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Comparison of the results of human embryo biopsy and outcome of PGD after zona drilling using acid Tyrode medium or a laser

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BACKGROUND: Zona pellucida opening for blastomere removal can be done by mechanical or chemical means, or by laser. So far, only limited data on the use of laser systems for zona drilling in cases of PGD are available. METHODS: Results of embryo biopsy and outcome of PGD in two periods were compared. In the first period, acid Tyrode medium was used for zona drilling. In the second period, zona drilling was performed by a 1.48 μm infrared laser. RESULTS: In the first period, 59 cycles resulted in 53 biopsy procedures with 356 biopsied embryos. In the second period, these numbers were 69 cycles, 69 biopsy procedures and 402 biopsied embryos. Fewer blastomeres were intact (95.2%) after zona drilling with acid Tyrode than after laser zona drilling (98.3%, *P* = 0.02). Rates of positive HCG (37.5% versus 35.5%), ongoing pregnancy rates (31.3% versus 25.0%) and ongoing implantation rates (18.9% versus 14.9%) did not differ. CONCLUSIONS: The use of a laser for zona drilling in cases of PGD is an easier procedure and results in more intact blastomeres. Since similar pregnancy rates are obtained, it is advantageous to use a laser for zona drilling. Further follow-up is necessary to prove the safety of this procedure.

Key words: acid Tyrode/embryo biopsy/laser/PGD/zona drilling

Introduction

PGD can be performed to avoid replacement of embryos affected by a monogenic disease or carrying chromosomal aberrations (Vandervorst *et al*., 2000). The diagnosis can be performed at different stages of embryo development. Opening the zona pellucida (ZP) is required irrespective of the stage of development. Before the introduction of PGD, different micromanipulation procedures had already been used to create an opening in the ZP. In mice and in humans, these procedures were used to increase fertilization rates in cases of poor semen quality (Gordon and Talansky, 1986; Garrisi *et al*., 1990; Wazzan *et al*., 1990) or to assist the hatching process (Cohen, 1991; Tucker *et al*., 1991; Khalifa *et al*., 1992). The opening in the ZP was usually made either mechanically or chemically. In humans, the same methods of zona drilling have also been used with PGD (Handyside *et al*., 1989, 1990; Verlinsky *et al*., 1990; Verlinsky and Cieslak, 1993).

In the last decade, the use of lasers as an alternative method of zona drilling has been evaluated and introduced into the IVF laboratory. Different laser systems have been tested. The first lasers worked in the UV range and were contact-mode lasers (Laufer *et al*., 1993; Antinori *et al*., 1994; Obruca *et al*., 1994). These systems have several disadvantages, such as possible DNA damage due to absorption of UV light or the need for a complex set-up that delivers the laser energy directly to the target rather than being absorbed by aqueous solutions (Tadir *et al*., 1994). Relatively larger wavelengths are not absorbed by DNA or water, so that they can be used in a non-contact mode (Tadir *et al*., 1994). Lasers working in the infrared (IR) range are highly suitable in this respect. In the mouse, Germond *et al*. (1995, 1996) showed that IR lasers can be used to assist the hatching process. The results from these studies do not indicate any negative effect on embryo development or on offspring. More recently, an application for human assisted hatching has been published (Germond *et al*., 1999; Montag and van der Ven, 1999; Baruffi *et al*., 2000; Mantoudis *et al*., 2001; Petersen *et al*., 2002), and the performance of laser zona drilling (LZD) was compared with other drilling procedures (Malter, 2001; Balaban *et al*., 2002; Hsieh *et al*., 2002). So far, however, data on the use of this type of laser for zona drilling with a view to embryo biopsy for PGD are limited (Veiga *et al*., 1997; Boada *et al*., 1998).

In this study, we evaluated if the use of a laser to open the ZP in cases of PGD has any influence on the results of the biopsy procedure, further embryo development *in vitro* or the outcome after embryo replacement, compared with chemical zona drilling.

Table I. Results of human embryo biopsy after zona drilling with acid Tyrode (ATD) or a laser (LZD)

	ATD	LZD	
Cycles with biopsy	53	69	
Biopsied embryos	356	402	
Successfully biopsied embryos[a]	356	402	
Aspirated blastomeres (A)	681	775	
Intact blastomeres (% of A)	648 (95.2)	762 (98.3)	$P = 0.02$
Cells lysed during zona drilling	19	0	
Cells lysed during blastomere manipulation	14	13	

[a]According to the definition of the ESHRE PGD Consortium.

Materials and methods

Fertilization and embryo culture

Oocyte denudation was carried out as described previously (Van de Velde *et al*., 1997) using 80 IU of hyaluronidase per ml. ICSI was performed after 2 p.m. on the day of oocyte retrieval, allowing assessment of fertilization within 18 h after ICSI early the next morning. After the injection procedure, oocytes were incubated in G1.2 medium (Vitrolife, Goteborg, Sweden). Further evaluations of embryo development were performed on the morning of the second day and the third day after oocyte retrieval. Where possible, the number of blastomeres was counted and recorded for each individual embryo. In other cases, the term compaction was used.

Embryos with <50% of their volume filled with anucleated fragments were considered suitable to undergo the biopsy procedure.

Preparation of microtools

Home-made microtools with the following characteristics were prepared from washed and sterilized capillaries. The holding pipette had an outer diameter of 100 µm and an opening of 25–30 µm. The biopsy pipette had an inner diameter of 35–40 µm. The opening was fire polished. The drilling pipette had a more tapered shape, with an outer diameter of 10–12 µm and an opening of ~5 µm. The microtools were sterilized the day before the biopsy procedure.

Embryo biopsy

Except for zona drilling, all laboratory procedures were similar for all PGD cycles included. Zona drilling using acid Tyrode (ATD) was performed between October 1998 and May 1999. From June 1999 until December 1999, LZD was used.

Dishes with 25 µl droplets of Ca^{2+}–Mg^{2+}-free medium (EB-10, Vitrolife) covered with mineral oil (M-8410, Sigma, St Louis, MO) were prepared and incubated overnight in an environment of 6% CO_2, 5% O_2 and 89% N_2. Just before the biopsy procedure, embryos selected for biopsy were incubated in this medium for between 5 and 10 min, depending on the degree of compaction, unless there were obvious signs of total absence of compaction. The biopsy procedure was performed in 50 µl droplets of HEPES-buffered Earle's medium supplemented with 0.5% (w/v) human serum albumin (HSA) covered with mineral oil. In cases of ATD (ZD-10, Vitrolife), one extra droplet with acid Tyrode solution was present.

The microtools were fixed and aligned on an inverted microscope (Diaphot 300, Nikon, Tokyo, Japan) equipped with Hoffman Modulation Contrast optics and a heated stage. A double tool holder carrying both the drilling and the aspiration pipette was used in cases with ATD. Needles were always replaced in cases of cell lysis. The embryo was visualized and fixed on the holding pipette after aspiration of the acid into the drilling pipette. Drilling was performed by releasing acid onto the ZP between two blastomeres. Strong aspiration was applied upon rupture of the ZP.

In cases with LZD (Fertilase, Octax, Herbron, Germany), embryos were fixed on the holding pipette in a similar way. Two, or exceptionally three, pulses of 5–8 ms (1.48 µm) were applied after correct positioning of the ZP using the target generator. Again, the opening was made between two blastomeres.

After zona drilling by either acid Tyrode or laser, blastomeres were aspirated gently, removed from the embryo and released into the medium. Biopsied embryos were transferred to 25 µl droplets of G2.2 medium (Vitrolife) and cultured until the time of diagnosis and possible replacement. Two blastomeres were aspirated from embryos containing at least seven cells. Exceptionally, a third blastomere was aspirated in cases where lysis of one of the two other cells occurred. Only one blastomere was aspirated from embryos with five or six blastomeres in some cases of fluorescence in-situ hybridization (FISH) analysis.

Outcome of embryo biopsy was presented in two different ways. The first is according to the definition of the ESHRE consortium on PGD (Harper and Thornhill, 2001). Here, the definition of successful biopsy was: 'the removal of a cell without lysis such that the cell could be used for analysis'. The second way presents the ratio of intact blastomeres to the total number of aspirated blastomeres.

The diagnosis was performed by PCR in order to avoid replacement of embryos affected by a monogenic disease, or by FISH (translocation carriers or sexing) to avoid replacement of embryos with an abnormal chromosomal constitution or affected by an X-linked disease (Vandervorst *et al*., 2000). After diagnosis, the best quality embryos considered normal for the tested chromosomes or mutation, which had developed further between the biopsy and time of the diagnosis, were replaced if selection was possible. Embryo replacement was performed on day 3 or day 4 after oocyte retrieval depending on the time necessary to obtain the results of the diagnosis.

Statistics

An unpaired *t*-test was performed to calculate differences in blastomere survival. The χ^2-test was applied to compare pregnancy and implantation rates. The Mantel–Haenszel test was applied to compare the outcome measures with regular ICSI cycles performed in the same period. Differences were considered significant for *P*-values <0.05.

Results

In the first period, 59 oocyte retrievals resulted in 53 biopsy procedures, 28 for FISH analysis and 25 for PCR. In the second period, all oocyte retrievals (69) resulted in a biopsy procedure, 31 for FISH and 38 for PCR. No biopsy was performed in six cycles from the first period because of the following reasons: (i) very poor quality of the embryos (>50% of the embryos were filled with anucleated fragments); (ii) none of the embryos reached the third mitotic division on day 3; and (iii) in one cycle, the patient opted not to proceed with the procedure after the recovery of only two oocytes.

An average of 1.91 blastomeres per embryo was aspirated in the first period. This figure was almost identical (1.93) in the second period. In both groups, all embryos were biopsied successfully according to the definition of the ESHRE PGD consortium. However, when evaluating outcome of individual blastomeres, significantly more intact blastomeres were obtained when performing LZD (95.2 versus 98.3%, P =

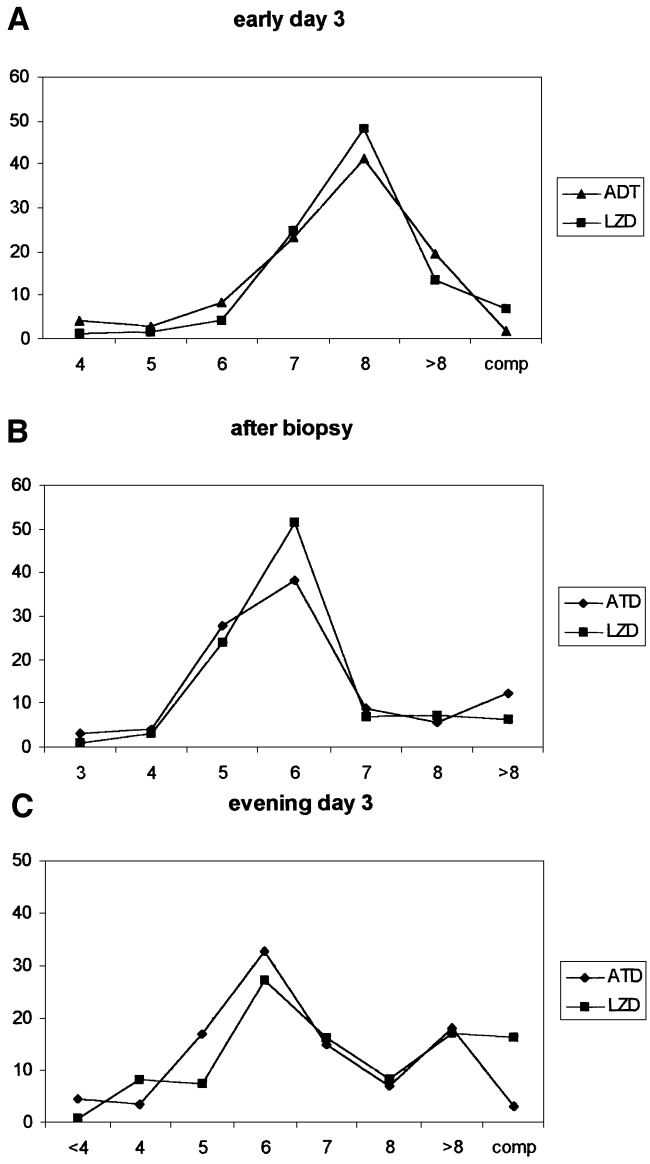

Figure 1. Embryo development early on day 3 (**A**), after embryo biopsy (**B**) and in the evening of day 3 (**C**). Points on the *x*-axis show the developmental stage (comp = compacted embryo). The *y*-axis represents the percentage distribution.

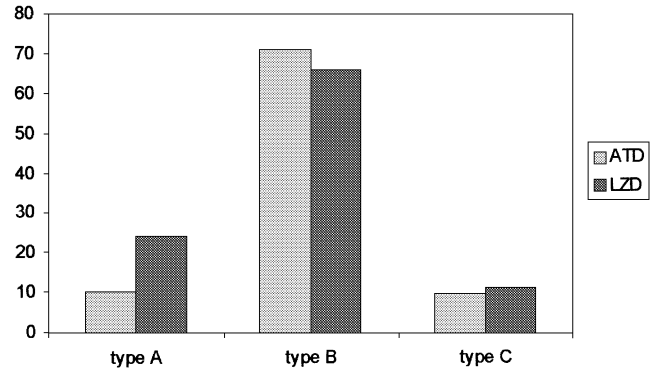

Figure 2. Embryo quality early on day 3 of embryos before biopsy: type A, embryos without fragmentation; type B, embryos with <20% anucleate fragments; and type C, embryos with 21–50% of their volume filled with fragments.

0.02). The numbers of biopsied embryos, aspirated blastomeres and intact blastomeres, as well as the causes of lysis, are summarized in Table I. Lysis of aspirated blastomeres after zona drilling did not occur when the laser was used, while ~60% of the lysed cells after ATD were a consequence of the drilling procedure only.

The actual time needed to create a hole in the ZP by blowing acid onto the zona or by activating the laser two or three times was in the same range for both procedures and usually took between 10 and 20 s. In practice, one embryologist performed all aspects of the biopsy procedure. The overall time required to perform the embryo biopsy procedure was in favour of LZD because (i) changing of needles in cases of cell lysis occurred less frequently; (ii) the alignment of two needles instead of three is easier and faster; and (iii) the extra manipulations necessary to (re)fill the drilling needle with acid and the necessary precautions to avoid unnecessary blowing of acid are absent.

After ATD, a third blastomere was aspirated in 23 embryos (23/356 = 6.5%) of which 15 were considered transferable after diagnosis. Nine of these embryos have been replaced (seven embryos) or cryopreserved (two embryos). Four pregnancies were established in the seven cycles where these embryos were used and, in two cases, one of which was with a single embryo transfer, there is evidence of implantation and development to term from these embryos. After LZD, 15 (15/402 = 3.7%) embryos were subject to aspiration of a third cell. From these, eight were considered genetically normal and six were used for replacement (five embryos) or cryopreservation (one embryo). Again, there is evidence of implantation of such an embryo in one of the two pregnancies where they were replaced. In this series, a third blastomere was not always aspirated because of lysis, but in a few cases because no DNA was found during fixation of the cells.

The developmental stage and the quality of the biopsied embryos early on day 3 were similar in both groups (Figures 1A and 2 respectively). A reduction in the number of blastomeres as a consequence of the biopsy procedure can clearly be observed in Figure 1B. Because of the higher damage rate after ATD and the resulting removal of a third blastomere in a number of embryos, fewer embryos were at the 6-cell stage and more embryos at the 5-cell stage after the biopsy procedure in this group. There was no impact of the biopsy procedure on embryo quality. Figure 1C represents the stage of development in the evening of day 3. The patterns of development between the moment of biopsy and the evening of day 3 were similar in both groups, except for the degree of compaction. After LZD, more embryos compacted earlier than after ATD. Figure 3 represents only those cycles with embryo replacement on day 4 (47 cycles). Again, similar patterns in overall development can be observed. After ATD, 63.3% of the biopsied embryos had more than eight cells or were at the morula stage. This rate was very similar after LZD (58.2%). However, the difference in initiation of compaction already observed on the evening of day 3 was even more pronounced

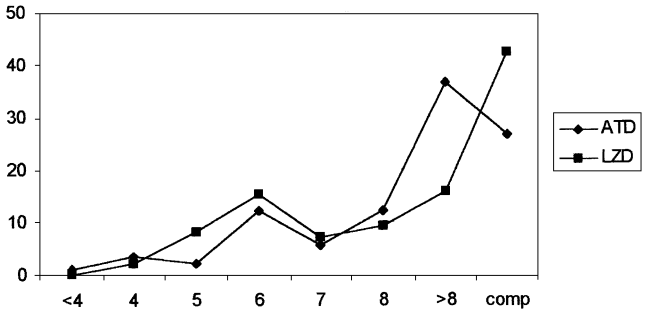

Figure 3. Developmental stage on day 4 for those cycles where embryo replacement took place on day 4.

on day 4 (26.7% of biopsied embryos after ATD versus 42.4% after LZD show compaction). When comparing developmental stages of biopsied embryos between pregnant and non-pregnant patients, a trend towards a higher number of 8-cell embryos irrespective of the mode of zona drilling was observed in pregnant patients (70 versus 52.6% after ATD, and 75.4 versus 67.7% after LZD respectively).

The patients' age and rank of trial were similar in both periods, i.e. 32.3 ± 4.4 years and 2.1 ± 1.4 respectively, in the group with ATD, and 32.2 ± 4.9 years and 2.0 ± 1.3 for LZD. A total of 111 embryos considered normal for the tested monogenic disease or for chromosomes were replaced in the first period (mean, 2.09 per transfer; range, 1–5). In the second period, 126 embryos were replaced (mean, 2.03 per transfer; range, 1–5). The technical requirements for obtaining the diagnosis determined whether embryo replacement was performed on day 3 or day 4 after oocyte retrieval. Sixty-three embryo replacements were performed on day 3 and 47 on day 4.

The numbers of embryo replacements and the numbers of positive hCG are listed in Table II. Comparison of outcome measures between the different subgroups is difficult because of the size of the subgroups and the heterogeneity of the patient population. However, outcomes of embryo replacements in relation to the day of embryo replacement and the procedure for diagnosis used are summarized in Table III. In general, embryo replacement on day 3 or day 4 did not influence the outcome. Initial and ongoing pregnancy rates were 36.5 and 29.0% for day 3 embryo replacements and 36.1 and 25% for day 4 embryo replacements respectively. In the second period, two pregnancies were lost to follow-up. In the first period, two biochemical pregnancies and one miscarriage (3/18 = 16.7%) occurred after FISH diagnosis, while one biochemical pregnancy after FISH diagnosis and four miscarriages (one after FISH diagnosis and three after PCR diagnosis) were recorded in the second period (5/20 = 25%). Ongoing implantation rates in cycles with known outcome were 18.9% (21/111) after ATD and 14.9% (18/121) after LZD. These results are not statistically different.

Initial pregnancy rates per embryo transfer, delivery rates of cycles with known outcome and ongoing implantation rates in all regular ICSI cycles performed in both study periods served as a control group. A total of 685 ICSI cycles were performed in the first period and 593 ICSI cycles in the second period. These resulted in 252 and 242 positive hCG respectively. The three outcome parameters for the control groups were 36.8, 27.8 and 13.2% for the period with ATD, and 40.8, 28.6 and 15.8% for the period with LZD, respectively. No differences were observed in any of the three parameters.

Table II. Rates of positive hCG and outcome of pregnancies

	ATD	LZD	
Embryo replacements	48	62	
Positive hCG (%)	18 (37.5)	22 (35.5)	NS
Clinical pregnancies	15	15[a]	
Deliveries (%)	15 (31.3)	15 (25.0[a])	NS
Singletons	9	12	
Twins	6	3	
Number of embryos replaced	111	126	
Number of embryos included	111	121[a]	
Ongoing implantations	21	18	
Implantation rate	18.9	14.9	NS

[a]Two pregnancies were lost to follow-up.

Table III. Embryo replacements according to the procedure for diagnosis and the day of embryo replacements after ATD and LZD

	ATD				LZD			
	Day 3		Day 4		Day 3		Day 4	
	FISH	PCR	FISH	PCR	FISH	PCR	FISH	PCR
Embryo replacements	12	21	12	3	9	21	17	15
Positive hCG	4	7	5	2	3	9	4	6
% positive hCG	33.3	33.3	41.7	66.6	33.3	42.9	23.5	40.0

A total of 39 children was born from 30 deliveries, 21 children in the group with ATD and 18 after LZD. In both groups, one major malformation was observed. Gestational age for singleton and twins deliveries was 39.2 weeks (range 37.6–40.4) and 34.6 weeks (range 29.1–37.0) after ATD, and 39.0 weeks (range 37.3–40.2) and 35.1 weeks (range 31.5–37.3) after LZD respectively. Average birth weights were 3408 g (range 2710–4380) and 2243 g (890–3010) for singletons and twins after ATD, and 3065 g (range 2600–3595) and 2334 g (range 1495–2910) for singletons and twins after LZD respectively.

Discussion

In this study, we aimed to evaluate the possible impact of a newly introduced procedure of zona opening (LZD) in PGD. Although there is evidence that neither in-vitro (Hardy *et al*., 1990) nor in-vivo development (Ao *et al*., 1996) is impaired after aspiration of up to two blastomeres from 8-cell human embryos, little attention has been paid to the possible impact of the way the opening in the ZP is made. Over the years, zona opening to assist the hatching process has been performed using different procedures. Recently, improved pregnancy and implantation rates after LZD have been observed by Hsieh *et al*. (2002), while others found similar results irrespective of the procedure of zona drilling (Malter, 2001; Balaban *et al*., 2002). In PGD, zona drilling with acid Tyrode has been used most often (ESHRE PGD Consortium Steering Committee, 1999,

2000) but the use of a laser for zona drilling is increasing (ESHRE PGD Consortium Steering Committee, 2002). In our PGD programme, ATD was applied until May 1999. LZD for clinical PGD was introduced in June 1999 after evaluation of the procedure on human embryos originating from abnormal fertilization. However, limited to in-vitro development, this preliminary testing did not indicate a deleterious impact of laser drilling on embryo development.

In our hands, a somewhat easier set-up together with fewer manipulations when using a laser for zona drilling meant that embryo biopsy with LZD took less time. This advantage of LZD has been confirmed by others (Balaban *et al*., 2002; Hsieh *et al*., 2002). Nevertheless, differences in procedures (Joris *et al*., 1999) or differences in experience between operators may also influence outcome or the time needed.

So far, no single uniform definition of success rates of embryo biopsy exists. It can be defined per embryo (Harper and Thornhill, 2001) or per individually aspirated blastomere. We think that the latter provides the most detailed information. Using this definition, the present study shows that LZD resulted in more intact cells than ATD. This difference was the result of the impact of acid Tyrode during the drilling procedure. Damage to blastomeres during aspiration into or expulsion from the biopsy pipette was similar in both groups. Easier blastomere lysis after ATD may be related to the influence of the acid solution (Depypere and Leybaert, 1994). Studies on zona drilling with acid Tyrode in assisted hatching usually do not discuss possible lysis of blastomeres during the procedure. Yet, early studies of zona drilling with acid Tyrode in assisted fertilization present oocyte lysis rates of 10% (Malter and Cohen, 1989) up to 33% (Gordon *et al*., 1988; Garissi *et al*., 1990). Although limited data on lysis rates of individual blastomeres during embryo biopsy are available in the literature, lysis rates reported after ATD (Chen *et al*., 1998; Inzunza *et al*., 1998) and after LZD (Lamb *et al*., 2002) are similar to those obtained in the present study.

Apart from technical failures related to the diagnostics procedure, lysis of blastomeres during embryo biopsy may be the cause of conflicting results when diagnosis is attempted on lysed cells (Sermon *et al*., 1998). We consider the availability of two intact blastomeres mandatory for PCR analysis and preferential for FISH analysis. As demonstrated in this series and also reported earlier (Van de Velde *et al*., 2000), occasional aspiration of three blastomeres from 8-cell embryos may result in ongoing pregnancies in humans. Nevertheless, this should be avoided as much as possible because aspiration of more than two cells results in a reduced developmental capacity *in vitro* as well as *in vivo* (Liu *et al*., 1993).

Safety aspects of LZD are currently being discussed, and, in particular, thermal effects should be minimized. Therefore, short pulse durations ($\leqslant$5 ms) and an appropriate distance from adjacent blastomeres (>8 μm) are recommended (Chatzimeletiou *et al*., 2001; Douglas-Hamilton and Conia, 2001) in order to avoid damage. From the start, we applied similar safety measurements in our LZD procedure: we use short pulse times (5–8 ms) in an area between cells. In most cases, two pulses are enough to create a hole that is large enough for blastomere removal. In addition, the lack of damage at the ultrastructural and biological level has been demonstrated by Germond *et al*. (1995) using the same laser system for zona drilling. Even if membrane damage without lysis may occur to the cells close to the site of drilling, those cells are removed and manipulated immediately for diagnosis. Although the use of acid Tyrode is considered safe, a temporary but significant cytoplasmic acidification in oocytes following zona drilling has been demonstrated by Depypere and Leybaert (1994). Similar to LZD, ATD may damage blastomeres close to the drilling site (Van Golde *et al*., 1996).

The total rates of embryos that developed further after the biopsy procedure were similar in the two study groups. However, a tendency towards lower compaction rates at these developmental stages after ATD can be observed. This difference in development is also observed in mouse embryos (Chatzimeletiou *et al*., 2001). In humans, when using sequential culture media, compaction may occur already on day 3 (Van Langendonckt *et al*., 2001). Zona drilling with acid Tyrode thus seems to influence embryo development *in vitro* more than LZD does. Chatzimeletiou *et al*. (2001) also show that, in the mouse, the impact of ATD on blastocyst formation is greater than that of LZD. This finding needs to be confirmed on human embryos. However, in humans, this impact may be less pronounced since pregnancy rates after ATD in assisted hatching (Cohen *et al*., 1992; Malter 2001; Balaban *et al*., 2002) or PGD (ESHRE PGD Consortium Steering Committee, 1999, 2000) are acceptable. When comparing patterns of embryo development between pregnant and non-pregnant patients within and between the two study groups, a tendency towards a higher speed of development both before and after the biopsy procedure was observed in pregnant patients, irrespective of the procedure used for zona drilling. This indicates that intrinsic embryo quality influences the chances of success, rather than the manipulation procedure *per se*.

PGD is a complex procedure in which the embryo biopsy procedure is just one step in a cascade of events leading to the replacement of embryos healthy for the tested disease or chromosomal aberration. It is therefore very difficult to determine the impact of just the embryo biopsy procedure. Initial pregnancy rates in this study were similar in both groups, indicating that further development to the blastocyst stage was similar irrespective of the zona drilling procedure. Considering that pregnancy rates after PGD with PCR are similar irrespective of the day of embryo replacement (day 3 or day 4) and the method of zona drilling performed (ATD or LZD) (Table III), other variables such as age, indication for PGD and the number of embryos available for diagnosis, rather than the biopsy procedure *per se*, influence the results. This explains the difference observed in results obtained after FISH diagnosis. In this group, more translocation cases were performed when LZD was used. Since the number of transferable embryos is lower in this specific patient population (ESHRE PGD Consortium Steering Committee, 2002), this explains the trend towards a lower number of replaced embryos on day 4 (1.71) compared with day 3 embryo replacements (2.22), and may thus affect outcome.

Over the years, after PGD, we have performed embryo replacements on different days after oocyte retrieval. Similar

pregnancy rates have been established from periods where embryo replacement took place on day 3, day 4 or day 5 (data not shown). Additionally, preliminary results of a prospective randomized controlled trial for aneuploidy screening in which all embryo transfers are performed on day 5 do not indicate a negative effect of extended culture on biopsied embryos compared with the non-treated control group (Staessen *et al*., 2002). Thus, the relevance of a possible impact of the day of embryo transfer on the results of the present study is probably minimal.

Overall, the implantation potential of transferred embryos in cycles with known outcome did not differ between the study groups. Although no statistical differences were observed, we acknowledge that the numbers in both groups are small. Moreover, the implantation rate obtained after ATD in the study period was somewhat increased compared with results obtained earlier (Vandervorst *et al*., 2000). After LZD, initial pregnancy rates and ongoing implantation rates in a recent 8 month period where all culture conditions were similar to those in the study period were 32.7 and 14.7% respectively. Nevertheless, a final conclusion on the impact of the drilling procedure on ongoing pregnancy rates requires a longer follow-up period. Up till now, we have continued to use the laser for zona drilling, replacing the ATD procedure. So far, no evidence of a possible negative effect of LZD has appeared. Our findings have been confirmed by other studies where a comparison of two (Malter, 2001) or more (Balaban *et al*., 2002) different procedures of zona drilling in assisted hatching did not show different pregnancy and implantation rates.

Twenty-one children were born from 15 deliveries after ATD. Of these, 12 children were from twin pregnancies and nine from singleton pregnancies. Three of these children were stillborn: one from a singleton pregnancy born at 23 weeks and two from a monozygotic monochorionic monoamniotic twin pregnancy born at 21 weeks. This leaves us with 18 live-born children from 13 deliveries. No malformations were recorded in the singletons, but two of these children now have a developmental delay at 2 years of age. From the 10 twin children, one child died at a few days of age from a chylothorax, and one child (birthweight 890 g) has diplegia. In the second period, 18 children were born from 15 pregnancies. Of these, 12 children were from singleton pregnancies and six from twin pregnancies. They were all live-born. One of the singleton children presented with an inherited and isolated syndactyly between fingers 4 and 5.

Of course these numbers are too small to provide any general conclusions but mean gestational age and birth weight are comparable between the two groups and with the data reported in our ICSI follow-up study (Bonduelle *et al*., 2002). With regard to malformations and complications, very few detailed data on PGD children are available with which to make any comparison (ESHRE PGD Consortium Steering Committee, 2000, 2002; Strom *et al*., 2000).

In conclusion, we confirm that the use of the 1.48 μm IR laser system results in an easier drilling procedure, but more important is the observation that fewer blastomeres are damaged. LZD does not influence further development *in vitro* and gives rise to similar pregnancy and implantation rates as after ATD. Although the current data are reassuring, more data on pregnancy and implantation rates as well as on pregnancy outcome and health of the children born have to be collected to prove the safety of this procedure.

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