Micromanipulation in human assisted reproduction program: Historical perspectives and advances

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Abstract

Among the main components of assisted reproductive technology (ART) are the micromanipulation of sperm, oocyte and embryo. Micromanipulation techniques have contributed to major advances and achievements in the treatment of infertility. Sperm handling can be performed for different purposes before intracytoplasmic sperm injection (ICSI) to improve outcomes in especial cases. Oocyte micromanipulation can be performed for diagnostic purposes and also for improving fertilization rate. Majority of the manipulation techniques were devised for embryos. ICSI was the earliest technique for embryo micromanipulation, which can be performed at zygote, cleavage-stage, morula and blastocysts stages. The purpose of the present review is to discuss and provide a wide overview on the development of micromanipulation techniques in human ART.

Disclaimer: The authors have no conflicts of interest.
J Reprod Biotechnol Fertil 11:11-31, 2022
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Compliance acknowledgement: This article was proofread by the Australian Editorial Services (www.nativeenglisheditor.com)
Keywords: manipulation, cleavage-stage embryo, blastocyst, pregnancy, infertility, assisted reproductive technology.

Background

Intracytoplasmic sperm injection (ICSI) was first suggested in 1990 by Ng et al. the pioneer of subzonal insemination (SUZI;Ng et al., 1990) of the National University of Singapore but at that time regulatory issues prevented its routine application in the human. Ng called this technique MIMIC (Ng et al., 1990). Routine application of ICSI commenced two years later in 1992 when Palermo and coworkers reported successful ICSI in the human (Palermo et al., 1992). ICSI brought considerable hope for treatment of male factor infertility and in the ensuing years proved its usefulness in overcoming most instances of male infertility. ICSI was one of many pioneer micromanipulation techniques introduced with the aim of improving the success rates in the treatment of male infertility.

Many techniques have been introduced after ICSI to help the ART professional treat the infertile couple. Some of the micromanipulation techniques focused on the oocyte are partial zona dissection (PZD), SUZI and polar body (PB) biopsy. The majority of micromanipulation techniques that have been proposed for embryos were performed on the embryo at the zygote, cleavage-stage, morula, and blastocyst stages. Zona hatching, blastomere biopsy, fragment removal and removal of lysed blastomere(s) are the main micromanipulation techniques at the cleavage-stage embryo (Halvaei et al., 2018).

Today, many clinics have moved the day of embryo transfer from 3 to 5 due to low risk of multiple pregnancy and increased rate of implantation. Two main micromanipulation procedures of blastocysts are trophectoderm (TE) biopsy and artificial shrinkage before vitrification. In this review, the micromanipulation techniques in human ART are discussed comprehensively.

Oocyte micromanipulation

Oocyte micromanipulation techniques were among the oldest proposed in order to increase fertilization rate. PZD is the first oocyte micromanipulation technique introduced in the late 80s (Malter and Cohen, 1989b). This method was used to facilitate zona pellucida (ZP) penetration to improve fertilization rate. SUZI was introduced by Ng et al. to facilitate fertilization in oligozoospermia. In this technique a few motile spermatozoa were placed in the perivitelline space (PVS). Only a few spermatozoa were used to prevent polyspermy (Ng et al., 1988). The next step was injection of a single spermatozoon into oocyte cytoplasm with specially designed microtools or needles, the ICSI injection and holding pipettes. This technique came to be known as ICSI (Palermo et al., 1992). ICSI was a major achievement which overcame the pitfalls of previous oocyte manipulation procedures. ICSI involved the manipulation of both gametes that resulted in increased fertilization rate especially in male factor infertility. History of ICSI, its indications and factors affecting fertilization rate are discussed elsewhere (Haddad et al., 2021, Palermo et al., 2017, Halvaei and Esfandiari, 2021).

Laser-assisted ICSI was introduced for opening the ZP in case of fragile oolema or when there was difficulty in penetration of the ZP with the injection needle. ICSI in fragile oolema leads to oocyte degeneration due to ooplasm outpour into PVS. ZP resistance to penetration may also be associated with cytoskeletal damage in the oocyte and subsequent degeneration. To overcome this, laser is used for drilling a small hole in the ZP at 3 o'clock making the entry of needle into the oocyte easier (Davidson et al., 2019). It was shown that the rates of oocyte survival and embryo development were significantly higher in laser-assisted ICSI compared to conventional ICSI (Abdelmassih et al., 2002). Although, higher fertilization rate was reported in cases undergoing first ICSI cycles (Verza et al., 2013), routine practice of this technique is not usually suggested (Richter et al., 2006). Fawzy and colleagues in a recent randomized controlled trial showed that using laser-assisted ICSI in patients undergoing first or second ICSI attempts improved the oocyte survival rate. However, after adjustment for oocyte survival, laser assisted ICSI did not affect the embryo development and pregnancy rates (Fawzy et al., 2020). It seems that this technique should be applied in selected cases only, like in cases with history of previous ICSI failure due to abnormal oolemma damage (Rienzi et al., 2001, Rienzi et al., 2004). Therefore, routine use of laser-assisted ICSI was not recommended in ART.

Ooplasm manipulation is another oocyte micromanipulation technique introduced to improve treatment outcome. Cytoplasmic defects in the oocyte may impair embryo development and infertility (Blerkom et al., 1995). Ooplasm transfer from a healthy oocyte or ooplasm modification can be used to improve ART outcomes. Flood et al. showed the beneficial effects of ooplasm transfer of mature oocytes into immature oocytes in the monkey (Flood et al., 1990). It was observed that the transfer of small amount of ooplasm from a normal oocyte into a 1-cell mouse embryo improved implantation rate (Levron et al., 1996). Cohen and colleagues were the first to report the birth of a girl following ooplasm transfer (Cohen and Scott, 1997). Mitochondria are the most important organelles in the ooplasm that may produce the desired effect after ooplasm transfer (Sansinena et al., 2011). González-Grajales et al. showed the positive effects of ooplasm transfer on embryos derived by interspecies somatic cell nuclear transfer (González-Grajales et al., 2016). It was shown that supplementation of autologous ooplasm-transfer did not affect embryo development (Lee et al., 2017). Mitochondrial replacement therapy can also be performed by ooplasm transfer and is a candidate for clinical application (Jiang and Shen, 2021). It is of interest to note that in the clinical setting the transfer of ooplasm from donor to recipient oocytes will alter the mitochondrial genetic makeup of the oocyte and the resulting progeny. This may not be acceptable in some communities.

Sperm micromanipulation

Selecting viable sperm for ICSI is an important issue for successful fertilization in cases of asthenozoospermia. Mechanical touch technique or sperm tail flexibility test was proposed to select viable spermatozoa (de Oliveira et al., 2004, Soares et al., 2003). In this

test, sperm tail is touched by injection needle. If sperm tail is moves independent of sperm head, the sperm is viable and if both tail and head moved together, the sperm is dead. The beneficial effects of this technique on rates of fertilization and pregnancy were shown in two retrospective studies (de Oliveira et al., 2004, Soares et al., 2003). However, touching the tail of the spermatozoa does not need any equipment and is a cost-effective and non-invasive. However it needs an expert embryologist and is not suitable for ejaculated frozen-thawed spermatozoa (Nordhoff, 2015). Clinical efficacy of this technique should be approved by well-design studies. Another sperm manipulation technique is laser-assisted sperm selection (LASS). This is a rapid, safe, user-friendly and repeatable technique to detect viable immotile spermatozoa (Nordhoff, 2015). A single laser pulse (200 µJ, approximately 2 mS) is shot to the sperm tail leading to curling of viable sperm tail (Aydos and Aydos, 2021). Aktan and colleagues reported, for the first time, the efficacy of LASS is similar to hypo-osmotic swelling test (Aktan et al., 2004). Furthermore, it was proposed as an alternative of hypo-osmotic swelling test (Gerber et al., 2008). One of the oldest of the sperm viability tests, the hypo-osmotic sperm tail swelling test (Jayendran et al. 1984) remains one of the cheapest and simplest technique of identification of potentially viable spermatozoa to this day. The effectiveness of LASS in a case of total immotile spermatozoa following cryopreservation or in Kartagener's syndrome was reported recently (Chen et al., 2017, Ozkavukcu et al., 2018).

Cleavage-stage embryo manipulation

Assisted hatching

One of the most common micromanipulation technique is assisted hatching (AH) in which the ZP is opened artificially. The embryo at the blastocyst stage should be able to hatch out of the ZP to be ready for implantation. There are several factors to induce ZP hatching, including enzymes released from the embryo or endometrium and blastocyst expansion. Factors like abnormality in the ZP, thick ZP and zona hardening can impair this phenomenon. It seems that in vitro oocyte or embryo culture reduces embryo quality (Friedler et al., 2007) and oocyte or embryo cryopreservation thickens/hardens the ZP (Petersen et al., 2006). These are the main reasons for using AH. In addition, there are several indications for AH, including advanced maternal age (≥40 years), high levels of FSH, thick ZP (>15µm), after cryopreservation, and history of implantation failure (≥2) (Cohen et al., 1992, Schoolcraft et al., 1994, Tao and Tamis, 1997, Mansour et al., 2000, Hammadeh et al., 2011). It is of interest to note Ali et al. (Ali et al, 2003) observed that ZP thickness is not an indication for AH but its texture. The texture of the ZP confers hardness or softness to it. They observed a thin ZP could be extremely hard and completely impervious to repeated laser bombardment during AH whereas the thick ZP could be extremely soft such that the use of single pulse of laser could damage the embryo by creating too large an opening in the ZP. It was shown that routine use of AH has no beneficial effects on ART outcomes (Razi et al., 2013) .

Some mechanisms have been proposed to explain how AH can increase the implantation potential including facilitating the utilization of nutrients, metabolite and growth factor transfer from the culture medium and earlier endometrium contact (Malter and Cohen, 1989b, Wan et al., 2014). It seems that the method and extend of AH are two important factors in success rate of AH (Le et al., 2018). One additional concern with regard to the use of AH is the possible increase in the implantation of abnormal embryos. There is limited data on the increase in the risk of congenital abnormalities and more RCTs are required for final conclusion. A recent meta-analysis evaluating AH in advanced maternal age also did not find any relationship between AH and congenital anomalies (He et al., 2018). Multiple pregnancies following AH is matter of debate. A monochorionictriamnionic triplet associated with monoamniotic twins following AH was reported in 2009 (Pantos et al., 2009). In a systematic review and meta-analysis, it was concluded that AH does not improve multiple pregnancies in fresh embryo transferred to non-poor prognosis patients (Martins et al., 2011). Different methods of AH are presented in Table 1.

Laser assisted hatching (LAH)

Laser was applied for the first time for trapping sperm in ART (Tadir et al., 1989). The same group then used laser for opening the ZP by drilling (Tadir et al., 1991). Rink et al. descri-

Table 1. Different assisted hatching (AH) methods in ART

Method of AH	Description	Pros and cons	Reference
PZD	Opening of ZP by microneedle	- is more physiological and natural in comparison with other methods - needs an expert embryologist - size of hole is not under control	(Malter and Cohen, 1989a)
Mechanical expansion of ZP	Increasing internal pressure by injecting medium	- is similar to natural process - preventing blastomere loss by preventing ZP thinning and hole creation - ultrastructural change	(Hammadeh et al., 2011, Fang et al., 2010)
Chemically assisted hatching	Dissolving the ZP by acid Tyrode's leading to ZP thinning or creation of a hole in ZP	- may face the embryo with abnormal pH and several washing steps after hatching are needed to reduce harmful effects of acid - The amount of acid solution and the time of acid exposure are very important - needs a rapid manipulation and a well-experienced embryologist	(Cohen et al., 1992, Tucker et al., 1993)
Pronase treatment	Enzymatically dissolve the ZP leading to ZP thinning or creation of a hole	- not a routine practice - the concentration of pronase and time of pronase exposure are two important factors affecting the outcome - needs several washings after treatment and an expert embryologist	(Fong et al., 1998)
Laser assisted hatching	Creating a hole in the ZP or thinning ZP by laser	- user friendly, safe, efficient, and has reproducibility potential - heat production during laser is a concern	(Tadir et al., 1991, Tadir and Douglas-Hamilton, 2007)
Piezo micromanipulation	Using piezo micromanipulation to create a hole in the ZP or zona thinning	- the position of the vibrating needle is the key factor - is very quick	(Nakayama et al., 1999)

-bed non-contact mode laser using 1480 nm wave length for drilling mouse ZP which is currently used in the clinic (Rink et al., 1996). In a small study population, a dramatic decline in clinical pregnancies was noted when the laser beam is above 250ms (60% vs 29%) in day 2 cleavage-stage embryos but this effect was not statistically significant (Ali et al., 2003). This finding suggests higher laser beam lengths could be harmful to the embryo but this remains to be proven in a larger study population. Laser is user friendly, safe, efficient, and has potential for reproducibility (Hammadeh et al., 2011). Balaban et al. showed that the rate of implantation and pregnancy rate were similar between four methods of AH in poor prognosis patients (Balaban et al., 2002). Later, Lanzendorf et al. in a prospective randomized study found no beneficial effects of LAH compared to acid Tyrods' method (Lanzendorf et al., 2007). There are three methods of LAH including complete LAH by creating a hole through the ZP, partial LAH by creating a hole in outer membrane of ZP, and quarter LAH by drilling a quarter of embryo (Davidson et al., 2019).

An earlier study showed that zona thinning had higher pregnancy rate compared to partial or fully ZP hatching (Mantoudis et al., 2001). Ali et al observed that the inner-most layer of the ZP is the hardest and most impervious to laser suggesting that complete drilling of the ZP is preferred to partial drilling (Ali et al., 2003). Wang et al. recently compared the ART outcomes of drilling and thinning using LAH on day 4 embryos during on FET cycles. They showed that the rates of miscarriages, multiple pregnancies, ectopic pregnancies, preterm births, live birth and congenital anomalies were similar between two groups. However, the rates of implantation and pregnancy were higher in thinning group compared to drilling in women under age of 35 years, patients with history of failed cycle and when endometrial thickness is 8-10 mm (Wang et al., 2021). Schimmel et al. using time-lapse study showed that incomplete AH may induce incomplete hatching (Schimmel et al., 2014).

The data on the efficacy of AH in ART is controversial. Sallam et al. in a meta-analysis showed that AH can improve implantation rate, clinical and ongoing pregnancy in poor prognosis patients (Sallam et al., 2003). Kissin et al. in a large retrospective study evaluated the efficacy of AH on ART outcomes. They found that application of AH increased in a period of ten years, but AH of both day-3 and day-5 embryos had no beneficial effects on implantation, clinical pregnancy, and live birth rates even in poor prognosis patients (Kissin et al., 2014). A recent systematic review evaluated 39 RCTs (7,249 women) showed that live birth rate in control was similar to AH group (28% vs 27-34%, respectively) (Lacey et al., 2021). However, they reported the quality of evidenced was very low to low. Razi et al. showed that routine use of LAH may not improve the ART outcomes and ASRM also did not recommend routine use of AH for patients (Razi et al., 2013, Medicine, 2014). In contradiction however, an earlier report (Ali et al., 2003) demonstrated significantly improved clinical pregnancy rate (about 65% vs 33%) and improved implantation rate (38% vs 18%) for day 2 cleavage-stage embryos in women below 36 years of age following routine LAH. It was shown that LAH may increase pregnancy rate in advanced maternal age and frozen-thawed cycles (Elhussieny et al., 2013).

A recent study showed that LAH can increase the rates of total and usable blastocyst, but the rate of pregnancy was similar between LAH and non-LAH groups (Xu et al., 2021). A very recent mouse model study showed that ZP drilling by laser may change embryo transcriptome and subsequently metabolic pathways (Liu et al., 2021). Association between epigenetic modifications and manipulations in ART has been an important tissue. A mouse model study has shown that cleavage-stage embryos subjected to LAH may have altered epigenetic pattern (Honguntikar et al., 2017). Huo and colleagues also found that DNA methylation of IGF2/H19 imprinting control region was reduced in the thinning group in comparison with control and drilling group. Zona thinning also increased DNA methylation level in the placenta and expression level of H19 mRNA in the offspring (Huo et al., 2020).

Embryo fragment removal

Mitosis division can induce embryo fragmentation in cleavage-stage embryos. Fragments are membrane-bound cytoplasm

near the blastomeres inside the embryo (Fujimoto et al., 2011). As the fragments are parts of embryo cytoplasm, different organelles may be seen inside the fragments. Our group showed that mitochondria, mitochondria-vesicle complexes, Golgi apparatus, primary lysosomes, and vacuoles could be found within the fragments (Halvaei et al., 2016a). Furthermore, the same group also found that the majority of mitochondria found in the fragments are intact, rounded to oval with few cristae (Safari et al., 2017).

Fragment removal is one of the most invasive micromanipulation techniques that is performed on the embryos at the cleavage-stage to increase ART outcomes. Alikani et al. was the first group that reported the beneficial effects of fragment removal (Alikani et al., 1999). Briefly, in this technique, the embryo should be initially hatched near the fragments and a special microneedle with 10-12 µm inner diameter is inserted into the embryo (Halvaei et al., 2016a). The fragments around the blastomeres then are gently removed. Only highly-skilled embryologists can perform fragment removal. This technique needs patience for preventing mechanical embryo damage while at the same time must be performed with speed to minimize the risks associated with the embryo being out of the incubator. There were assumptions that blastomere fragment removal has beneficial effects including improving cell junctions, increasing relationships and contact between blastomeres, to prevent the release of potentially harmful degradation material from apoptotic fragments. The percent of fragmentation may vary in embryos from 0-100%.

Theoretically, fragment removal can be performed in all fragmented embryos. But, low (0-10%) and severe (>50%) fragmented embryos do not benefit from this technique, because low fragmentation has no detrimental effects on embryo development and severe fragmentation is associated with reduced pregnancy rate and anomaly (Ebner et al., 2001, Racowsky et al., 2009). There are controversial results on efficacy of fragment removal on ART outcomes. Alikani et al., in a retrospective study, showed that implantation rate was significantly decreased in embryos with >15% fragmentation even after fragment removal (Alikani et al., 1999).

Later, Eftekhari-Yazdi et al., in a prospective study showed that fragment removal on 4-6 cell embryos increased the number of blastocyst cell and decreased the apoptotic index of derived blastocyst in the experimental groups compared to the control (Eftekhari-Yazdi et al., 2006). In a retrospective study, Keltz and teammate found that fragment removal in poor quality embryos resulted in improvement of ART outcomes (Keltz et al., 2006). The same group, in another prospective randomized study, showed that fragment removal on day 3 embryos decreased the rate of fragmentation on day 5, but had no effects on compaction and blastocyst rate (Keltz et al., 2010). However, this technique did not increase rates of implantation and live birth in embryos with 10-20% fragmentation derived from young mothers (Sözen et al., 2012). We showed that fragment removal may increase the success rate in patients with implantation failure (Halvaei et al., 2015). Kim et al. retrospectively evaluated the effects of early fragment removal on embryo development and pregnancy outcomes. They found that fragment removal on day 2 can increase the grade of embryo at the following day and implantation and pregnancy rates as well (Kim et al., 2018). Sordia-Hernandez et al., in a prospective observational small study (13 patients in each group), showed that defragmentation of moderately fragmented embryos can increase pregnancy rate compared to the top-quality embryo with increased chance of abortion (Sordia-Hernandez et al., 2020).

Fragment removal can improve the grade of embryo, but has no effects on intrinsic defects of the embryo. Fragment removal does not change the fate of severely fragmented embryos of poor quality and could result in ART failure. It is highly recommended to use this technique for selected cases like repeated implantation failure (Halvaei et al., 2015). The authors' group did not find any beneficial effects on ART outcomes after fragment removal in fresh or vitrified-warmed embryos (Halvaei et al., 2016a, Safari et al., 2017).

Debris removal

There are some reports of removing debris in PVS of mature oocytes. It seems this technique may have beneficial effects on selected patients such as those with a history of implantation

failure (Halvaei et al., 2015). Routine use of debris removal in unselected patients is matter of debate (Halvaei et al., 2016a). It is an invasive technique and needs a high level of expertise.

Removal of lysed cells

Cryopreservation of low-grade embryos or sub-optimal vitrification/warming may be related to blastomere lysis and/or necrosis. Presence of necrotic blastomeres could impair embryo development by interrupting cell communication and could release toxic materials. Lysed blastomere removal is performed like fragment removal. In a mouse model, it was shown that the presence of degenerated blastomeres impaired hatching process and removing these improved hatching and embryo viability (Alikani et al., 1993). Rienzi et al. in a prospective study showed that removing degenerated blastomeres increased rates of implantation and pregnancy (Rienzi et al., 2002). Another study from Europe showed that removal of necrotic blastomeres in partially damaged embryos and in embryos that had cleaved post-thaw helped increased delivery rate (Rienzi et al., 2005). Nagy et al. showed that the embryo vitality and pregnancy rate increased, when lysed blastomeres were removed on the day of embryo transfer in frozen-thawed cycles (Nagy et al., 2005). The same group also reported embryo morphology and development were improved following removal of lysed blastomere in a mouse model (Elliott et al., 2007). Positive effects of the removal of necrotic blastomeres at the 4-cell stage in mouse embryo were apparent by improvement in the blastocyst quality and in reducing incidence of cell death (Fathi et al., 2008). Significant advances made in cryopreservation resulted in a reduction in blastomere damage. This led to a decline in the use of this technique and because of which this technique is no longer recommended or performed in the recent years.

Embryo biopsy

Blastomere biopsy

Preimplantation genetic testing (PGT) is performed on the oocytes and embryos for HLA-typing or detecting genetic abnormalities. There are three types of PGT including PGT-A (aneuploidies), PTG-M (monogenic diseases), and PTG-SR (structural rearrangements) (Zegers-Hochschild et al., 2017). Embryos can be biopsied at different stages for PGT including the zygote (polar body), cleavage-stage (blastomere) and blastocyst stage (TE; Cimadomo et al., 2016). Most of embryos are biopsied at the cleavage-stage (Moutou et al., 2014). Historically, embryo biopsy began in the 1960s and early 1970s for farm animals (Cimadomo et al., 2020). Handyside et al. reported the first blastomere biopsy for sex determination for prevention of a X-linked disorder (Handyside et al., 1990). ZP should be opened for embryo biopsy. There are different methods for opening ZP including mechanical, acid Tyrode's, and laser. It was shown that there is no difference in the blastocyst development between acid Tyrode's solution and laser, suggesting that laser could be an alternative method to chemical assisted hatching (Jones et al., 2006).

It is recommended to perform biopsy on the morning of day 3 on embryos that has at least 6 blastomeres, low amount of fragmentation with these two requirements being the inclusion criteria for the biopsy procedure (Harton et al., 2011). ZP should be drilled to reach the blastomeres with the methods described in the previous section. The opening in the ZP created for embryo biopsy is bigger than that created in AH. The most usable technique for ZP hatching in embryo biopsy is LAH (Moutou et al., 2014). Ca^{++}/Mg^{++} free medium is used to make blastomere biopsy easy by decreasing the adhesion of cells during the procedure. This medium may deplete the blastomeres of Ca^{++}. Animal studies have shown that Ca^{++} depletion may change the cytoskeleton and compaction process (Pey et al., 1998). Using Ca^{++}/Mg^{++} free medium for embryo biopsy may cause the biopsied embryos to delay compaction and blastulation (Bar-El et al., 2016). After opening the ZP, biopsy pipette is gently entered into the embryo and blastomere/s with visible nuclei is/are removed.

Timing of blastomere biopsy seems to be a factor that could predict the subsequent development of the biopsied embryo. Kalma and colleagues showed that embryos that were biopsied in the last quarter of 8-cell stage were less affected by blastomere biopsy and had more implantation potential (Kalma et al., 2018).

Also, it was shown that the hatching process may be affected by blastomere biopsy (Kirkegaard et al., 2012). Another important factor is the number of blastomeres removed. It was shown that removing two blastomeres can increase the biopsy success rate with no negative effects on embryo development (Brodie et al., 2012). The limited number of blastomeres that could be removed is one of the limitations of cleavage-stage biopsy. Goossens et al. in a prospective study, found that polymerase chain reaction (PCR) efficacy was significantly higher following two blastomeres removal in comparison with one cell removal, but the likelihood of blastocyst formation may decrease. However, they found that day 3 developmental stage was more important than removing blastomere/s to predict further embryo development (Goossens et al., 2008). It seems the quality of embryos and expertise of embryologists are two important factors that predict the embryo viability and development after biopsy (Munné et al., 2007). If the embryo quality is high and an expert embryologist performs blastomere biopsy, removing two blastomeres had no detrimental impact on embryo development.

Another limitation of blastomere biopsy is chromosomal mosaicism that occurs during the first mitotic divisions and is higher in cleavage-stage embryos compared to blastocysts (van Echten-Arends et al., 2011). TE biopsy could be a good candidate to overcome this limitation.

Mosaicism has been reported for 2-13% blastocysts compared to 50% for whole embryos (Popovic et al., 2020). Re-biopsy is recommended when the removed blastomere is lost, anucleate blastomere is removed or diagnosis had failed (Harton et al., 2011). Animal studies have shown that blastomere biopsy was not associated with alterations in global patterns of gene expression (Duncan et al., 2009). Removing one blastomere in an 8-cell embryo did not affect genes participating in the implantation (Naseri et al., 2019). Nevertheless, increasing cDNA following biopsy was reported which may be due to stress response (Jones et al., 2015). A recent time-lapse study showed that blastomere biopsy may affect morphokinetics of biopsied embryos (Lammers et al., 2021).

Morula biopsy

Blastomere biopsy is preferred on day 3 because the compaction had not started and removing blastomeres is easier than at subsequent stages. But, blastomere biopsy on day 4 could also be performed with more numbers of blastomeres removed. Zakharova and colleagues were the first that proposed blastomere biopsy at the morula stage (Zakharova et al., 2014). They showed that embryo biopsy on day 4 had no detrimental effects on further embryo development. Application of day 6 morula biopsy is also suggested in women < 40 years for PGT-A, however, the rates of implantation and live birth were significantly lower in euploid morula in comparison with euploid blastocysts (Irani et al., 2018). Embryos that are not suitable for biopsy on day 3 are good candidates for morula biopsy. Orvieto and colleagues compared clinical outcomes of PGT-M for embryos biopsied on day 3 and day 4. They reported a significantly higher ongoing pregnancy rate for embryos biopsied at morula stage compared to cleavage-stage (33.3 vs 20.5%, $P<0.03$, respectively; Orvieto et al., 2020).

Trophectoderm biopsy

Summers and associates reported the first TE biopsy on monkey blastocyst (Summers et al., 1988) which was followed in humans (Dokras et al., 1990). TE cells have a low rate of mosaicism making TE biopsy a good alternative for blastomere biopsy (Harton et al., 2011, Vera-Rodriguez and Rubio, 2017). Developing or developed blastocyst are the optimum for TE biopsy (Aoyama and Kato, 2020). There are three approaches for TE biopsy including pulling, flicking and sequential ZP opening and TE biopsy (Cimadomo et al., 2020). In the pulling approach, some TE cells are pulled by biopsy pipette and detached from blastocyst; while, in the flicking approach, TE cells are detached from blastocyst by vigorous movement of biopsy pipette which is suitable for hatched blastocyst (McArthur et al., 2005). In the last approach, the blastocyst was hatched on the day of biopsy and some media were blown by biopsy pipette in the hole beneath the ZP and then some TE cells were pulled by a pipette and were detached by laser shots (Capalbo et al., 2014).

Opening the ZP on days 3, 4 or 5-6 followed by TE biopsy have been proposed as well (De Boer et al., 2004, Veiga et al., 1997, Capalbo et al., 2016a). A smaller ZP hole is suggested to avoid less constriction of the hatching site to facilitate cutting (Aoyama and Kato, 2020). Inner cell mass (ICM) should be avoided to prevent damage. It is recommended to hatch the ZP on day 5-6 rather than day 3-4 (Aoyama and Kato, 2020). Time taken for a single TE biopsy is generally less than 3 minutes (Capalbo et al., 2016b).

Regarding the comparison between cleavage-stage and TE biopsy, Kokkali et al. reported a lower diagnostic efficiency for blastomere biopsy in comparison to TE biopsy for beta-thalassemia (75.2 vs 94.3%, respectively; Kokkali et al., 2007). Scott et al. showed that positive predictive value of comprehensive chromosome screening for clinical outcomes was significantly lower in blastomere biopsy in comparison with TE biopsy (29.2 vs 48.2%, respectively); while, negative predictive value was similar between two techniques (Scott Jr et al., 2012). The same group also reported that blastomere biopsy resulted in a relative reduction of 39% in the live birth rate, while implantation rate was similar in TE biopsy group and controls (Scott Jr et al., 2013). Zhao et al. found that TE biopsy without ZP hatching on day 3 resulted in a higher frozen blastocyst rate in comparison with the hatching group (53.96 vs 47.94%, respectively) (Zhao et al., 2019).

The numbers of TE cells that has to be biopsied is a matter of debate. It seems when the number of biopsied TE cells was <15, the pregnancy was not affected by the technique (Neal et al., 2017). Also, it was shown that TE biopsy did not compromise the cryosurvival of blastocysts (Cimadomo et al., 2018). TE biopsy is associated with high efficacy and accuracy (Greco et al., 2015). Regarding safety and neonatal outcomes of TE biopsy, a recent meta-analysis showed that time of biopsy (day 3 vs day 5) was not correlated with clinical pregnancy and live birth rates without affecting early childhood diseases (Natsuaki and Dimler, 2018). He and colleagues in a large study followed 1,721 children born after TE biopsy. They found no relationship between increased risk of neonatal outcomes and blastocyst biopsy (He et al., 2019). The effect of TE biopsy has been proposed on embryo gene expression. TE biopsy may down regulate the expression of different genes in the placenta like placental growth factors by disruption of tight junctions between TE cells (Tocci, 2021). One concern is the relationship between embryo biopsy and increased risk of monozygotic splitting following embryo biopsy (Kamath et al., 2020). Sellers et al. in a recent retrospective study showed an increased risk of monozygote twining following TE biopsy (Sellers et al., 2021).

Blastocentesis

Blastocentesis is a micromanipulation technique performed at the blastocyst stage. This technique is a part of a non-invasive PGT (niPGT) procedure (Leaver and Wells, 2020). To do this, a very low amount of blastocoel fluid (≈ 1µl) is aspirated from the blastocyst with an injection pipette (Cimadomo et al., 2020). Rule and colleagues showed that cell-free DNA that is present in the blastocoel fluid is associated with embryo quality (Rule et al., 2018). Nuclear and mitochondrial DNA found in the blastocoel fluid may be used for PGT (Hammond et al., 2016). Recently, Magli et al. evaluated the presence of DNA in blastocoel fluid with the ensuing pregnancy rate (Magli et al., 2019). They concluded that blastocoelic DNA was associated with blastocyst ploidy and could be considered as a predictor of a viable pregnancy. Further studies are required to clinically approve this minimally invasive technique.

Polar body biopsy

Since introduction of PB biopsy in 1990 (Verlinsky et al., 1990), several indications have been proposed for this technique including detection of single-gene disorders, X-linked disorders, analysis of translocations, HLA matching and aneuploidy (Verlinsky et al., 1997, Verlinsky et al., 2001, Munné et al., 1998). First PB is extruded following first meiotic division and second PB is extruded following second meiotic division.

First and second PB can be safely removed for estimation of oocyte genetic material without any detrimental effects on fertilization or embryo development. First and second PB biopsies are considered as alternative methods for reading genome to embryo biopsy. This method can be chosen in countries with restrictions on embryo biopsy and cryopreservation. However, only

maternal contribution can be analyzed and the role of sperm is neglected. To do this, ZP should be opened (usually by laser). Following zona drilling, the oocyte is held firmly by the holding pipette. A biopsy needle (18 µm diameter) is introduced into the oocyte to remove the PB (Montag, 2019). It is less invasive compared to blastomere and TE biopsy. Two PBs can be removed simultaneously or sequentially. In the latter, the first PB is removed from the oocyte and the second PB is removed after fertilization (Gianaroli, 2000).

The optimum time for first PB biopsy is before or just after ICSI and for second PB is 16 hours after insemination (Montag, 2019, Greco et al., 2020). Simultaneous first and second PB removal is less invasive compared to separate removal of each PB, and is suggested this be performed at 8-14 hours after fertilization (Montag, 2019). If the sequential approach is selected, second PB should be removed from the zona opening created for first PB biopsy. The zona opening must be as small as possible to avoid hatching problems. Evaluating both PBs can improve the abnormality detection rate (Schmutzler, 2019).

Verpoest et al. in a multicentric, multinational randomized clinical trial showed that embryo selection according to PGT-A in first and second PB did not impact on live birth rate in advanced maternal age (36-40 years; Verpoest et al., 2018). PB errors are dependent on female age and it seems that first PB analysis is more suitable for young women while the first and second PB analyses more suited for older women (Fragouli et al., 2011). More caution should be exercised if the PB is fragmented. If PB is fragmented, the fragments should also be removed and analyzed for increasing test accuracy. Missing PB fragments may lead to misdiagnosis (Montag, 2019). There are controversies with regards to the clinical application of PB biopsy (Christopikou et al., 2013, Salvaggio et al., 2014).

Artificial shrinkage

Embryos can be cryopreserved at any stage from zygote to blastocyst. Blastocyst cryopreservation is the most efficient stage (Nagy et al., 2020) but day 4 compacted morula may be more resistant to damage during cooling but day 4 cooling has been less attempted in the human. This remains to be investigated.

Cryopreservation of blastocysts may be associated with an increased risk of formation of ice crystals due to presence of a high amount of water in the blastocoele cavity. Artificial shrinkage or blastocyst collapse refers to fluid removal from the blastocoel cavity before vitrification which was anticipated to reduce the risk of ice crystal formation. There are different methods of artificial shrinkage including blastocyst puncture, aspiration of blastocoele fluid and use of high osmotic media (Hiraoka et al., 2004, Davidson et al., 2019).

Application of laser pulse is also performed for artificial shrinkage (Boyard et al., 2020). There are several studies about the beneficial effects of blastocyst collapse on ART outcomes (Van Landuyt et al., 2015b, Pooyanfar et al., 2018, Hiraoka et al., 2004). Vanderzwalmen and colleagues reported live birth following artificial shrinkage (Vanderzwalmen et al., 2002) and numerous studies have been performed to evaluate efficacy of this technique.

It seems retrospective studies are in favor of beneficial effects of this technique on pregnancy and implantation rates. Mukaida et al. used mechanical or laser for blastocyst collapse and showed a significant increase in survival and clinical pregnancy rates in the experimental group compared to the control (Mukaida et al., 2006). In a retrospective study, it was found that using artificial shrinkage by laser or osmotic shock increased survival and implantation rates compared to controls, however, there was no significant difference between laser and osmotic shock (Iwayama et al., 2011). Wang and colleagues compared two methods of artificial shrinkage and found that the rate of neonatal outcomes was the same for laser and micro-needle method; while, the rates of implantation, pregnancy and live birth were higher in the laser group (Wang et al., 2017). Nevertheless, Van Landuyt in a randomized controlled trial showed that artificial shrinkage by laser could not significantly increase the implantation rate, although the survival rate was significantly increased (Van Landuyt et al., 2015a). It was shown that the delivery rate could also be increased following artificial shrinkage in a retrospective study (Levi-Setti et al., 2016).

In a prospective observational study, Kovačič et al. showed that re-expansion speed of artificially collapsed embryos may be different with no impact on live birth rate (Kovačič et al., 2018). However, a recent systematic review and meta-analysis showed that artificial shrinkage of blastocyst before vitrification can improve rates of survival and clinical pregnancy, but has no impact on the live birth rate (Boyard et al., 2020).

Recently, an animal study showed that a combination of artificial shrinkage and melatonin supplementation may increase the hatching rate, reduce apoptotic cells and could be an alternative method to improve cryotolerance (Marques et al., 2021). It seems there is no impact on neonatal outcomes of artificial shrinkage (Mitsuhata et al., 2019).

Embryo splitting

Embryo splitting or embryo twinning is a natural method to copy organisms. The term "cloning" is used for embryo splitting due to producing several embryos with the identical genomes (Medicine, 2004). Compared to another method of copying organisms, somatic cell nuclear transfer, embryo splitting is more cost-effective and less invasive. Embryo splitting has several indications like producing more embryos for poor responders, producing genetically identical embryos for research and producing more embryos to generate new pluripotent stem cell lines (Noli et al., 2017, Omidi et al., 2019). Indeed, this technique can increase the number of embryos that can be used for infertility treatment or research purposes. Mainly, this technique is performed at cleavage-stage or post-compaction embryo.

Research on animal embryo splitting was started in the 19th century followed by human reports about one hundred years later in the 90s (Driesch, 1894, Hall et al., 1993). For embryo splitting/blastomere separation at the cleavage-stage embryo, one or two blastomere/s is/are removed from donor embryo and inserted into an empty ZP producing split twin and supposedly identical embryo designated by the authors as “recipient” embryos. If more cells are removed, the technique is more invasive but the success rate is increased. This technique is similar to blastomere biopsy. The embryo should be incubated in Ca^{++}/Mg^{++} free medium for 10 minutes. After opening the ZP (35-40 µm), the biopsy needle (ID: 35 µm) is gently inserted into the embryo and the blastomeres removed one by one and placed in a host empty ZP (Omidi et al., 2019). Empty ZP has several indications in ART and the method for preparing empty ZP has been previously described (Halvaei et al., 2016b). Splitting at the post-compaction embryo (bisection) is performed with a surgical microblade to halve the embryo. In case of blastocyst, ICM and TE cells are divided into two segments (Noli et al., 2017). There is a lack of data on the bisection method on human embryos. Wood, in a cattle model, reported a 30-40% increase in the chance of conception and concluded that embryo splitting may reduce cost, stress, time and complications for patients who are seeking ART treatment (Wood, 2001). It was shown that embryo splitting at the cleavage-stage embryo could result in producing viable twin blastocysts. Van de Velde split six 4-cell embryos and cultured each blastomere individually. They found that each blastomere can produce a blastocyst with ICM and TE (Van de Velde et al., 2008).

Although a blastomere can produce a blastocyst, the quality of blastocyst is not satisfactory (Tang et al., 2012). However, Noli and colleagues showed that the quality of blastocysts derived from blastomere separation was low. There was a delay in detection of ICM in twin blastocysts with small size and low quality (Noli et al., 2015). Illmensee et al. evaluated the effects of serial cleavage-stage embryo splitting in a mouse model. They found that up to the second splitting, the rate of blastulation was satisfactory (Illmensee et al., 2006). The size of twin blastocyst is dependent on the number of blastomeres used for its creation (Noli et al., 2015). Splitting efficacy was higher at 6- to 8-cell embryos compared to 2- to 5-cell stage (Illmensee et al., 2010). Splitting success rate is dependent on the morphological quality of embryo before splitting (Illmensee et al., 2010).

Metabolic profiles of twin embryos may have different patterns compared to controls. Noli et al. showed that split embryos secrete a low level of miRNA-30c, a marker of blastocyst implantation, and 22.9% miRNAs detected in split embryo culture medium were not secreted

in the control blastocysts (Noli et al., 2017). Omidi et al. found that blastocyst rate was significantly higher in donor embryos compared to the “recipient” (its split twin) embryos. It is suggested to place the blastomeres inside the empty ZP near each other to facilitate the re-establishment of cell–cell contact which is necessary for further development. Omidi et al. tried to derive human embryonic stem cells from twin 3PN embryos with their splitting protocol (Omidi et al., 2019). Morphokinetics of embryo may be affected by splitting (Noli et al., 2015). Omidi et al. found that there were significant differences in attaining compaction and expansion stages between the twin and control embryos while the rate of aneuploidy and mosaicism was the same between different groups (Omidi et al., 2020). The same group evaluated splitting efficacy and developmental potential of in vitro splitting at cleavage-stage embryos into different groups. They showed a high splitting and developmental efficacy for frozen-warmed donated embryos followed by aneuploid, parthenogenic and in vitro maturation-derived embryos.

The total cell number and the proportion of dead cells were significantly lower in twin embryos compared to controls (Omidi et al., 2021). As splitting is an invasive technique, it could be anticipated that blastomeres respond to this stress at the molecular level. The transcriptome and gene expression are also affected by blastocyst splitting in animal models (Velasquez et al., 2016, Ventura-Juncá et al., 2015). The embryo quality is an important predictor of splitting efficacy and developmental potential of derived blastocysts. Therefore, good quality embryos with more cell numbers are recommended for splitting. The ASRM has approved research on embryo splitting, but the concerns that remain to be addressed is its clinical application (Medicine, 2004). The clinical usage of this technique needs further investigation and strict guidelines need be defined to avoid abuse.

Conclusion

A successful ART procedure needs normal and healthy sperm, oocyte and embryo. When confronted with abnormal gametes and embryos, different strategies can be applied to improve the success rate including the use of appropriate micromanipulation techniques. The ingenuity and technical skills of embryologists are paramount to introduce and perform these techniques. Each technique has its pros and cons, and should be carefully selected for special cases and routine application is not recommended. It is cautioned that the concerns about genomic and epigenomic alterations induced by micromanipulations exists. Patients should be aware of any probable side effects and comprehensive consultation is necessary when less common and less utilized micromanipulation techniques are applied. It is anticipated that many more micromanipulation techniques will be introduced in the future to overcome infertility problems in ART program.

References

Abdelmassih S, Cardoso J, Abdelmassih V, Dias JA, Abdelmassih R, Nagy ZP. Laser-assisted ICSI: a novel approach to obtain higher oocyte survival and embryo quality rates. Human Reprod. 2002; 17(10):2694-2699.

Aktan T, Montag M, Duman S, Gorkemli H, Rink K, Yurdakul TJA. Use of a laser to detect viable but immotile spermatozoa. Andrologia. 2004; 36(6):366-369.

Ali J, Rahbar S, Burjaq H, Sultan AM, Al Flamerzi, Shahata MAM. Routine laser assisted hatching results in significantly increased clinical pregnancies. J. Asst Reprod. Genetics. 2003; 20(5):177-181

Alikani M, Cohen J, Tomkin G, Garrisi GJ, Mack C, Scott RT. Human embryo fragmentation in vitro and its implications for pregnancy and implantation. Fertil Steril. 1999; 71(5):836-842.

Alikani M, Olivennes F, Cohen J. Fertilization and early embryology: Microsurgical correction of partially degenerate mouse embryos promotes hatching and restores their viability. Human Reprod. 1993; 8(10):1723-1728.

Aoyama N, Kato K. Trophectoderm biopsy for preimplantation genetic test and technical tips: A review. Reprod Med Biol. 2020; 19(3):222-231.

Aydos K, Aydos OSJJoCM. Sperm Selection Procedures for Optimizing the Outcome of ICSI in Patients with NOA. J Clin Med. 2021; 10(12):2687.

Balaban B, Urman B, Alatas C, Mercan R, Mumcu A, Isiklar A. A comparison of four

different techniques of assisted hatching. Human Reprod. 2002; 17(5):1239-1243.

Bar-El L, Kalma Y, Malcov M, Schwartz T, Raviv S, Cohen T, Amir H, Cohen Y, Reches A, Amit A, Ben-Yosef D. Blastomere biopsy for PGD delays embryo compaction and blastulation: a time-lapse microscopic analysis. J Assist Reprod Genet. 2016; 33(11):1449-1457.

Blerkom JV, Davis P, Lee J. ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. Human Reprod. 1995; 10(2):415-424.

Boyard J, Reignier A, Chtourou S, Lefebvre T, Barrière P, Fréour T. Should artificial shrinkage be performed prior to blastocyst vitrification? A systematic review of the literature and meta-analysis. Hum Fertil. 2020:1-9.

Brodie D, Beyer C, Osborne E, Kralevski V, Rasi S, Osianlis T. Preimplantation genetic diagnosis for chromosome rearrangements–one blastomere biopsy versus two blastomere biopsy. J Assist Reprod Genet. 2012; 29(8):821-827.

Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, Nagy ZP, Ubaldi FM. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. Human Reprod. 2014; 29(6):1173-1181.

Capalbo A, Romanelli V, Cimadomo D, Girardi L, Stoppa M, Dovere L, Dell'Edera D, Ubaldi FM, Rienzi L. Implementing PGD/PGD-A in IVF clinics: considerations for the best laboratory approach and management. J Assist Reprod Genet. 2016; 33(10):1279-1286.

Capalbo A, Ubaldi FM, Cimadomo D, Maggiulli R, Patassini C, Dusi L, Sanges F, Buffo L, Venturella R, Rienzi L. Consistent and reproducible outcomes of blastocyst biopsy and aneuploidy screening across different biopsy practitioners: a multicentre study involving 2586 embryo biopsies. Human Reprod. 2016; 31(1):199-208.

Chen H, Feng G, Zhang B, Zhou H, Shu J, Gan X. A successful pregnancy using completely immotile but viable frozen-thawed spermatozoa selected by laser. Clin Exp Reprod Med. 2017; 44(1):52-55.

Christopikou D, Tsorva E, Economou K, Shelley P, Davies S, Mastrominas M, Handyside AH. Polar body analysis by array comparative genomic hybridization accurately predicts aneuploidies of maternal meiotic origin in cleavage stage embryos of women of advanced maternal age. Human Reprod. 2013; 28(5):1426-1434.

Cimadomo D, Capalbo A, Levi-Setti PE, Soscia D, Orlando G, Albani E, Parini V, Stoppa M, Dovere L, Tacconi L. Associations of blastocyst features, trophectoderm biopsy and other laboratory practice with post-warming behavior and implantation. Human Reprod. 2018; 33(11):1992-2001.

Cimadomo D, Capalbo A, Ubaldi FM, Scarica C, Palagiano A, Canipari R, Rienzi L. The Impact of Biopsy on Human Embryo Developmental Potential during Preimplantation Genetic Diagnosis. Biomed Res Int. 2016; 2016.

Cimadomo D, Rienzi L, Capalbo A, Rubio C, Innocenti F, García-Pascual CM, Ubaldi FM, Handyside A. The dawn of the future: 30 years from the first biopsy of a human embryo. The detailed history of an ongoing revolution. Hum Reprod Update. 2020; 26(4):453-473.

Cohen J, Alikani M, Trowbridge J, Rosenwaks Z. Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. Human Reprod. 1992; 7(5):685-691.

Cohen J, Scott R. Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. The Lancet. 1997; 350(9072):186-187.

Davidson LM, Liu Y, Griffiths T, Jones C, Coward K. Laser technology in the ART laboratory: a narrative review. Reprod Biomed Online. 2019; 38(5):725-739.

De Boer KA, Catt JW, Jansen RP, Leigh D, McArthur S. Moving to blastocyst biopsy for preimplantation genetic diagnosis and single embryo transfer at Sydney IVF. Fertil Steril. 2004; 82(2):295-298.

de Oliveira NM, Sánchez RV, Fiesta SR, Salgado TL, Rodríguez R, Bethencourt JCA, Zamora RB. Pregnancy with frozen–thawed and fresh testicular biopsy after motile and immotile sperm microinjection, using the mechanical touch technique to assess viability. Human Reprod. 2004; 19(2):262-265.

Dokras A, Sargent IL, Ross C, Gardner RL, Barlow DH. Trophectoderm biopsy in human blastocysts. Human Reprod. 1990; 5(7):821-825.

Driesch H (1894). Analytische theorie der organischen entwicklung. Leipzig, Engelmann.

Duncan FE, Stein P, Williams CJ, Schultz RM. The effect of blastomere biopsy on

preimplantation mouse embryo development and global gene expression. Fertil Steril. 2009; 91(4, Supplement):1462-1465.

Ebner T, Yaman C, Moser M, Sommergruber M, Pölz W, Tews G. Embryo fragmentation in vitro and its impact on treatment and pregnancy outcome. Fertil Steril. 2001; 76(2):281-285.

Eftekhari-Yazdi P, Valojerdi MR, Ashtiani SK, Eslaminejad MB, Karimian L. Effect of fragment removal on blastocyst formation and quality of human embryos. Reprod Biomed Online. 2006; 13(6):823-832.

Elhussieny A, El Mandouh M, Hanafi S, Mansour GM, El-Kotb A. Effect of laser assisted hatching on outcome of assisted reproductive technology. Open J Obstet Gynecol. 2013; 3(5A2):34290.

Elliott TA, Colturato LFA, Taylor TH, Wright G, Kort HI, Nagy ZP. Lysed cell removal promotes frozen–thawed embryo development. Fertil Steril. 2007; 87(6):1444-1449.

Fang C, Li T, Miao BY, Zhuang GL, Zhou C. Mechanically expanding the zona pellucida of human frozen thawed embryos: a new method of assisted hatching. Fertil Steril. 2010; 94(4):1302-1307.

Fathi R, Valojerdi MR, Eftekhari-Yazdi P. Effect of laser-assisted hatching and necrotic blastomere removal on the development of vitrified–warmed four-cell mouse embryos. J Assist Reprod Genet. 2008; 25(7):333-339.

Fawzy M, Emad M, Mahran A, Abdelrahman MY, Fetih AN, Abdelghafar H, Elsuity MA, Rashed H, Sabry M. A randomized controlled trial of laser-assisted ICSI. Human Reprod. 2020; 35(12):2692-2700.

Flood JT, Chillik CF, van Uem JF, Iritani A, Hodgen GD. Ooplasmic transfusion: prophase germinal vesicle oocytes made developmentally competent by microinjection of metaphase II egg cytoplasm. Fertil Steril. 1990; 53(6):1049-1054.

Fong CY, Bongso A, Ng SC, Kumar J, Trounson A, Ratnam S. Blastocyst transfer after enzymatic treatment of the zona pellucida: improving in-vitro fertilization and understanding implantation. Human Reprod. 1998; 13(10):2926-2932.

Fragouli E, Alfarawati S, Goodall N-n, Sánchez-García JF, Colls P, Wells D. The cytogenetics of polar bodies: insights into female meiosis and the diagnosis of aneuploidy. Mol Hum Reprod. 2011; 17(5):286-295.

Friedler S, Schachter M, Strassburger D, Esther K, Ron El R, Raziel AJ. A randomized clinical trial comparing recombinant hyaluronan/recombinant albumin versus human tubal fluid for cleavage stage embryo transfer in patients with multiple IVF-embryo transfer failure. Human Reprod. 2007; 22(9):2444-2448.

Fujimoto VY, Browne RW, Bloom MS, Sakkas D, Alikani M. Pathogenesis, developmental consequences, and clinical correlations of human embryo fragmentation. Fertil Steril. 2011; 95(4):1197-1204.

Gerber PA, Kruse R, Hirchenhain J, Krüssel JS, Neumann NJ. Pregnancy after laser-assisted selection of viable spermatozoa before intracytoplasmatic sperm injection in a couple with male primary cilia dyskinesia. Fertil Steril. 2008; 89(6):1826.e1829-1812.

Gianaroli L. Preimplantation genetic diagnosis: polar body and embryo biopsy. Human Reprod. 2000; 15 Suppl 4:69-75.

González-Grajales LA, Favetta LA, King WA, Mastromonaco GF. Lack of effects of ooplasm transfer on early development of interspecies somatic cell nuclear transfer bison embryos. BMC Dev Biol. 2016; 16(1):36.

Goossens V, De Rycke M, De Vos A, Staessen C, Michiels A, Verpoest W, Van Steirteghem A, Bertrand C, Liebaers I, Devroey P. Diagnostic efficiency, embryonic development and clinical outcome after the biopsy of one or two blastomeres for preimplantation genetic diagnosis. Human Reprod. 2008; 23(3):481-492.

Greco E, Biricik A, Cotarelo RP, Iammarone E, Rubino P, Tesarik J, Fiorentino F, Minasi MG. Successful implantation and live birth of a healthy boy after triple biopsy and double vitrification of oocyte-embryo-blastocyst. Springerplus. 2015; 4(1):22.

Greco E, Litwicka K, Minasi MG, Cursio E, Greco PF, Barillari P. Preimplantation genetic testing: where we are today. Int J Mol Sci. 2020; 21(12):4381.

Haddad M, Stewart J, Xie P, Cheung S, Trout A, Keating D, Parrella A, Lawrence S, Rosenwaks Z, Palermo GD. Thoughts on the popularity of ICSI. J Assist Reprod Genet. 2021; 38(1):101-123.

Hall JL, Engel D, Gindoff PR, Mottla G, Stillman R. Experimental cloning of human polyploid embryos using an artificial zona pellucida. Fertil Steril. 1993; 61:S1.

Halvaei I, Esfandiari N (2021). Factors affecting intarcytoplasmic sperm injection fertilization rate. Intarcytoplasmic sperm injection (ICSI), cliical considerations, outcomes and

potential health effects. M. Javed. New York, Nova Science Publishers, Inc.

Halvaei I, Ghazali S, Nottola SA, Khalili MA. Cleavage-stage embryo micromanipulation in the clinical setting. Syst Biol Reprod Med. 2018; 64(3):157-168.

Halvaei I, Khalili MA, Esfandiari N, Safari S, Talebi AR, Miglietta S, Nottola SA. Ultrastructure of cytoplasmic fragments in human cleavage stage embryos. J Assist Reprod Genet. 2016 ;33(12):1677-1684.

Halvaei I, Khalili MA, Nottola SA. A novel method for transmission electron microscopy study of cytoplasmic fragments from preimplantation human embryos. Micros Res Tech. 2016; 79(6):459-462.

Halvaei I, Khalili MA, Safari S, Esfandiari N. Ongoing pregnancies following cosmetic micromanipulation of preimplantation embryos in patients with implantation failure. Case Rep Med. 2015; 2015.

Hammadeh ME, Fischer-Hammadeh C, Ali KR. Assisted hatching in assisted reproduction: a state of the art. J Assist Reprod Genet. 2011; 28(2):119-128.

Hammond ER, Shelling AN, Cree LM. Nuclear and mitochondrial DNA in blastocoele fluid and embryo culture medium: evidence and potential clinical use. Human Reprod. 2016; 31(8):1653-1661.

Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature. 1990; 344(6268):768-770.

Harton G, Magli M, Lundin K, Montag M, Lemmen J, Harper J. ESHRE PGD Consortium/Embryology Special Interest Group—best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). Human Reprod. 2011; 26(1):41-46.

He F, Zhang CY, Wang LS, Li SL, Hu LN. Assisted Hatching in Couples with Advanced Maternal Age: A Systematic Review and Meta-analysis. Curr Med Sci. 2018; 38(3):552-557.

He H, Jing S, Lu CF, Tan YQ, Luo KL, Zhang SP, Gong F, Lu GX, Lin G. Neonatal outcomes of live births after blastocyst biopsy in preimplantation genetic testing cycles: a follow-up of 1,721 children. Fertil Steril. 2019; 112(1):82-88.

Hiraoka K, Hiraoka K, Kinutani M, Kinutani K. Blastocoele collapse by micropipetting prior to vitrification gives excellent survival and pregnancy outcomes for human day 5 and 6 expanded blastocysts. Human Reprod. 2004; 19(12):2884-2888.

Honguntikar SD, Salian SR, D'souza F, Uppangala S, Kalthur G, Adiga SK. Epigenetic changes in preimplantation embryos subjected to laser manipulation. Lasers Med Sci. 2017; 32(9):2081-2087.

Huo P, Deng K, Wang L, Li M, Yao J, Le J, Lei X, Zhang S. The effect of laser-assisted hatching on the methylation and expression pattern of imprinted gene IGF2/H19 in mouse blastocysts and offspring. J Assist Reprod Genet. 2020; 37(12):3057-3067.

Illmensee K, Kaskar K, Zavos PM. In vitro blastocyst development from serially split mouse embryos and future implications for human assisted reproductive technologies. Fertil Steril. 2006; 86(4):1112-1120.

Illmensee K, Levanduski M, Vidali A, Husami N, Goudas VT. Human embryo twinning with applications in reproductive medicine. Fertil Steril. 2010; 93(2):423-427.

Irani M, Zaninovic N, Canon C, O'Neill C, Gunnala V, Zhan Q, Palermo G, Reichman D, Rosenwaks Z. A rationale for biopsying embryos reaching the morula stage on Day 6 in women undergoing preimplantation genetic testing for aneuploidy. Human Reprod. 2018; 33(5):935-941.

Iwayama H, Hochi S, Yamashita M. In vitro and in vivo viability of human blastocysts collapsed by laser pulse or osmotic shock prior to vitrification. J Assist Reprod Genet. 2011; 28(4):355-361.

Jayendran RS, Van der Ven HH, Perz- Paleaz M, Crabo BG, Zaneveld LJD. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other sperm characteristics. J Reprod Fertil 1984; 70:19-29

Jiang Z, Shen H. Mitochondria: emerging therapeutic strategies for oocyte rescue. Reprod Sci. 2021:1-12.

Jones AE, Wright G, Kort HI, Straub RJ, Nagy ZP. Comparison of laser-assisted hatching and acidified Tyrode's hatching by evaluation of blastocyst development rates in sibling embryos: a prospective randomized trial. Fertil Steril. 2006; 85(2):487-491.

Jones D, Paczkowski M, Kuehl T. Embryo manipulation techniques alter pre-implantation development and gene expression in mouse

embryos. Reprod Fertil Dev. 2015; 28(2):182-182.

Kalma Y, Bar-El L, Asaf-Tisser S, Malcov M, Reches A, Hasson J, Amir H, Azem F, Ben-Yosef D. Optimal timing for blastomere biopsy of 8-cell embryos for preimplantation genetic diagnosis. Human Reprod. 2018; 33(1):32-38.

Kamath MS, Antonisamy B, Sunkara SK. Zygotic splitting following embryo biopsy: a cohort study of 207 697 single-embryo transfers following IVF treatment. BJOG. 2020; 127(5):562-569.

Keltz M, Fritz R, Gonzales E, Ozensoy S, Skorupski J, Stein DJ. Defragmentation of low grade day 3 embryos resulted in sustained reduction in fragmentation, but did not improve compaction or blastulation rates. Fertil Steril. 2010; 94(6):2406-2408.

Keltz MD, Skorupski JC, Bradley K, Stein DJ. Predictors of embryo fragmentation and outcome after fragment removal in in vitro fertilization. Fertil Steril. 2006; 86(2):321-324.

Kim SG, Kim YY, Park JY, Kwak SJ, Yoo CS, Park IH, Sun HG, Kim JW, Lee KH, Park HD. Early fragment removal on in vitro fertilization day 2 significantly improves the subsequent development and clinical outcomes of fragmented human embryos. Clin Exp Reprod Med. 2018; 45(3):122-128.

Kirkegaard K, Hindkjaer JJ, Ingerslev HJ. Human embryonic development after blastomere removal: a time-lapse analysis. Hum Reprod. 2012; 27(1):97-105.

Kissin DM, Kawwass JF, Monsour M, Boulet SL, Session DR, Jamieson DJ. Assisted hatching: trends and pregnancy outcomes, United States, 2000-2010. Fertil Steril. 2014; 102(3):795-801.

Kokkali G, Traeger-Synodinos J, Vrettou C, Stavrou D, Jones GM, Cram DS, Makrakis E, Trounson AO, Kanavakis E, Pantos K. Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of β-thalassaemia: a pilot study. Hum Reprod. 2007; 22(5):1443-1449.

Kovačič B, Taborin M, Vlaisavljević V. Artificial blastocoel collapse of human blastocysts before vitrification and its effect on re-expansion after warming–a prospective observational study using time-lapse microscopy. Reprod Biomed Online. 2018; 36(2):121-129.

Lacey L, Hassan S, Franik S, Seif MW, Akhtar MA. Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)). Cochrane Database Syst Rev. 2021; 3(3):Cd001894.

Lammers J, Reignier A, Loubersac S, Chtourou S, Lefebvre T, Barrière P, Fréour T. Modification of late human embryo development after blastomere removal on day 3 for preimplantation genetic testing. Syst Biol Reprod Med. 2021; 67(2):121-126.

Lanzendorf SE, Ratts VS, Moley KH, Goldstein JS, Dahan MH, Odem RR. A randomized, prospective study comparing laser-assisted hatching and assisted hatching using acidified medium. Fertil Steril. 2007; 87(6):1450-1457.

Le MT, Nguyen TTA, Nguyen TTT, Nguyen VT, Le DD, Nguyen VQH, Cao NT, Aints A, Salumets A. Thinning and drilling laser-assisted hatching in thawed embryo transfer: A randomized controlled trial. Clin Exp Reprod Med. 2018; 45(3):129-134.

Leaver M, Wells D. Non-invasive preimplantation genetic testing (niPGT): the next revolution in reproductive genetics? Hum Reprod Update. 2020; 26(1):16-42.

Lee WJ, Lee JH, Jeon RH, Jang SJ, Lee SC, Park JS, Lee SL, King WA, Rho GJ. Supplement of autologous ooplasm into porcine somatic cell nuclear transfer embryos does not alter embryo development. Reprod Domest Anim. 2017; 52(3):437-445.

Levi-Setti PE, Menduni F, Smeraldi A, Patrizio P, Morenghi E, Albani E. Artificial shrinkage of blastocysts prior to vitrification improves pregnancy outcome: analysis of 1028 consecutive warming cycles. J Assist Reprod Genet. 2016; 33(4):461-466.

Levron J, Willadsen S, Bertoli M, Cohen J. The development of mouse zygotes after fusion with synchronous and asynchronous cytoplasm. Hum Reprod. 1996; 11(6):1287-1292.

Liu Y, Jones C, Coward K. An investigation of the effects of laser-assisted zona pellucida drilling on the preimplantation mouse embryo and the competency of embryo implantation. F&S Science. 2021.

Magli MC, Albanese C, Crippa A, Tabanelli C, Ferraretti AP, Gianaroli L. Deoxyribonucleic acid detection in blastocoelic fluid: a new predictor of embryo ploidy and viable pregnancy. Fertil Steril. 2019; 111(1):77-85.

Malter HE, Cohen J. Blastocyst formation and hatching in vitro following zona drilling of mouse

and human embryos. Gamete Res. 1989; 24(1):67-80.

Malter HE, Cohen J. Partial zona dissection of the human oocyte: a nontraumatic method using micromanipulation to assist zona pellucida penetration. Fertil Steril. 1989; 51(1):139-148.

Mansour R, Rhodes C, Aboulghar M, Serour G, Kamal A. Transfer of zona-free embryos improves outcome in poor prognosis patients: a prospective randomized controlled study. Hum Reprod. 2000; 15(5):1061-1064.

Mantoudis E, Podsiadly BT, Gorgy A, Venkat G, Craft IL. A comparison between quarter, partial and total laser assisted hatching in selected infertility patients. Hum Reprod. 2001; 16(10):2182-2186.

Marques TC, Santos ECdS, Diesel TO, Martins CF, Cumpa HCB, Leme LdO, Dode MAN, Alves BG, Costa FPH, Oliveira EBd, Gambarini ML. Blastocoel fluid removal and melatonin supplementation in the culture medium improve the viability of vitrified bovine embryos. Theriogenology. 2021; 160:134-141.

Martins WP, Rocha IA, Ferriani RA, Nastri CO. Assisted hatching of human embryos: a systematic review and meta-analysis of randomized controlled trials. Hum Reprod Update. 2011; 17(4):438-453.

McArthur SJ, Leigh D, Marshall JT, de Boer KA, Jansen RP. Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertil Steril. 2005; 84(6):1628-1636.

Medicine PCotASfR. Role of assisted hatching in in vitro fertilization: a guideline. Fertil Steril. 2014; 102(2):348-351.

Medicine TECotASfR. Embryo splitting for infertility treatment. Fertil Steril. 2004; 82:256-257.

Mitsuhata S, Endo Y, Hayashi M, Fujii Y, Motoyama H. Effect on clinical and neonatal outcomes of blastocelic microsuction prior to vitrification. 2019; Reprod Med Biol. 18(3):284-289.

Montag M (2019). Polar body biopsy. In Vitro Fertilization. Z. P. Nagy, A. C. Varghese A. Agarwal, Springer: 583-590.

Moutou C, Goossens V, Coonen E, De Rycke M, Kokkali G, Renwick P, SenGupta S, Vesela K, Traeger-Synodinos J. ESHRE PGD Consortium data collection XII: cycles from January to December 2009 with pregnancy follow-up to October 2010. Hum Reprod. 2014:deu012.

Mukaida T, Oka C, Goto T, Takahashi K. Artificial shrinkage of blastocoeles using either a micro-needle or a laser pulse prior to the cooling steps of vitrification improves survival rate and pregnancy outcome of vitrified human blastocysts. Hum Reprod. 2006; 21(12):3246-3252.

Munné S, Fung J, Cassel MJ, Márquez C, Weier HUG. Preimplantation genetic analysis of translocations: case-specific probes for interphase cell analysis. Hum Genet. 1998; 102(6):663-674.

Munné S, Gianaroli L, Tur-Kaspa I, Magli C, Sandalinas M, Grifo J, Cram D, Kahraman S, Verlinsky Y, Simpson JL. Substandard application of preimplantation genetic screening may interfere with its clinical success. Fertil Steril. 2007; 88(4):781-784.

Nagy ZP, Shapiro D, Chang C-C. Vitrification of the human embryo: a more efficient and safer in vitro fertilization treatment. Fertil Steril. 2020; 113(2):241-247.

Nagy ZP, Taylor T, Elliott T, Massey JB, Kort HI, Shapiro DB. Removal of lysed blastomeres from frozen–thawed embryos improves implantation and pregnancy rates in frozen embryo transfer cycles. Fertil Steril. 2005; 84(6):1606-1612.

Nakayama T, Fujiwara H, Yamada S, Tastumi K, Honda T, Fujii S. Clinical application of a new assisted hatching method using a piezo-micromanipulator for morphologically low-quality embryos in poor-prognosis infertile patients. Fertil Steril. 1999; 71(6):1014-1018.

Naseri F, Hosseini S, Ghaffari Novin M, Hosseini A, Heidari Mh, Salehi M. Does blastomere removal alter the expression level of miR-Let7a and its target genes following mouse embryo biopsy? J Cell Biochem. 2019; 120(6):9430-9436.

Natsuaki MN, Dimler LM. Pregnancy and child developmental outcomes after preimplantation genetic screening: a meta-analytic and systematic review. World J Pediatr. 2018; 14(6):555-569.

Neal SA, Franasiak JM, Forman EJ, Werner MD, Morin SJ, Tao X, Treff NR, Scott Jr RT. High relative deoxyribonucleic acid content of trophectoderm biopsy adversely affects pregnancy outcomes. Fertil Steril. 2017; 107(3):731-736. e731.

Ng SC, Bongso A, Ratnam S, Sathananthan H, Chan C, Wong P, Hagglund L, Anandakumar C, Wong Y, Goh V. Pregnancy after transfer of

sperm under zona. Lancet. 1988; 2(8614):790-790.

Ng, S.C., Bongso, T.A., Sathananthan, A.H., Ratnam, S.S., 1990, Micro-manipulation: its relevance to human IVF. Fertil.Steril. 1990; 53: 203-219

Noli L, Dajani Y, Capalbo A, Bvumbe J, Rienzi L, Ubaldi FM, Ogilvie C, Khalaf Y, Ilic D. Developmental clock compromises human twin model created by embryo splitting. Hum Reprod. 2015; 30(12):2774-2784.

Noli L, Ogilvie C, Khalaf Y, Ilic D. Potential of human twin embryos generated by embryo splitting in assisted reproduction and research. Hum Reprod Update. 2017 ;23(2):156-165.

Nordhoff V. How to select immotile but viable spermatozoa on the day of intracytoplasmic sperm injection? An embryologist's view. Andrology. 2015; 3(2):156-162.

Omidi M, Aflatoonian B, Tahajjodi SS, Khalili MA. Attempts for generation of embryonic stem cells from human embryos following in vitro embryo twinning. Stem Cells Dev. 2019; 28(5):303-309.

Omidi M, Khalili MA, Agha-Rahimi A, Nottola S, Anbari F, Faramarzi A, Palmerini MG. Efficacy of the in vitro splitting of human preimplantation embryos from ART programs. Turk J Med Sci. 2021; 51(1):68-75.

Omidi M, Khalili MA, Halvaei I, Montazeri F, Kalantar SM. Quality of blastocysts created by embryo splitting: a time-lapse monitoring and chromosomal aneuploidy study. Cell J. 2020; 22(3):367-374.

Orvieto R, Feldman B, Wiesel M, Shani H, Aizer A. Is Day-4 morula biopsy a feasible alternative for preimplantation genetic testing? PloS one. 2020; 15(9):e0238599.

Ozkavukcu S, Celik-Ozenci C, Konuk E, Atabekoglu C. Live birth after Laser Assisted Viability Assessment (LAVA) to detect pentoxifylline resistant ejaculated immotile spermatozoa during ICSI in a couple with male Kartagener's syndrome. Reprod Biol Endocrinol. 2018; 16(1):1-9.

Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992; 340(8810):17-18.

Palermo G, O'Neill C, Chow S, Cheung S, Parrella A, Pereira N, Rosenwaks Z. Intracytoplasmic sperm injection: state of the art in humans. Reproduction. 2017; 154(6):F93-F110.

Pantos K, Kokkali G, Petroutsou K, Lekka K, Malligiannis P, Koratzis A. Monochorionic triplet and monoamniotic twins gestation after intracytoplasmic sperm injection and laser-assisted hatching. Fetal Diagn Ther. 2009; 25(1):144-147.

Petersen CG, Mauri AL, Baruffi RLR, Oliveira JBA, Felipe V, Massaro FC, Franco Jr JG. Laser-assisted hatching of cryopreserved—thawed embryos by thinning one quarter of the zona. Reprod Biomed Online. 2006; 13(5):668-675.

Pey R, Vial C, Schatten G, Hafner M. Increase of intracellular Ca2+ and relocation of E-cadherin during experimental decompaction of mouse embryos. 1998; Proc Natl Acad Sci U S A. 95(22):12977-12982.

Pooyanfar F, Foroutan T, Dashtizad M. Effects of blastocyst artificial collapse prior to vitrification on hatching and survival rates and the expression of klf4 gene in mouse embryos. Vet Res Forum. 2018; 9(1):87-92.

Popovic M, Dhaenens L, Boel A, Menten B, Heindryckx BJ. Chromosomal mosaicism in human blastocysts: the ultimate diagnostic dilemma. Hum Reprod Update. 2020; 26(3):313-334.

Racowsky C, Ohno-Machado L, Kim J, Biggers JD. Is there an advantage in scoring early embryos on more than one day? Hum Reprod. 2009; 24(9):2104-2113.

Razi MH, Halvaei I, Razi Y. Laser assisted zona hatching does not improve live birth rate in patients undergoing their first ICSI cycles. Iran J Reprod Med. 2013 ;11(12):1021-1026.

Richter KS, Davis A, Carter J, Greenhouse SJ, Mottla GL, Tucker MJJJoe, reproduction ca. No advantage of laser-assisted over conventional intracytoplasmic sperm injection: a randomized controlled trial [NCT00114725]. J Exp Clin Assist Reprod. 2006; 3(1):1-7.

Rienzi L, Greco E, Ubaldi F, Iacobelli M, Martinez F, Tesarik J. Laser-assisted intracytoplasmic sperm injection. Fertil Steril. 2001; 76(5):1045-1047.

Rienzi L, Nagy ZP, Ubaldi F, Iacobelli M, Anniballo R, Tesarik J, Greco E. Laser-assisted removal of necrotic blastomeres from cryopreserved embryos that were partially damaged. Fertil Steril. 2002; 77(6):1196-1201.

Rienzi L, Ubaldi F, Iacobelli M, Minasi MG, Romano S, Ferrero S, Sapienza F, Baroni E,

Tesarik J, Greco E. Developmental potential of fully intact and partially damaged cryopreserved embryos after laser-assisted removal of necrotic blastomeres and post-thaw culture selection. Fertil Steril. 2005; 84(4):888-894.

Rienzi L, Ubaldi F, Martinez F, Minasi MG, Iacobelli M, Ferrero S, Tesarik J, Greco E. Clinical application of laser-assisted ICSI: a pilot study. Eur J Obstet Gynecol Reprod Biol. 2004; 115 Suppl 1:S77-79.

Rink K, Delacrétaz G, Salathé RP, Senn A, Nocera D, Germond M, De Grandi P, Fakan S. Non-contact microdrilling of mouse zona pellucida with an objective-delivered 1.48-microns diode laser. Lasers Surg Med. 1996; 18(1):52-62.

Rule K, Chosed RJ, Chang TA, Wininger JD, Roudebush WE. Relationship between blastocoel cell-free DNA and day-5 blastocyst morphology. J Assist Reprod Genet. 2018; 35(8):1497-1501.

Safari S, Khalili MA, Barekati Z, Halvaei I, Anvari M, Nottola SA. Cosmetic micromanipulation of vitrified-warmed cleavage stage embryos does not improve ART outcomes: An ultrastructural study of fragments. Reprod Biol. 2017 ;17(3):210-217.

Sallam HN, Sadek SS, Agameya AF. Assisted hatching--a meta-analysis of randomized controlled trials. J Assist Reprod Genet. 2003; 20(8):332-342.

Salvaggio C, Forman E, Garnsey H, Treff N, Scott R. Polar body based aneuploidy screening is poorly predictive of embryo ploidy and reproductive potential. J Assist Reprod Genet. 2014; 31(9):1221-1226.

Sansinena MJ, Lynn J, Bondioli KR, Denniston RS, Godke RA. Ooplasm transfer and interspecies somatic cell nuclear transfer: heteroplasmy, pattern of mitochondrial migration and effect on embryo development. Zygote. 2011; 19(2):147-156.

Schimmel T, Cohen J, Saunders H, Alikani M. Laser-assisted zona pellucida thinning does not facilitate hatching and may disrupt the in vitro hatching process: a morphokinetic study in the mouse. Hum Reprod. 2014; 29(12):2670-2679.

Schmutzler AG. Theory and practice of preimplantation genetic screening (PGS). Eur J Med Genet. 2019; 62(8):103670.

Schoolcraft WB, Schlenker T, Gee M, Jones GS, Jones HW. Assisted hatching in the treatment of poor prognosis in vitro fertilization candidates. Fertil Steril. 1994; 62(3):551-554.

Scott Jr RT, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil Steril. 2012; 97(4):870-875.

Scott Jr RT, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril. 2013; 100(3):624-630.

Sellers R, Castillo JC, Ten J, Rodríguez A, Ortiz JA, Sellers F, Llácer J, Bernabeu R. Monozygotic twinning following embryo biopsy at the blastocyst stage. JBRA Assist Reprod 2021; 25(1):122-127.

Soares JB, Glina S, Antunes N, Jr., Wonchockier R, Galuppo AG, Mizrahi FE. Sperm tail flexibility test: a simple test for selecting viable spermatozoa for intracytoplasmic sperm injection from semen samples without motile spermatozoa. Rev Hosp Clin Fac Med Sao Paulo. 2003; 58(5):250-253.

Sordia-Hernandez LH, Morales-Martinez FA, Frazer-Moreira LM, Villarreal-Pineda L, Sordia-Piñeyro MO, Valdez-Martinez OH. Clinical pregnancy after elimination of embryo fragments before fresh cleavage-stage embryo transfer. J Family Reprod Health. 2020; 14(3):198-204.

Sözen E, Vicdan K, Akarsu C, Tuncay G, Buluc B, Colak M. A prospective, randomized, controlled study of fragment removal in women who have moderate fragmentated embryos. Ham Rep. 2012; 27.

Summers PM, Campbell JM, Miller MW. Normal in-vivo development of marmoset monkey embryos after trophectoderm biopsy. Ham Reprod. 1988; 3(3):389-393.

Tadir Y, Douglas-Hamilton DH. Laser effects in the manipulation of human eggs and embryos for in vitro fertilization. Methods Cell Biol. 2007; 82:409-431.

Tadir Y, Wright W, Vafa O, Liaw L, Asch R, Berns M. Micromanipulation of gametes using laser microbeams. Hum Reprod. 1991; 6(7):1011-1016.

Tadir Y, Wright WH, Vafa O, Ord T, Asch RH, Berns MW. Micromanipulation of sperm by a laser generated optical trap. Fertil Steril. 1989; 52(5):870-873.

Tang HH, Tsai YC, Kuo CT. Embryo splitting can increase the quantity but not the quality of

blastocysts. Taiwan J Obstet Gynecol. 2012; 51(2):236-239.

Tao J, Tamis R. Application of assisted hatching for 2-day-old, frozen-thawed embryo transfer in a poor-prognosis population. J Assist Reprod Genet. 1997; 14(2):128-130.

Tocci A. Hypothesis: human trophectoderm biopsy downregulates the expression of the placental growth factor gene. J Assist Reprod Genet. 2021.

Tucker MJ, Luecke NM, Wiker SR, Wright G. Chemical removal of the outside of the zona pellucida of day 3 human embryos has no impact on implantation rate. J Assist Reprod Genet. 1993; 10(3):187-191.

Van de Velde H, Cauffman G, Tournaye H, Devroey P, Liebaers I. The four blastomeres of a 4-cell stage human embryo are able to develop individually into blastocysts with inner cell mass and trophectoderm. Hum Reprod. 2008; 23(8):1742-1747.

van Echten-Arends J, Mastenbroek S, Sikkema-Raddatz B, Korevaar JC, Heineman MJ, van der Veen F, Repping S. Chromosomal mosaicism in human preimplantation embryos: a systematic review. Hum Reprod Update. 2011; 17(5):620-627.

Van Landuyt L, Polyzos N, De Munck N, Blockeel C, Van de Velde H, Verheyen G. A prospective randomized controlled trial investigating the effect of artificial shrinkage (collapse) on the implantation potential of vitrified blastocysts. Hum Reprod. 2015; 30(11):2509-2518.

Van Landuyt L, Polyzos NP, De Munck N, Blockeel C, Van de Velde H, Verheyen G. A prospective randomized controlled trial investigating the effect of artificial shrinkage (collapse) on the implantation potential of vitrified blastocysts. Hum Reprod. 2015; 30(11):2509-2518.

Vanderzwalmen P, Bertin G, Debauche C, Standaert V, van Roosendaal E, Vandervorst M, Bollen N, Zech H, Mukaida T, Takahashi K, Schoysman R. Births after vitrification at morula and blastocyst stages: effect of artificial reduction of the blastocoelic cavity before vitrification. Hum Reprod. 2002; 17(3):744-751.

Veiga A, Sandalinas M, Benkhalifa M, Boada M, Carrera M, Santalo J, Barri P, Menezo Y. Laser blastocyst biopsy for preimplantation diagnosis in the human. Zygote. 1997; 5(4):351-354.

Velasquez AE, Castro FO, Veraguas D, Cox JF, Lara E, Briones M, Rodriguez-Alvarez L. Splitting of IVP bovine blastocyst affects morphology and gene expression of resulting demi-embryos during in vitro culture and in vivo elongation. Zygote. 2016; 24(1):18-30.

Ventura-Juncá P, Irarrázaval I, Rolle AJ, Gutiérrez JI, Moreno RD, Santos MJ. In vitro fertilization (IVF) in mammals: epigenetic and developmental alterations. Scientific and bioethical implications for IVF in humans. Biol Res. 2015; 48(1):1-13.

Vera-Rodriguez M, Rubio C. Assessing the true incidence of mosaicism in preimplantation embryos. Fertil Steril. 2017 ;107(5):1107-1112

Verlinsky Y, Ginsberg N, Lifchez A, Valle J, Moise J, Strom CM. Analysis of the first polar body: preconception genetic diagnosis. Hum Reprod. 1990; 5(7):826-829.

Verlinsky Y, Rechitsky S, Cieslak J, Ivakhnenko V, Wolf G, Lifchez A, Kaplan B, Moise J, Walle J, White M. Preimplantation diagnosis of single gene disorders by two-step oocyte genetic analysis using first and second polar body. Biochem Mol Med. 1997; 62(2):182-187.

Verlinsky Y, Rechitsky S, Schoolcraft W, Strom C, Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA matching. JAMA. 2001; 285(24):3130-3133.

Verpoest W, Staessen C, Bossuyt PM, Goossens V, Altarescu G, Bonduelle M, Devesa M, Eldar-Geva T, Gianaroli L, Griesinger G, Kakourou G, Kokkali G, Liebenthron J, Magli MC, Parriego M, Schmutzler AG, Tobler M, van der Ven K, Geraedts J, Sermon K. Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies in advanced maternal age: a randomized clinical trial. Hum Reprod. 2018; 33(9):1767-1776.

Verza S, Schneider D, Siqueira S, Esteves S. Laser-assisted intracytoplasmic sperm injection (LA-ICSI) is safe and less traumatic than conventional ICSI. Fertil Steril 2013; 100(3):S236.

Wan CY, Song C, Diao LH, Li GG, Bao ZJ, Hu XD, Zhang HZ, Zeng YJRbo. Laser-assisted hatching improves clinical outcomes of vitrified–warmed blastocysts developed from low-grade cleavage-stage embryos: a prospective randomized study. Reprod Biomed Online. 2014; 28(5):582-589.

Wang C, Feng G, Zhang B, Zhou H, Shu J, Lin R, Chen H, Wu Z. Effect of different artificial

shrinkage methods, when applied before blastocyst vitrification, on perinatal outcomes. Reprod Biol Endocrinol. 2017; 15(1):32.

Wang Y, Chen C, Liang J, Fan L, Liu D, Zhang X, Liu F. A comparison of the clinical effects of thinning and drilling on laser-assisted hatching. Lasers Med Sci. 2021.

Wood C. Embryo splitting: a role in infertility? Reprod Fertil Dev. 2001; 13(1):91-93.

Xu W, Zhang L, Zhang L, Jin Z, Wu L, Li S, Shu J. Laser-assisted hatching in lower grade cleavage stage embryos improves blastocyst formation: results from a retrospective study. J Ovarian Res. 2021; 14(1):1-9.

Zakharova EE, Zaletova VV, Krivokharchenko AS. Biopsy of human morula-stage embryos: outcome of 215 IVF/ICSI cycles with PGS. PloS one. 2014; 9(9):e106433.

Zegers-Hochschild F, Adamson GD, Dyer S, Rcowsky C, De Mouzon J, Sokol R, Rienzi L, Sunde A, Schmidt L, Cooke ID. The international glossary on infertility and fertility care, 2017. Human Reprod. 2017; 32(9):1786-1801.

Zhao H, Tao W, Li M, Liu H, Wu K, Ma S. Comparison of two protocols of blastocyst biopsy submitted to preimplantation genetic testing for aneuploidies: a randomized controlled trial. Arch Gynecol Obstet. 2019; 299(5):1487-1493.