Multiple micromanipulations for preimplantation genetic diagnosis do not affect embryo development to the blastocyst stage

To determine the impact of multiple micromanipulation procedures for preimplantation genetic diagnosis (PGD) on embryo development, a retrospective analysis was performed of 9,925 embryos (862 PGD cycles), which were compared with 28,126 nonbiopsied embryos (2,751 consecutive intracytoplasmic sperm injection [ICSI] cycles) from the same time period. Because fertilization rates, the proportion of embryos with ≥ 6 cells on day 3, and blastocyst rates were similar in the PGD and control groups, we conclude that multiple micromanipulations on oocytes and embryos can be performed safely for PGD. (Fertil Steril® 2006;85: 1826–9. ©2006 by American Society for Reproductive Medicine.)

Preimplantation genetic diagnosis (PGD) has become an established clinical option in reproductive medicine for the identification of single-gene mutations, human leukocyte antigen matching, chromosome rearrangements, and aneuploidy in oocytes and embryos. Currently, the number of apparently healthy children born after PGD has exceeded 1,000 (1, 2). The majority of cases was performed for improved reproductive outcome in poor-prognosis IVF patients showing a positive clinical outcome after PGD in terms of improved implantation and decreased miscarriage rates (3, 4). However, despite these findings, the debate regarding the benefits of PGD for aneuploidy testing, in particular, is ongoing (5). More recently, investigators have reported a significant improvement in implantation and take-home-baby rates after PGD for chromosome abnormalities when compared with the previous obstetric history of the same patients before undergoing PGD (6, 7).

In addition to the many variables that impact IVF treatment outcome (e.g., age, ovarian stimulation response, oocyte and sperm quality, and in vitro culture conditions), patients undergoing PGD have the additional influence of potentially deleterious effects of oocyte or embryo biopsy, because this is the basis of performing PGD. The mechanism used to create an opening in the zona pellucida (mechanical, with a microneedle; chemical digestion, by acidified Tyrode's solution; or heat ablation, with a noncontact infrared laser) may impact further embryo development (8). In addition, the cell type being investigated (polar bodies, blastomeres, or both) as well as the number of biopsy procedures can vary depending on the clinical indications.

If there is more than one clinical indication for PGD, such as the risk of passing on a genetic mutation and a chromosomal abnormality as a result of advanced maternal age, there may be a need to perform more than one biopsy procedure on the same oocyte or embryo. These micromanipulations may be performed in addition to ICSI because ICSI is a mandatory requirement for PCR-based testing to avoid sperm DNA contamination. Preimplantation genetic diagnosis for aneuploidy should include first and second polar-body analysis because the majority of errors are contributed maternally (9, 10) and because this allows avoidance of potential misdiagnosis caused by the prevalence of mosaicism at the cleavage stage (11–14). Thus, with embryo biopsy, the number of micromanipulations may vary from one to three, in addition to ICSI.

The goal of our study was to determine the potential impact on further embryo development in relation to the stage and number of micromanipulations performed for PGD. We performed a retrospective analysis of a large series of 9,925 oocytes and embryos, after one to three biopsy procedures in 862 PGD cycles, in comparison to 28,126 nonbiopsied embryos that occurred consecutively in house, from January 2002 to December 2004.

Only cases in which ICSI was used to achieve fertilization in both the PGD and control groups were included. Preimplantation genetic diagnosis embryos were divided into three groups: PGD 1, those in which embryo biopsy was performed on day 3, requiring one micromanipulation procedure; PGD 2, those in which simultaneous polar body removal was performed after fertilization assessment on day 1 specifically for aneuploidy studies and in which afterward, a second micromanipulation procedure was performed on day 3 in which a single blastomere was removed; and PGD 3, those in which so-called sequential polar body removal was performed. In the PGD 3 category,

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Reprint requests: Jeanine Cieslak-Janzen, B.A., M.L.T., Reproductive Genetics Institute, 2825 N. Halsted, Chicago, Illinois 60657 (FAX: 773-871-5221; E-mail: rgi@flash.net).

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the first polar body (PB1) was removed before ICSI for cases involving maternal chromosome rearrangements or maternally derived dominant disorders or to access the maternal contribution of a recessive disorder. The second polar body (PB2) removal occurred after fertilization assessment on day 1. This was followed by embryo biopsy on day 3; thus, a total of three micromanipulations were performed on the same oocyte or embryo. These three PGD groups and control group were further divided by age: those derived from female patients of ages <35 and ≥35 years.

Fertilization rates and embryo development on day 3 were studied in both PGD and ICSI cycles. Early blastocyst (grade 1) development by days 5–6 according to Gardner's criteria (15) was accessed for all PGD groups and for those ICSI cycles in which all embryos were cultured until embryo transfer day 5. Data were analyzed by using Microsoft Excel software (Redmond, WA). Chi-square analysis was performed for all group comparisons. A P value of $<.05$ was considered significant.

Before initiation of the PGD cycle, patients were informed of the strategy and all possible risks of PGD during genetic counseling, and all signed an institutional review board–approved informed consent.

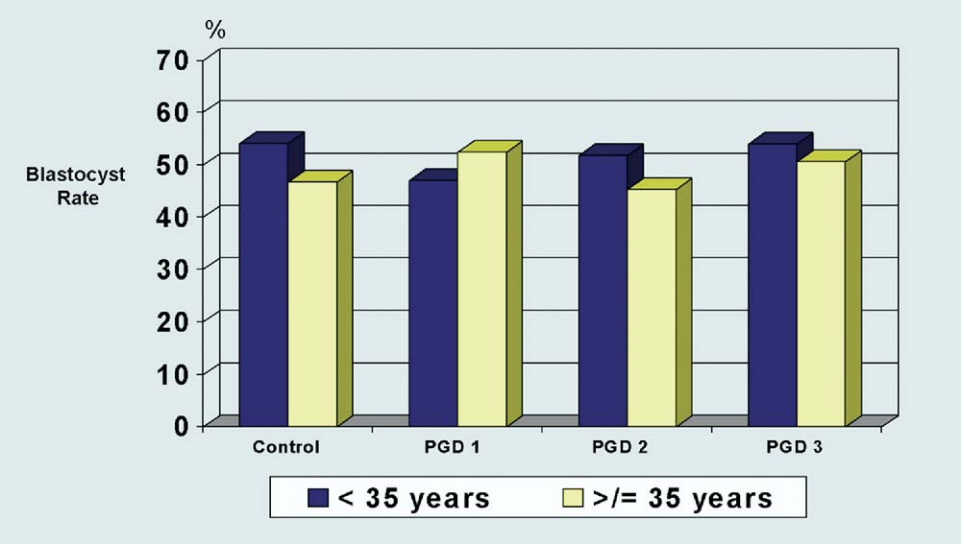
The microtool construction, the micromanipulation setup, and the procedures have been described elsewhere in detail (16). Oocyte retrieval was performed ~35 hours after hCG administration. Oocytes were denuded ~38–41 hours after hCG, taking into consideration that the premature removal of granulosa cells could cause artificial activation (17). Consideration also was given to the completion of cytoplasmic maturation (i.e., the spindle has moved from the point of polar-body extrusion) because removal of the PB1 too soon can result in enucleation of the oocyte as a result of the presence of a substantial cytoplasmic bridge (18).

Approximately 1 hour after denuding oocytes, 39–42 hours after hCG, the mechanical method of partial zona dissection was used to create an opening in the zona pellucida (19). A single slit was positioned so that after PB1 removal, ICSI could be performed through the opening in the zona pellucida at the three o'clock position away from the meiotic spindle. Immediately after PB1 removal, ICSI was performed, well within the time frame required for optimal fertilization results (20).

After 16–18 hours in culture, after fertilization assessment on day 1, PB2 removal also was performed through the same opening. If PB2 had been extruded away from the opening, a second intersecting slit was made in the zona pellucida (three-dimensional; partial zona dissection) to achieve access to PB2 (21). Both PB1 and PB2 were removed simultaneously after fertilization assessment on day 1 for aneuploidy testing. Embryo biopsy was performed on day 3 of embryo development by the creation of a flap opening with a second intersecting slit (16, 21).

FIGURE 1

Effect of one to three micromanipulations for PGD on oocytes and embryos on blastocyst development by female age. Bars indicate the percentage of blastocysts in each PGD group and in the control group (*blue bars,* patient ages <35 y; *yellow bars,* patient ages ≥ 35 y). No significant differences were found between groups.



Cieslak-Janzen. Blastocyst development after PGD. Fertil Steril 2006.

When early compaction was seen on day 3, calcium- and magnesium-free medium (Quinn's Advantage Medium with Hepes [Ca/Mg free]; Sage In-Vitro Fertilization, Inc, Trumbull, CT) was used to facilitate the disruption of intercellular adhesions and junction formation (16, 22). This allowed for the easy removal of a single blastomere to keep the amount of cellular mass removed to a minimum without damage to the embryo.

The mean fertilization rate was 82.8% (1,205/1,456) for 111 PGD cycles (90 patients; mean age ± SD, 32.8 ± 4.3 y) requiring PB1 removal before ICSI, compared with 79.5% (13,416/16,866) in 1,421 control cycles, with patient age of <35 years (1,137 patients; mean age, 30.7 ± 2.7 y) when only ICSI was performed ($P<.01$). There was no significant difference found in the percentage of one-pronuclear (4.3 vs. 3.4) and tri-pronuclear oocytes (4.1 vs. 3.6) between the PGD and control groups, respectively.

The proportion of embryos with six or more cells on day 3 in each group for all ages was compared. No significant difference was found between PGD 1 (57.8% [203/351]; mean age, 35.4 ± 4.8 y) and PGD 2 (56.8% [3,649/6,420]; mean age, 37.7 ± 3.8 y) compared with the control group (56.3% [12,468/22,134]; mean age, 34.3 ± 4.5 y). In PGD 3, 60% (723/1,205) of embryos had at least six cells on day 3, which was significantly higher ($P<.05$) compared with the control group. This may be attributed to the lower average maternal age of 32.9 ± 4.2 in group PGD 3.

Figure 1 shows the proportion of embryos reaching the early blastocyst stage for the three PGD groups and the control group for patients of ages <35 and ≥35 years. Early blastocyst rates by the morning of day 6 for patients in the younger age group for PGD groups 1, 2, and 3 and for the control group were 47.0 % (55/117), 51.8% (579/1,118), 54.0% (313/580), and 54.1% (5,693/10,526), respectively. Early blastocyst rates for patients ≥35 years of age, PGD groups 1–3, and the control group were 52.5 % (106/202), 45.3% (1338/2952), 50.6% (132/261), and 46.8% (2,669/5,701), respectively. No significant difference was found for each of the PGD groups when compared with the control group in both age categories.

When a narrower time frame was applied of approximately 114 hours after ICSI, the morning of day 5, once again, no significant difference was seen in early blastocyst development between the PGD groups (age <35 y; PGD 1, 39.3% [46/117]; PGD 2, 41.6% [465/1,118]; and PGD 3, 43.1% [250/580]) when compared with the control group (43.5% [4,583/10,526]) and between PGD groups (age ≥35 y; PGD 1, 33.2% [67/202]; PGD 2, 37.6% [1,109/2,952]; and PGD 3; 40.6% [106/261]) compared with the control group (38.7% [2,204/5,701]).

To our knowledge, this is the largest study regarding the development of oocytes and embryos after sequential or simultaneous PB1 and PB2 removal and embryo biopsy. Our results concur with a previous report by Magli et al. (23) demonstrating that the combination of polar body and embryo biopsy does not affect embryo viability.

Although this was a retrospective review, the large numbers of embryos render the data meaningful and useful. The timing of all micromanipulation procedures is considered important to fertilization and development. The nondetrimental effect of 1–3 biopsy procedures revealed in our study further supports the use of the mechanical method to create an opening in the zona pellucida. Moreover, the practice of single blastomere removal during embryo biopsy on day 3 also may have contributed to our results.

We conclude that sequential or simultaneous polar-body removal with subsequent embryo biopsy of a single blastomere does not compromise embryo development. Multiple micromanipulations can be performed safely on oocytes and embryos for PGD of more than one clinical indication and to increase accuracy with aneuploidy testing. These data are useful and important for all centers offering PGD.

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Ilan Tur-Kaspa, M.D.
Yuri Ilkevitch, Ph.D.
Alejandra Bernal, M.S.
Randy Morris, M.D.
Yury Verlinsky, Ph.D.
IVF and Preimplantation Genetics Diagnosis Program, Reproductive Genetics Institute, Chicago, Illinois

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