.81)	15 (23.79)	36 (27.27)	18 (23.38)	54.32	43.04
Debrock et al. (2009)[21]	50	44	AMA	73/241 (30.29)	2	1.6	84	63	10 (20.00)	6 (13.64)	11 (13.10)	7 (11.11)	43.25	25.69
Hardarson et al (2008)[19]	53	56	AMA	98/302 (32.45)	1.34	1.39	95	70	10 (18.87)	3 (5.36)	12 (12.63)	3 (4.29)	38.92	11.02
Meyer et al, (2009)[22]	24	23	Good prognosis	78/163 (47.85)	1.79	1.78	38	33	15 (62.50)	6 (26.09)	21 (55.56)	8 (24.24)	100	24.24
The percent of euploid embryo <30%														
Schoolcraft et al, 2009a[20]	30	32	AMA	99/352 (28.13)	2.7	2.2	81	68	20 (66.67)	25 (78.13)	20 (24.69)	25 (36.76)	87.77	41.88

AMA = advanced maternal age, FISH = fluorescence in situ hybridization, IVF = in-vitro fertilization, PGS = preimplantation Genetic Screening, RIF = repeated implantation failure.

significant difference in live birth rate per embryo between the PGS group (36.8%, 25/68) and the control group (12.3%, 10/81) (n = 149; RR = 2.98, 95%, CI: 1.54–5.75) (Fig. 2). This date indicated that when the percent of euploid embryo was <30%, PGS could increase live birth rate per embryo ().

3.5. Embry tolerance after PGS

With age, the quality and euploid of embryo decrease and the chromosome mosaicism increases, which weaken the embryos to tolerance of additional operations. In this article, we combined 9 RCTs to analyze the relationship between the percent of euploid embryo and embryo tolerance after PGS (Table 3).[14–22] Here, we investigated there was a positive correlation between them ($r = 0.80$, $P = .010$). Although the date was not from the same laboratory, the results provided some message that the embryos' damage should occur in the PGS. The result needs larger sample analysis to further verify.

4. Discussion

A biopsy of PGS is not a noninvasive examination and those additional operations for the embryos make them damage. In the process of biopsy, those additional operations such as taking an embryo out of the incubator, making a hole in the zona pellucid and aspirating one more cell and so on often occur.[8] Only embryo enduring those additional operations has the opportunity grow as a healthy live baby. It is a consensus that biopsy can influence the efficiency of PGS.[11,29] In medicine, a side effect occurs along with diagnosis and treatment, for example, drug therapy and radiodiagnosis. Gonal-F of main effects is stimulating follicle develop and its side effect is ovarian cyst and ovarian hyperstimulation syndrome (OHSS). PGS is also a method of diagnosis and treatment and its side effect exists naturally which is caused by biopsy, failure rate, and embryo mosaicism.[10] The biopsy is an additional and noninvasive operation. The age-related decline in fertility is attributable to both a decrease in conception rates and an increase in pregnancy loss rates.[30,31] This decline begins at around age 30, and accelerates after age 35, such that fertility is close to zero by the time a woman reaches age 45.[31] The percentage of aneuploid embryos is increased with AMA, which was the main reason of implantation failure and miscarriage. With the decreasing percent of euploid embryo, the tolerance of additional operations also decreases. However, the effect of biopsy alone on pregnancy rates has never been properly studied.

The indication of PGS is the high aneuploid rates in the transferred embryos. With higher aneuploid rates, the PGS efficiency is higher. Up to now, there was no article that reported the relationship between the percent of aneuploid embryos and the effect of PGS. All researchers paid much attention to the groups, AMA or RIF, who perhaps produced higher percent of aneuploid embryos.[10] However, the aneuploid rate was 30% to 50% in AMA, according to 8 articles included in this study, whereas in good prognosis patients, it is about 60%, and not all of AMAs have high aneuploid rates, so there was a cross-section between AMA and good prognosis patients in the aneuploid rate. So, it is unreasonable that AMA and RIF are regarded as the indications of PGS. Here, we first concentrate our attention on the percent of aneuploid embryos and PGS of efficiency. The subgroup was performed via the percent of euploid embryos. When the percent of euploid embryos is at the lowest level
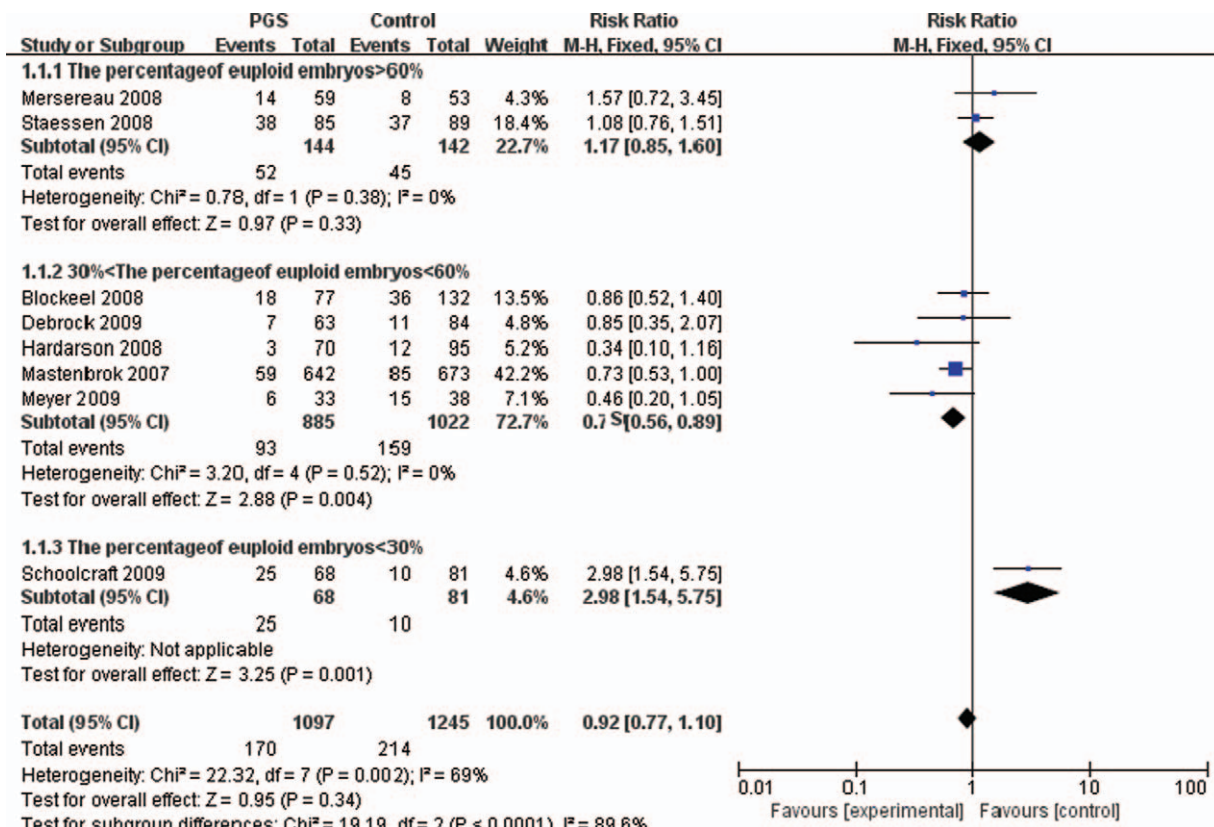
Figure 3. The effect of PGS on the live birth rate per embryo in different percent of euploid embryo. CI = confidence interval, PGS = preimplantation genetic screening

Table 3

The relationship between the percent of euploid embryo and embryo tolerance after PGS.

First author/ Publication year	Staessen et al, (2008)[16]	Mersereau et al, (2008)[17]	Staessen et al, (2004)[14]	Mastenbroek et al, 2007ba[15]	Blockeel et al, (2008)[18]	Debrock et al, (2009)[21]	Hardarson et al, (2008)[19]	Meyer et al, (2009)[22]	Schoolcraft et al. 2009a[20]	r	P
Embry tolerance (%)	65.52	100	54.08	27.95	43.04	25.69	11.02	24.24	41.88	0.80	.010
Euploid embryo (%)	60.92	67.82	42.64	38.41	50.2	30.29	32.45	47.85	28.13		

PGS = preimplantation genetic screening.

(<30%), there is a beneficial effect of PGS on live birth rates per embryo. But the percent of euploid embryos is difficult to foreknow based on AMA and RIF.

In a conclusion, with the decreasing percent of euploid embryo, embryo tolerance after PGS also decreased, which influenced the effect of PGS. However, the percent of euploid embryo is from 28.3% to 50.2% and is difficult to assess in AMA and RIF before biopsy, so PGS is used with caution. This finding was helpful to better understand the effect of PGS in different percent of euploid embryo.

Author contributions

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Supervision: Xinglu Jiang, Chenggui Zhao.
Validation: Rui Zhang.
Writing – original draft: Xinglu Jiang, Chenggui Zhao, Wei Xu.
Writing – review & editing: Xinglu Jiang, Chenggui Zhao, Wei Xu.

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