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REVIEW

Method of Embryo Biopsy for Preimplantation Genetic Diagnosis

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ABSTRACT

Preimplantation genetic diagnosis (PGD) has been developed and offered the opportunity of transferring only the detected normally genetic embryos to the couples at risk of inherited disease transmission. Various methods of isolating the genetic material from preconception oocytes and embryos in PGD have been developed. Polar bodies, blastomeres and trophectoderm cells were isolated at various stages of preimplantation development for PGD. Several biopsy techniques have been introduced, for instance, slitting, piercing and drilling with acid Tyrode's solution or laser. The zona pellucida drilling with acid Tyrode's solution followed by aspiration method has been eventually world-wide used for blastomere removal in PGD. Ca^{2+} and Mg^{2+} free medium has been used to loosen the gap junction between blastomeres without any adverse effects in the biopsy procedure. More researches in PGD including the techniques of genetic material isolation, however, should be carried out to define its effectiveness and safety. The long-term development of children derived from PGD should also be monitored to assure that PGD is useful and safe for universal practise.

Key words: preimplantation genetic diagnosis, embryo biopsy, acid Tyrode's solution, laser, Ca^{2+} and Mg^{2+} free medium

Preimplantation genetic diagnosis (PGD) has provided a capacity of detecting genetic abnormalities occurring in the embryos before transferring them to the uterine cavity in in vitro fertilisation (IVF).[1-3] This early genetic diagnosis has allowed the selection of unaffected embryos to the couples at risk of inherited disease transmission to their offspring and avoiding of termination of affected pregnanciese.[4] PGD consists of three important processes, which are in vitro fertilisation, isolation of genetic material for further genetic diagnosis, and molecular genetic diagnosis based on polymerase chain reaction (PCR) or fluorescent in situ hybridisation (FISH). With regard to genetic material isolation or embryo biopsy, there are many important factors to be concerned, such as stage at biopsy, number of cells to be removed, methods of zona pellucida opening and methods of blastomere removal.

Genetic material isolation from various stages of preimplantation development

Isolation of genetic material has been performed in preconception oocytes or embryos at various stages of preimplantation development. Currently, polar

bodies and cleavage stage embryos as well as blastocysts have been biopsied for further genetic diagnosis in PGD.

Polar body biopsy

Polar body biopsy has been introduced for PGD for the last decade.[5-8] Since some aneuploidies can arise in meiosis II,[9] analysis of both the first and second polar body has been suggested.[10] The disadvantages of this technique involve providing only maternal genetic information,[11] labour intensive of technique, and failure to diagnose the possibility of recombination and post-zygotic events.[12,13] Currently, some centres have performed polar body biopsy.

Cleavage stage embryo biopsy

Cleavage stage embryo biopsy has been the preferred technique for most groups performing PGD.[3] The 8-cell stage was suggested to be the most suitable stage for embryo biopsy because of its higher mitotic index and totipotency.[14] Wilton et al. removed a single blastomere from the four-cell stage mouse embryo and proposed that the removal did not compromise the developmental potential both in vitro and vivo.[15] Twenty-five percents of the cleavage-stage mouse embryos could be removed without affecting rates of implantation and fetal development.[14-17] Since one cell analysis for the diagnosis of chromosomal abnormalities and age-related aneuploidy may be insufficient due to the occurrence of mosaicism,[18] two blastomeres analysis has been suggested to improve diagnostic accuracy.[7] Sago et al. has also suggested that dual blastomere biopsy and independent blastomere analysis dramatically improved preimplantation diagnosis reliability.[19]

Blastocyst biopsy

Blastocyst biopsy offers a higher number of cells to be obtained and genetically analysed.[20,21] The higher number of cell to be analysed may decrease the likelihood of misdiagnosis, which can occur from the limitation of the number of cells obtained from the biopsy, and possibly allow more diagnostic techniques to be performed.[20,21] The removal of trophectoderm cells from blastocyst has been thought to be unlikely to have an affect on the development of the embryo proper[11] and avoids ethical concerns over removal of totipotent cells.[22] Moreover, the chances of pregnancy also increase due to the higher implantation potential of blastocyst.[23-25] The disadvantage of blastocyst biopsy, however, is that a limited number of embryos can reach the blastocyst stage.[26] Vandervorst et al. reported that at least 9 oocytes cumulus complexes were required for a successful PGD as high number of embryos are required for PGD.[27] The possibility of confined placental mosaicism (CPM), where the trophectoderm may possess different genetic material from the inner cell mass, has been considered to be another disadvantage.[28] Ruangvutilert et al. have reported that the high prevalence of mosaicism found in the blastocyst stage would offer the same problems of mosaicism in cleavage stage embryos.[29]

Methods of zona pellucida opening

Several biopsy techniques for PGD have been introduced for the last decade. Mechanical biopsy methods, which were partial zona dissection,[30] slitting[31,32] and piercing,[32] were firstly reported. Chemical dissolution by acid Tyrode's solution has eventually been introduced for embryo biopsy.[33] The use of laser was a recently developed technique in embryo biopsy for PGD.[21,34,35]

Zona pellucida slitting

The embryo was stabilised with a holding micropipette and a slit on the zona pellucida was made by a sharpened dissection micropipette (Figure 1a). The biopsy micropipette was inserted through the slit into the perivitelline cavity with subsequent removal of a single blastomere by aspiration[36] or pushing against the part of the zona adjacent to extrude one or two blastomere escaping from the slit.[32]

Zona pellucida piercing

The embryo was immobilised with a holding micropipette while the biopsy micropipette was used

to puncture the zona pellucida and withdraw one or two blastomeres by aspiration (Figure 1b). The blastocyst formation and hatching of the embryos following this method were similar to zona intacted embryos.[32]

Zona pellucida drilling with acid Tyrode's solution

Zona drilling using the glycoprotein-digestive properties of acid Tyrode's solution has been clearly popular and used by most centres within the ESHRE PGD Consortium.[37] The embryo was immobilised by gentle suction on a holding micropipette controlled by a micromanipulator. A second micromanipulator with a double holder system controlled a drilling micropipette containing acid Tyrode's solution and a biopsy micropipette. An opening was created in the zona pellucida by moving the drilling micropipette into contact with the zona pellucida and gently expelling the acid Tyrode's solution, as shown in Figure 1c. The drilling micropipette was removed and the biopsy micropipette was inserted through the opening to remove one or two blastomeres.[38]

The main drawback of acid Tyrode's solution drilling was its toxicity and cytoplasmic acidification that changed intracellular pH, leading to cytoplasmic degeneration.[39] Exposure of 4-cell stage embryos to acid Tyrode's solution has been reported to reduce their viability[40,41] and preimplantation development.[30,40] However, Cui et al. and Viville et al. have reported that no significant adverse effect was found in the embryos, which were biopsied by acid Tyrode's solution.[42-44]

Zona pellucida drilling with laser

Several clinical and basic applications for laser systems in IVF involving micromanipulation on gametes and embryos have been introduced over the last decade (Figure 1d). Lasers operating at a variety of wavelengths, which were 532, 366, 355 and 266 nm, have been used for photoablation of the zona in mouse and human oocytes.[45] Mouse oocytes cleaved normally after manipulation with a 193 nm ArF-excimer laser[46], a 248 nm [47] and a 308 nm XeCl excimer laser.[48,49] The hatching of mouse embryos occurred earlier in laser exposured embryos but abnormal development was also noted in the embryos.[48] This adverse effect has been thought to be the result of thermal change caused by too high frequency of laser shooting. Decreasing in pulse repeat rate combined with a higher energy setting per pulse might prevent this event.[48] The laser system of wavelength of 366 nm has been reported to affect the genetic structure of the oocyte.[45] The 308 nm non-contact excimer laser transmits well through high purity quartz objectives and water and offers efficient zona ablation. However, this laser may cause sister chromatid exchange in Chinese hamster ovary cells.[50] Strohmer and Feichtinger have suggested that it was possible to achieve a pregnancy using assisted hatching by an erbium:yttrium aluminium garnet (Er:YAG) laser to create an opening in the zona pellucida of human embryos to facilitate the embryonic hatching after the embryo transfer.[51]

Laser assisted opening of the zona pellucida by a non-contact infrared 1.48 μm diode laser has eventually been introduced.[52-54] The laser, focused through a microscope objective, has allowed a rapid, easy and non-touch microdrilling of mouse and human zona pellucidae while maintaining a high degree of accuracy under conventional culture conditions.[54] The lysis of the zona pellucida has been thought to be the result of either a greater susceptibility of the zona pellucida glycoprotein matrix to lytic disruption or a higher absorption of the laser wavelength by the zona pellucida glycoproteins.[53] The safety of microdrilling the zona pellucida of mouse oocytes with a infrared 1.48 μm diode laser has been reported both in vitro and in vivo.[52-54] No alteration in oocyte cytoplasmic structure was observed at the light or ultrastructural levels.

Laser assisted opening of the zona pellucida by a non-contact 1.48 μm diode laser was recently introduced for polar body[35] and blastocyst biopsy. Laser assisted blastocyst biopsy has been suggested to be an alternative tool in PGD because the reliability and possibility of the diagnosis could be offered by the higher number of cells obtained from the biopsy. However, this technique has not

been to date clinically used.[37]

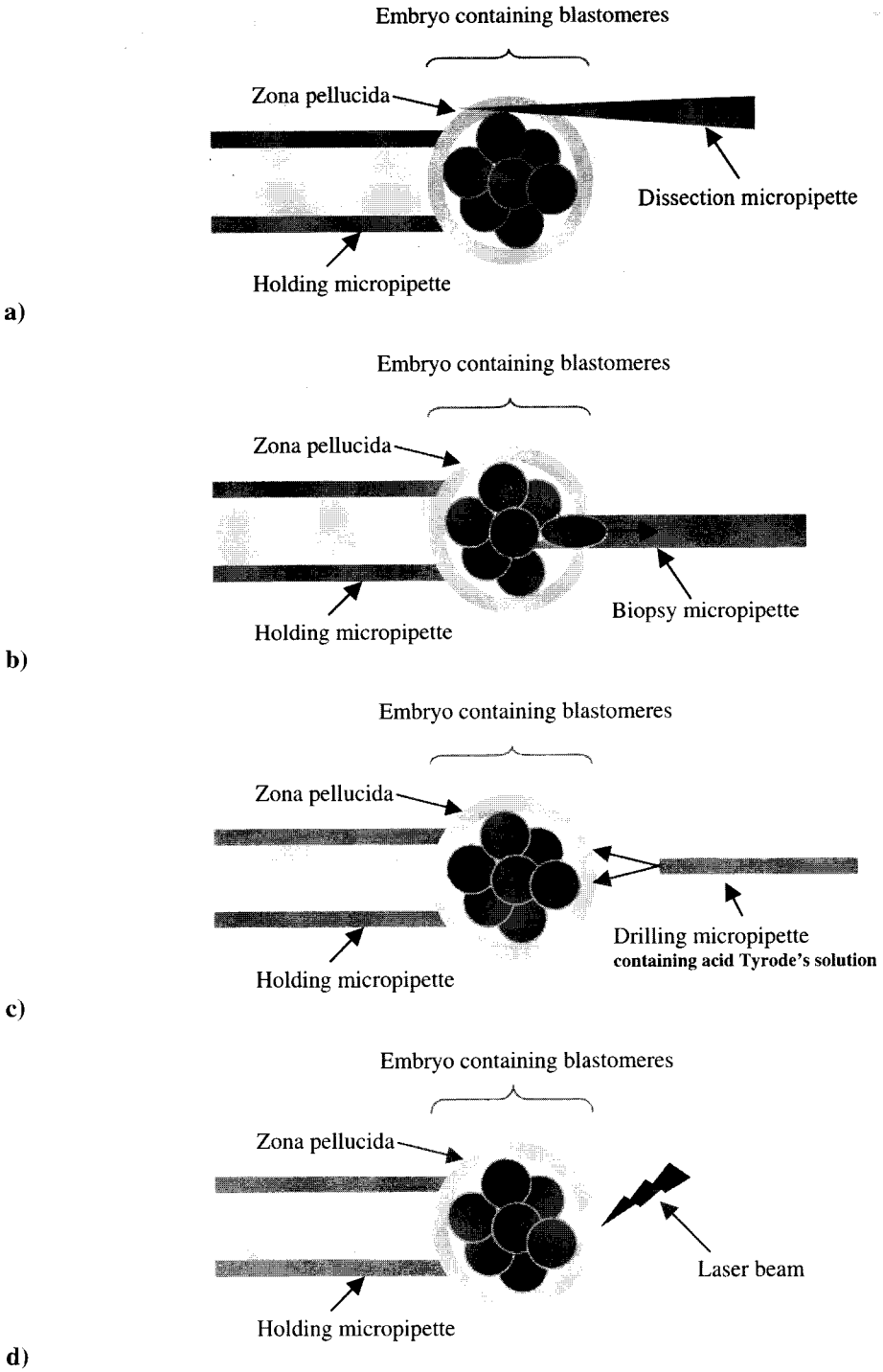

Fig. 1. Diagrams of methods of zona pellucida opening
a) Zona pellucida slitting
b) Zona pellucida piercing
c) Zona pellucida drilling with acid Tyrode's solution
d) Zona pellucida drilling with laser

Methods of blastomere removal

Displacement method

Blastomeres can be displaced through the zona opening with a flow of medium from a micropipette inserted through the zona pellucida, as shown in Figure 2a.[17,55] Roudeboush et al. reported that the survival rate of biopsied mouse embryos after the displacement method was higher than that of the aspiration method.[55] The displacement method has also been suggested to be more suitable than the stitch and pull and aspiration methods for cleavage-stage embryo biopsy.[11] Recently, the displacement biopsy method was claimed to be relatively rapid compared with other methods because it did not require changing and manual repositioning of micropipettes as well as the use of a third micromanipulation.[56]

Stitch and pull method

The stitch and pull method has been described in the review by Tarin and Handyside.[11] The biopsy is carried out by drilling an opening in the zona pellucida with acid Tyrode's solution or partial zona dissection. One or two blastomeres are removed using stitching movements with a microneedle, as shown in Figure 2b.

Push method

The blastomeres were extruded through the opening by pushing against the zona pellucida with a micropipette at some distance from the opening, as shown in Figure 2c.[57] The push method has also been proposed to be more suitable than the stitch and pull, and aspiration methods for cleavage-stage embryo biopsy.[11]

Aspiration method

A bevelled biopsy micropipette may either be forced through the zona pellucida like a needle [14,15,17,55,58,59] or directly contact with the blastomere through the opening or slit in the zona pellucida previously created by either acid Tyrode's solution[60-62] or a sharpened dissection pipette.[36] The blastomere was sucked into the biopsy micropipette with gently suction controlled with a micromanipulator (Figure 2d). The majority of centres within the ESHRE PGD Consortium also use this method for clinical PGD.[37]

Effect of Ca^{2+} and Mg^{2+} free medium on blastomeres

Ca^{2+} and Mg^{2+} free medium has been frequently used to loosen the membrane adhesion mechanism between blastomeres.[15,64-66] The use of Ca^{2+} and Mg^{2+} free medium has no detrimental effect on the viability of the biopsied embryos and their biopsied cells when used during relatively short times of exposure Santalo et al.[67] The efficiency of the biopsy procedure seems to be the same using Ca^{2+} and Mg^{2+} containing medium or Ca^{2+} and Mg^{2+} free medium because no statistically significant differences were observed in the number of embryos efficiently biopsied under these conditions. Van Blerk et al. have shown that exposing mouse embryos at the morula stage to Ca^{2+} and Mg^{2+} free medium affected their survival rate.[59] The difference in developmental stage of the studied embryos and the decompacting process occurring at the morula stage might provide the different outcomes of the studies.[67] Furthermore, these studies were performed in a mouse model and might not be definitely extrapolated to human embryos. Recently, the use of Ca^{2+} and Mg^{2+} free medium has also demonstrated not to affect subsequent development of human embryos.[68] An easier biopsy procedure with less damage of blastomere being removed has also offered.[68] However, the 8-cell non-compacted embryos could be biopsied effectively without the need for preincubation in Ca^{2+} and Mg^{2+} free medium or medium containing cytochalasin to loosen cell-cell contact.[69] Some centres have preferred to perform biopsy procedure in Ca^{2+} containing medium.[61,63] About half of PGD centres within ESHRE PGD Consortium currently use Ca^{2+} and Mg^{2+} free medium to decompact embryos prior to biopsy.[37]

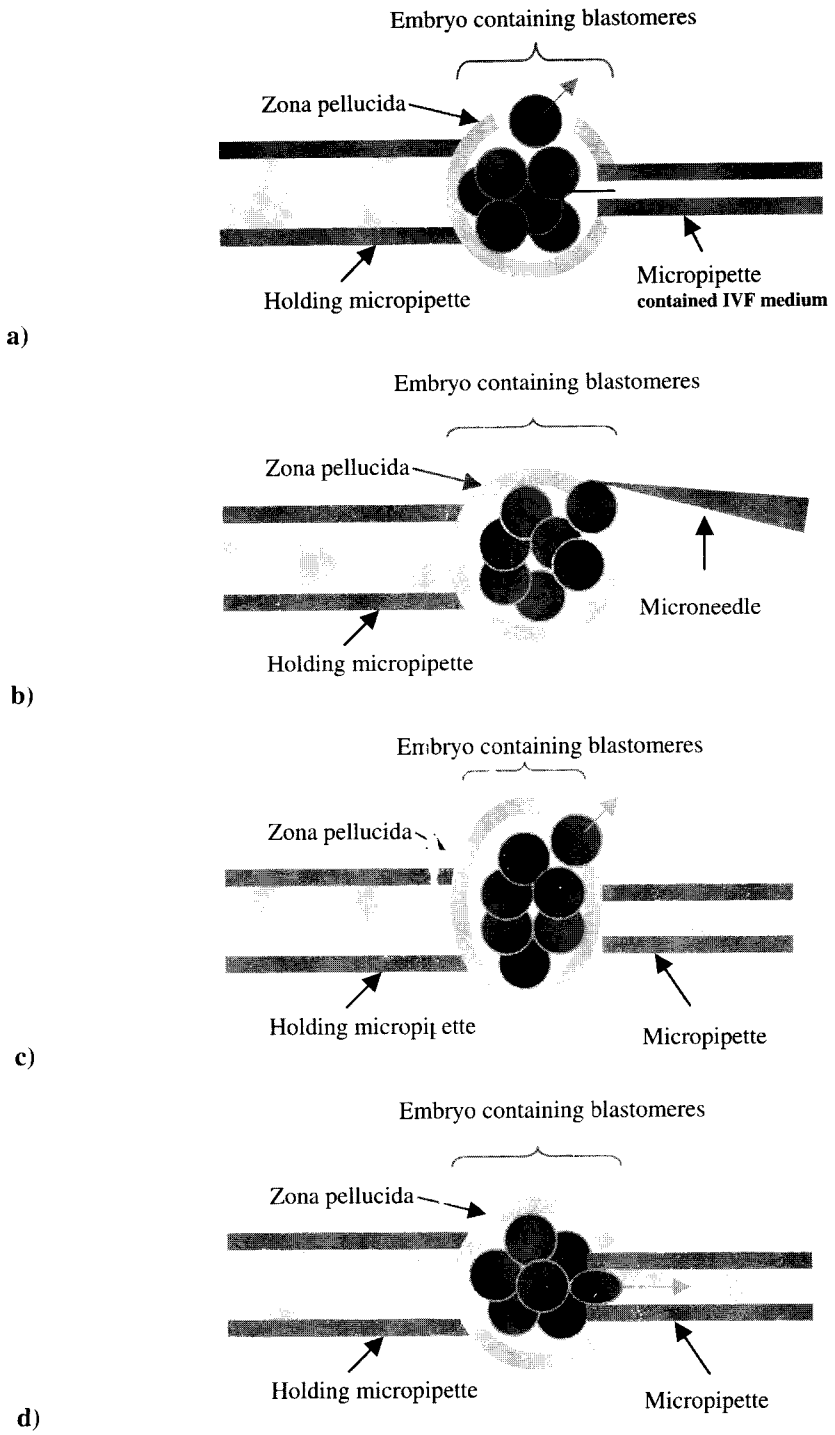

Fig. 2. Diagrams of methods of blastomere removal
a) Displacement method
b) Stitch and pull method
c) Push method
d) Aspiration method

In conclusion, isolation of genetic materials from preconception oocytes and embryos at various stages of development has been definitely considered as an important process in PGD. Many techniques including mediums have been introduced in order to provide the capability of further development of the embryos post biopsy and the accuracy of diagnostic techniques. Although cleavage stage embryo has been accepted and performed in most centres, with more advanced molecular genetic diagnostic techniques and assisted reproductive technology, other stages such as blastocyst may be alternative in the near future. Similarly, introduction of developed biopsy techniques such as laser assisted biopsy may be substitute of the conventional acid Tyrode's solution. However, since PGD has been developed for just a decade, more researches should be carried out to define its effectiveness and safety. Intrauterine and after birth development until the puberty of the children following pregnancies with PGD should be close monitored to assure that PGD is both valuable and safe for mankind.

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