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Advances of microfluidic technology in reproductive biology

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ABSTRACT

According to World Health Organization (WHO) reports about 70 million couples suffer from infertility all over the world. A lot of research groups are working on this issue and have made therapeutic approaches by integrating biology, medicine, genetics, chemistry, psychology, mechanic, and many other branches of science. However, these methods have their own pros and cons. Assisted Reproductive Technologies (ART) has appeared to solve infertility problems. In Vitro Fertilization (IVF), Intracytoplasmic Sperm Injection (ICSI), Intrauterine Insemination (IUI) are the most common and conventional technologies in this regard. There are at least two characteristics of microfluidics, mechanical and biochemical, which can be influential in the field of mammalian gamete and preimplantation embryo biology. These microfluidic characteristics can assist in basic biological studies on sperm, oocyte and preimplantation embryo structure, function and environment. Using microfluidics in sorting sperm, conducting different steps of oocyte selection and preparation, and transferring embryo by passing sub-microliter fluid through microchannels results in low cost and short time. The size and shape of microchannels and the volume of used fluid differs from non-human cells to human cells. The most progressions have been seen in animal models. Results suggest that microfluidic systems will lead to improved efficiencies in assisted reproduction.

1. Introduction

Infertility is defined as inability to get pregnancy after at least one year of unprotected sex according to World Health Organization (WHO) definition [1,2]. More than 70 million couples suffer from infertility, worldwide [3]. Male factors are responsible for about 50% of infertilities among couples [4], and approximately, 40% of them have not clear etiology [5–7]. To experience conceive sperm must swim in a dens and viscous area to fertilize the oocyte [8]. Male infertility comes after different factors such as life style, obesity, smoking, drug abuse, and loss of nutritional and mineral factors [9]., Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations leading to congenital absence of the vas deferens, Y-chromosome microdeletions, karyotype abnormalities, oncological diseases, hormonal issues, hyperprolactinemia, hypothyroidism, congenial adrenal hyperplasia, hypogonadotropic hypogonadism, and panhypopituitarism, physical problems like varicocele, damaged sperm ducts, torsion, Klinefelter's syndrome, retrograde ejaculation, psychological problems such as erectile dysfunction, premature ejaculation, and ejaculatory incompetence [10] and oxidative stress [11] and sexual dysfunction [12].

Female factors are also responsible for 20% of couples infertility [13,14].Oocytes, as female cells, which feed the embryo, have critical roles in healthy fertilization, embryonic cell division, and pregnancy [15]. Poly Cystic Ovary Syndrome (PCOS) [16,17] as the usual endocrine disorder in woman reproductive system [18–22], psychological problems [23], low vitamin D levels [24,25], Gestational diabetes mellitus (GMD) [26] and many other factors can also lead to female infertility.

During last decades, the application of assisted reproductive technologies (ART) including Intrauterine Insemination (IUI) as the first solution in most individuals [27], and In Vitro Fertilization (IVF), and Intracytoplasmis Sperm Injection(ICSI) as the most useful methods [28] has been raised [29].

Microfluidics, as an emerging generation of technology, studies

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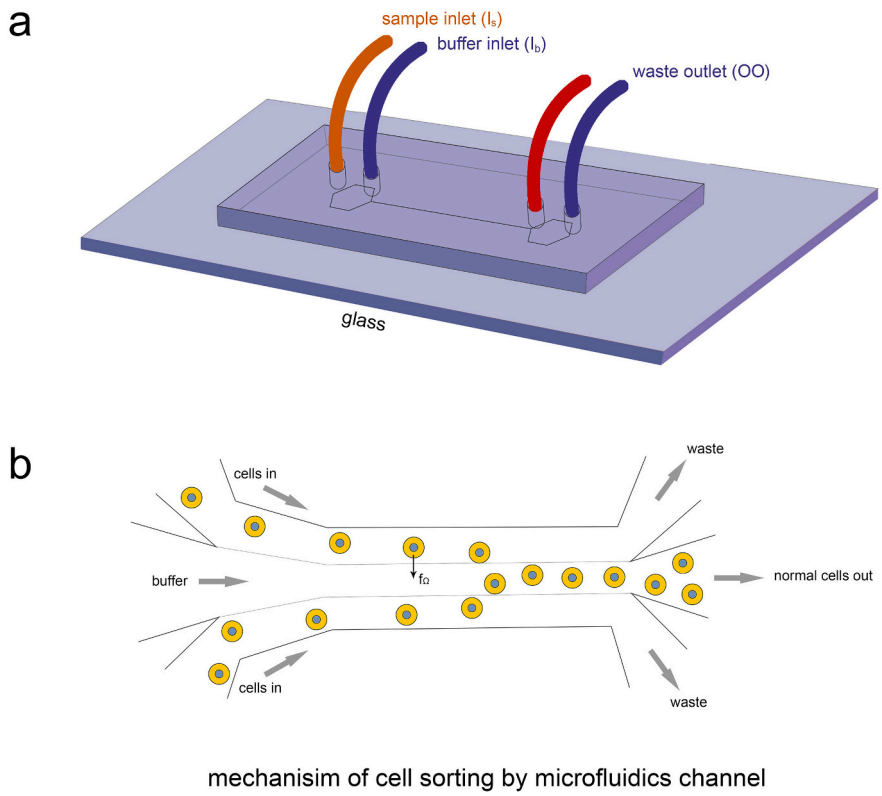

Fig. 1. Mechanism of cell sorting by Microfluidics channel. (a) A microfluidics system (b) magnified channel of microfluidics system: sample and buffer comes from one side after cell sorting during the channel waste and normal cells outcomes from two different channels.

liquid behavior at microliter levels in microchannels, with high technological applications [30]. In a way that from one side of microchannel fluid, which contains sample, comes in and along channel by turbulent or laminar flow cell sorting achieve and from the other side waste and aimed product come out. (Fig. 1) It is a valid platform to follow up both male and female gametes [31] and embryo growth [32,33]. This technology benefits physics, chemistry, biology and engineering to help people with their infertility complications [34].

From 1950s to 1970s, great efforts were conducted to design and utilize fluidic circuits for defense industry with electromagnetic pulses that have destructive effects on nucleuse [35]. In 1970s to 1990s reporting in micro-Total Analysis Systems (μ-TAS) for chemistry, microelectro-mechanical-systems for electronics and Lab-On-a-Chip [36] designs for chemical, molecular, and cellular manipulations was started and formed the foundation for Bio-Microfluidics [37]. Nowadays, it plays a key role in single cell isolation and sample preparation, electrophoresis, DNA, RNA, and epigenome analysis [38].

Over the last 3–4 decades, significant improvements in human ART were achieved and laboratory technologies, equipment, and medium and the collective environments progressed over time. So that, human IVF, embryo culture and analysis, and embryo cryopreservation processes have been affected by these progressions. IVF cycle is very important in elective Single Embryo Transfer (eSET), to increase pregnancy rates and decrease harmful influences of multiple implantations. The efficiency of each individual laboratory step in an IVF cycle becomes increasingly important for people who need these therapeutic approaches. [39]. A single successful offspring in an IVF cycle is influenced by the success of multiple IVF steps. However, none of them are completely efficient and attrition of each, has harmful effects on the main outcome resulting in a healthy pregnancy and neonate.

As another novel systems, “Organs-on-chip” (OoC) or three-dimensional (3D) cell culture applications help to reproduce tissue and organ-level functions of living organs and systems, in vitro. They are allowed to be used in special female reproductive functions [40]. Human placenta [41] and the uterus [42] have been made with this system.

In Vitro Fertilization-Embryo Transplantation (IVF-ET) is also an approach that mixes oocyte and sperm outside of female’s body to achieve fertilization [43].

This review aims to summarize the current ongoing research carried out with the purpose of standardizing different stages of assisted reproductive programs by microfluidic technologies, focusing on the benefits and shortcomings of these applications.

2. Microfluidics-based sperm selection

The Sperm Penetration Assay (SPA) is a process for the evaluation of sperms fertility potentials [44]. SPA plays a role in IVF to facilitate insemination [45]. Sperms with normal SPA test will be subjected for IVF. Any reduction in SPA may lead to Intracytoplasmic Sperm Injection (ICSI).

Reduced sperm count, motility, and abnormal morphology [46,47] are some factors that lead to sperm inefficiency. Motility is a key factor for fertility and over 80% of male infertilities are caused by abnormal motility [48]. Sperm morphology is also an important factor in sperm motility. A disordered morphology usually leads to male infertility. This abnormalities can be characterized by head, neck, mid-piece or tail phenotype of the cell [49,50]. So far, no drugs are available to improve male fertility disorders in vitro [51]. Additionally, several clinical studies have shown that suboptimal Ca2+ signaling in response to progesterone is linked to oligo asthenoteratozoo spermia, and IVF failure [52]. Reduced Ca2+ also causes decreased IVF rate [53]. Glycosylation, the metabolic mechanism required for sugar residues addition to proteins, is involved in maturation, function, and fertilization [54]. Within spermatogenesis, glycosylation directs the distribution of proteins to their structural and functional parts and pathways, and in the epididymis, it participates in spermatozoa maturation [55]. Any defect

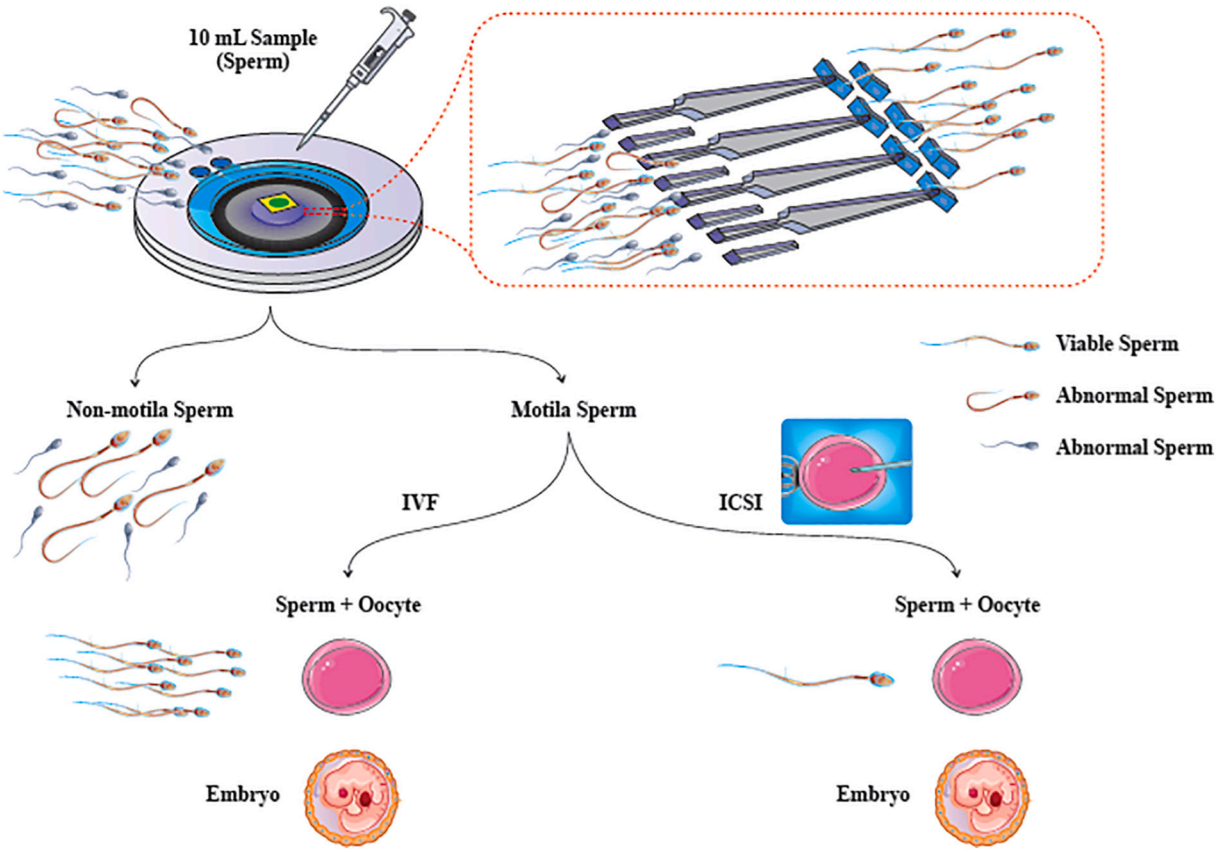

Fig. 2. Utilizations of microfluidics in human infertility treatment. Seminal fluid is pumped to the device. The sorted motile sperms are collected at the outlet. Non-motile sperms are as waste cells in this process. The cells are used in IVF or ICSI and fertile oocyte. IVF: In-Vitro Fertilization, ICSI: Intracytoplasmic Sperm Injection.

in these processes may result in male infertility.

In human IVF, an objective is to select and isolate high quality and quantity sperms as male gamete to facilitate oocyte fertilization as female gamete by either conventional insemination (IVF/CI) or ICSI (IVF/ICSI). Depending on semen characteristics (semen volume, sperm concentration, sperm motility, viability, morphology and presence/concentration of non-gamete cells) a technician can choose how to process or select sperm. The goal is to remove seminal plasma from sperm, sustain and/or enrich the sperm population for motility and inseminate with numerous (IVF/CI) or one (IVF/ICSI) motile sperm with normal morphology. According to WHO definition simple media washing, semen overlay with medium and sperm swim-up out of the seminal plasma, density gradient centrifugation or a combination of above methods are used to prepare sperm for IVF process [56].. To measure sperm motility, silicon/glass microfluidic applications have been appeared as diagnostic methods in 1990–1995. In 2003, for the first time, microfluidics came to help human semen processes and elect motile sperm [57]. In brief, through fluids mechanical behavior evaluation and their utilization in microfluidics systems, motile and non-motile sperms distinguish will be an easy process [58]. When compared, seminal plasma processed by microfluidics has 98% motile sperm, while unprocessed samples showed 44% motile sperms. In morphological characteristics microfluidics processing overweight too. In this method, seminal plasma, dead sperm and debris are considered as deposal medium. Recent researches revealed that microfluidics systems are able to isolate poor semen samples with high quality [59].. Additionally, by changing width and length of microchannel results will be altered too [60]. Employing new techniques especially microfluidics has recently been suggested, as novel applications for sperm sorting and in IVF or IVF/ICSI. Recently, a microfluidics system had promoted that selects sperm by their motility. This device shows 80% improvement in DNA integrity. Centrifuging in semen processing may damage the cells sub-lethally [61]. Moreover, using centrifugation, reactive oxygen species (ROS) may damage the sperm DNA [62,63]. Although, sperm motility is not a valid factor for DNA integrity evaluation, DNA damage has negative effects on the sperm motility [64,65]. In human ART, DNA damage cannot be recognized; however, low fertilization rate [66], low embryo quality [67], and reduced pregnancy rate [68] can be its consequences. Another merit of microfluidics systems in sperm isolation during ART, is the volume of semen. Some of microchannels can be set up just with 1 ml of semen [69].

Subsequently, combining new methods of sperm selection with classic ones may result in better conclusion such as low DNA injury and morphological abnormalities [70]. (Fig. 2).

3. Oocyte

Infertility has more negative social effects on woman than men [71]. infertility treatments and psychiatric disorders are closely linked to each other [72]. Therefore, emotional problems may lead to female infertility.

In fertilization, oocyte plays the role as maternal cell and feeds the embryo. A qualified oocyte is the one which can get fertile, becomes a blastocyte, and does implantation in the uterus [73]. So, it is important to investigate the efficient markers to select the most functional oocyte to be used in ART to have high pregnancy rate and a healthy embryo [74]. In IVF, oocyte selection depends on morphological factors of cytoplasm, polar body, and cumulus cells [75]. In clinic, mixturation of macro- and micro-processing using needles, tubing, vacuum, test tubes, petri-dishes and microscopic observations to hunt follicular aspirates and differentiate individual oocyte cumulus masses are referred to ART. Oocyte cumulus complex can also be sorted and part of theme can be

stored for IVF\ICSI, and the remaining can be omitted by mechanical or biochemical methods such as pipetting and enzymatic treatment, respectively. These procedures, are necessary to choose mature cell, efficient settling of the polar body in relation to sperm injection in IVF/ICSI. The ambient cumulus around the oocyte is also essential for fertilization in IVF\ICSI [76]. In the last 20 years, above method for cumulus cell removal has been effective in human IVF/ICSI and no significant side effects were identified in fertilization or subsequent embryo growth success. It was previously confirmed that microfluidics is a novel mechanical method for cumulus cell removal of bovine oocyte cumulus complexes without enzymatic interference. However, there is no report showing the advantages of microfluidics in removing oocyte cumulus [77].

4. IVF

IVF was first used in 1978 leading to live birth. Since then, IVF was greatly progressed [78]. During IVF, one oil-covered oocyte is transferred into an optimal concentration of sperm cells culture dish, then, one single sperm fertile the oocyte [79]. Based on CDC's 2017 Fertility Clinic Success Rates Report Approximately 1.7% of all infants born in the United States every year are conceived using ART. In the preliminary clinical trials, in order to higher implantation rates, several embryos were simultaneously transferred to obtain successful pregnancy. As time passed, this rate was improved, and the number of transferred embryos decreased to two [80,81]. However, The risk of twin pregnancies was still undeniable [82]. To minimize this risk, elective Single Embryo Transfer (eSET) was proposed as a feasible solution [81]. To use eSET, person should have criteria including age < 35, possessing more than one high-quality embryo for transfer, and first or second treatment cycle [82]. Extended culture was also replaced with the embryo selection. To experience further implantation potential, blastocysts were considered instead of cleavage-stage embryos [83]. Using single blastocyst transfer in pregnancy also showed efficient results than single cleavage-stage embryo transfer [84]. Additionally, using frozen embryo could be considered as a better alternative compared to fresh embryo to ameliorate uterine environment. This strategy is heavily suggested to provide a better environment to reach desirable embryo implantation. Because it recovers supraphysiological hormones and shedding of the exposed endometrium. New cryopreservation technologies have also augmented the survived embryos rate after thawing and increased pregnancy rate after frozen embryo transfer in comparison to fresh embryo transfer [85]. Recently, the use of frozen embryo transfer has been extensively increased [86].

One of the IVF challenges is polyspermic fertilization, in which one oocyte is fertilized with more than one sperm. This phenomenon leads to pre-embryo formation, decreased embryo number and IVF success rate [68].

Classically, macroscopic placement of sperm in the volume of 10ul up to 1 ml with single oocyte cumulus is a method of human IVF. If the number of oocytes become more than one, the utilized technique is called ICSI. The fertilization rate in these processes differs from 50% to 70% [1].

In IVF/ICSI cycle, a true fertilization rate cannot be exactly measured due to the presence of cumulus cells and ascertain mature oocyte cells weakness in cumulus mass. Although, cells can continue to metaphase II, after fertilization of immature oocyte by sperm, beside that there is not the potential to build pro-nuclei or a competent embryo. And it is opposite of true fertilization [38]. Microfluidics systems in porcine model IVF/CI, omitted polyspermic fertilization occurrence, and an efficient limitation in porcine model in vitro embryo generation was achieved. The comparison of microdrop, as a traditional method of insemination, and microfluidics, as a new technology, microfluidics showed more clear outcomes in monospermic fertilization [87]. On the other hand, in murine model, microfluidics-based insemination promoted fertilization rate when compared to the traditional processes with low sperm concentration (0.01–0.08 × 106 sperm/ml) [88]. The rate of polyspermic fertilization in human IVF/ICSI cycles is about 2–7%,; however, employment of microfluidics may further decrease this rate [87]. To overcome sperm low concentration in human ART, ICSI plays an important role in oocyte fertilization with one sperm. In this method there is a potential risk of oocyte lysis or cell degeneration because of glass needle injection mechanical pressure. Usually, a professional technician may consider 7% of these inefficiencies at the moment of injection [36]. By the microfluidics progression in human IVF with low sperm concentration and achieving acceptable results of high fertilization rate, the necessity of ICSI can be ignorable in most human cases and risks of degeneration and cell lyse will be resolved. Now the question is that, these researches will done and results the change of ICSI to microfluidics IVF/CI or not? Todays, studies are more designed on the use of female factors [37].

Despite the fact that in 2012 American Society of Reproductive Medicine Committee Opinion mentioned in an article that there is no evidence to defend using routine way of ICSI for non-male factor infertility, many infertility programs have been based on ICSI [89]. Matsuura et al. [90] in a porcine model showed that microfluidics could be used in ICSI process and the technician could save time for other steps. These proof-of-concept studies with animal models are the pioneers. Although, human ICSI has been used with acceptable outcomes, the need for several high technologies related to human mistaken or alteration seems to be a great limitation. Beside, human ICSI is an expensive method which could not be afforded by everyone who needs infertility treatments.

In 2018, Weng et al., was designed a microfluidics based device in which surrounding cumulus cell mass of mouse oocyte was deleted to improve oocyte quality to have better injection of sperm in ICSI [91]. Microfluidics IVF/CI have emerged to reduce human errors and costs by protecting the outcomes. However, in this regard more studies are still needed.

5. Embryo culture

The pre-implanted embryos of rodent, domestic species, non-human primate or human grow in fallopian tube and uterus dynamic environment (by constant change). In embryo culture, microfluidics can be used for equivalence, benefit or detriment of the test including microenvironment, dynamic fluid environment and dynamic chemical environment, respectively [59].

The cell surrounding microenvironment is combined by individual and interactive factors which impress the qualification of pre-implantation embryo cell. These factors affect embryo growth, structure, and function through biophysical or biochemical processes either in direct or indirect routs. Agar-mediated encapsulation for sheep embryo was the first research to regulate embryo microenvironment in 1979 [92].

Microfluidics was also first conducted to test microenvironment on mouse embryo in low fluid flow in microchannels [93]. Researches showed that culturing mouse embryos in microchannels had faster cleavage rate, more yield of blastocytes and less embryo degeneration than that of microdrop as a traditional culture method. However, when the used flow rate was 0.1 and 0.5 ml/h in microchannel, results were not acceptable. There was no progression in embryo development, and deleterious results of embryo growth and abnormalities of mouse embryo were achieved [94]. From this point of view, using microchannels in human embryo culture have some concerns that need great efforts to be solved. One of the concerns is salvaging the embryo from microchannels. Additionally, fluid flow may have injurious effects on embryo and its development. Takayama et al. [95] in 2004, on a computer-controlled refreshable Braille display platform clarified that vertical moving pins can pander integrated pumping of channel-contained fluids localized deformation of elastic made microchannels. Based on this result, it would be easy to plan an application by the help of computer

Table 1
Advantages and disadvantages of some different methods for sperm, oocyte, embryo selection.

	Method	Advantages	Disadvantages	References
Sperm isolation	Microfluidics	1.decreased time of experiment 2.decreased cost 3.differentiate motile and non-motile sperms 4.less volume of sample up to 10 5.isolate poor semen samples with more efficiency	1.less purity	[49,59,69,104]
	Centrifuge	1.enough yield 2.eliminate leukocyte in large extent 3.reduce reactive oxygen specie	1.damage the cells sub-lethally 2. expensive 3.damage sperm DNA by increased ROS and leads to low fertilization rate, low embryo quality	[61–63,66,67,105]
	Swim-up	1.Easy performing 2.recover motile sperm	1.low yield 2.damage sperm with ROS 3.decrease in normal choromatin-condense	[67]
Oocyte isolation	Microfluidics	1.Eliminate polyspermic fertilization of oocyte 2.save time	1.may damage morophology of the cell	[36,68]
	Microscope	1.detect LBCs and nuclear organelles	1. may damage morophology of the cell	[106]
Embryo culture and selection	Microfluidics	1.provide elective single embryo transfer 2.lessen risk of twin pregnancy rate 3.transfer frozen embryo instead of fresh embryo 3. provide dynamic fluid to have more yield of blactocyte 4. provides good microenvironment which qualifies embryo" growth 5. more yield of blastocytes	1.increase cleavage rate 2.in low rate of flow in channel results in bad embryo growth 3.hard salvaging of embryo from channel 4.injurios influences on embryo 4.fluid evaporation	[36,95,96]
	Microscope	1.control cleavage rate 2.count cell number of embryo	1.control cleavage rate 2.count cell number of embryo	[107]

programmers to punctually draw up fluid flow within microchannels, in which embryo culture will be possible. In this platform, thin elastomeric compounds are essential components that can be deformed by Braille pins. It results in mouse embryo culture progression in microchannels with fine elastomeric bottoms with precise and controlled fluid flow. These investigations demonstrated evaporation as a problem in sub-microliter volumes of fluid in elastomeric fine and flexible microchannels (PDMS) when environment was humid [96]. Media evaporation ends to fundamental osmolality changes. This kind of shifts can impact the mouse embryo growth. Mathematical analysis of the experiments results can predict evaporation and osmolality changes. Indeed, they tried an experiment on osmolality change (about 15 mmol/kg) and understood that this changes could not be due to water evaporation through PDMS. This evaporation happened at the time of media transfer from its macroscopic media vessel to a microdrop. This study showed that how different environmental conditions and different techniques can affect media osmolality and embryo culture [97].

PDMS-parylene-PDMS hybrid membrane was provided for two reasons: i) to sustain a thin and flexible membrane, ii) to omit evaporation and osmolality shifts which are against embryo survival. By this hybrid membrane, evaporation and fluid osmolality shifts decreased flexibility that is necessary to mediate the Braille deformation-based microfluidics system was sustained, the clarity needed for microscope optical function was approached, and mouse embryo development was provided [96].

When compared to microdrop, mouse embryo culture in dynamic microfluidics system results in faster growth of pre-implanted embryo, more blastocysts cells, and efficient implantation improvements [94].

To conclude this part, most experiments have suggested the culturing of mouse embryos in microchannels and microfluidics devices to help pre-implantation step.

6. Microfluidics in embryo selection

Over the last 3 or 4 decades, little changes have been done regarding human ART embryo evaluation and selection for transfer. On the other hand, Time-lapse micro-videography of morphometrics and morphokinetics have significantly been progressed [98], and pre-implantation genetic aneuploidy screening have greatly been improved [99].

Embryo selection for transfer is usually done using microscope. In this process researchers check cleavage rates, embryo cell number (in relation to development time), cellular fragmentation degree, blastocyst formation, stage of blastocyst development (early, full, expanding, expanded or hatching) and cellular contributions of the blastocyst inner cell mass and trophectoderm. Like other manual methods, it is vulnerable against the risks of laboratory condition or technician errors [100]. Selecting embryo with higher development competence and implantation potential, and gaining healthy offspring from an eSET require enough information on embryo morphology, morphokinetics, genetic normalcy and biochemical factors. To analyze biochemical non-invasive and embryo-selection health biomarkers and devices to measure that biomarkers are needed. Oxygen update [101], amino acid turnover [102], and energy metabolism [103] are such biomolecules. Measuring glucose consumption from media and lactate production, representing embryo glycolytic activity, can also help the human embryo selection [101,103].

In a research, Heo et al. [96] tried to bring microfluidics devices in clinic to measure embryo's metabolism. This research group used an automated computer-controlled application for both embryo culture and metabolic analysis in one device. (Table.1).

7. Cryopreservation with microfluidics

Cryopreservation is a low temperature process in which biological, chemical, and physical factors of the cell or tissue can be maintained for long-term purposes such as tissue transplantation [108].

Cryopreservation of oocytes, zygotes, and embryos has been extensively used in infertility treatment programs. Nowadays, oocyte cryopreservation allows women with the risk of losing their fertility by chronic disease, cancer, or genetic predispositions to protect their oocyte. There are two common methods in this field: slow-rate freezing and vitrification. Due to the faster cryosurvival rates in vitrification (more than 90%), it is better than the first one; however, it has its own concerns. Water of the cell exchanged with the Cryoprotectant Agents (CPAs) before cooling and come back to the water in warming process. CPAs are ignorable in this process because of their function in omitting of harmful intracellular ice crystals formation. CPAs cause osmotic stress after osmotic exchanges in inter\exteracellular pressure. Despite that lethal osmotic stress in vitrification and warming are solved, sub-lethal

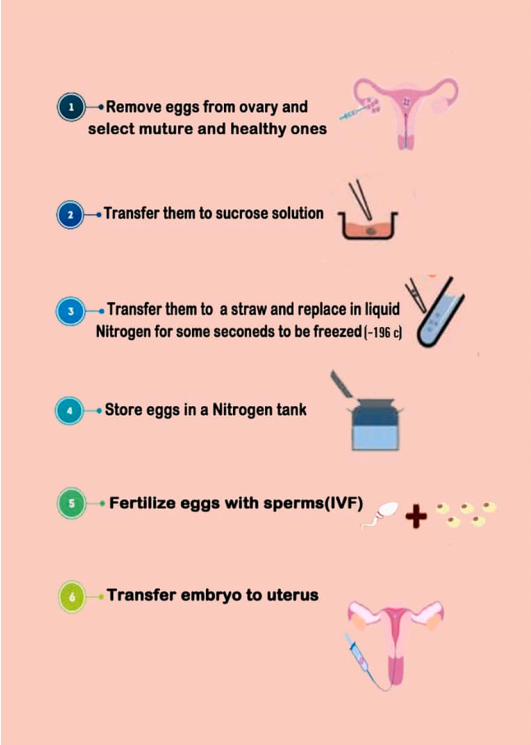

Fig. 3. Utilization of microfluidics in embryo Cryopreservation.

stress cannot be omitted and affects oocyte function, embryo development, and treatment outcomes. Osmotic stress mostly impacts oocyte and zygote cryopreservation. Their fluid volume is more than other mammalian cells and this water should be exchanged with CPAs during the process. High concentration of CPAs in vitrification is another problem in sub-lethal osmotic stress. Initial studies on microfluidics and cryopreservation were designed on hepatocellular carcinoma cell line (HepG2) and approved the benefits in cell storage and survival [109]. In both methods there are a lot of protocols for CPA exchange for cryopreservation, which varies in CPA concentrations, and exchange time. These days, computer-based models are used to pick the optimal CPA exchange protocols. Researches have demonstrated that the presence of a threshold minimum cell volume and cell shrinkage comes after that volume results in cell death [110,111]. To avoid critical minimum cell volume oocyte and zygote vitrification cells are manually pipetted into subsequently higher levels of permeating CPA concentrations in which cells are allowed to shrink in a specific interval [112,113]. (Fig. 3).

8. Conclusion

According to the latest researches, microfluidics can be considered as a clinical technique to help couples suffering infertility; however, more experiments on animal models are still needed. It has been confirmed that microfluidics can help to isolate human sperm in an efficient way, but there is no report demonstrating its efficiency in human oocyte process and embryo implantation. In spite of significant progressions during the last decade there is a lot to do yet. To make microfluidics as a retinue technique in infertility treatment, it is necessary to make better communication between medical group, engineers, and analyzers.

9. Future prospective

Growing new generation of laboratory techniques such as microfluidics applications can lead to more safety, less time, reduced cost, and more acceptable outcomes in the field of pregnancy and ART. The main question is that how much time and efforts are needed to make microfluidics to be clinically applied. Nevertheless, further investigation is required in this field. There are more concerns regarding human embryo culture than cells in this case. Retrieving cultured embryo in microfluidics microchannels is a main concern. On the other hand, fluid flow can harmfully affect the developed embryo. In this regard, more studies on fluid behavior are needed to solve these challenges.

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CRediT authorship contribution statement

Aylin Nikshad wrote the article. Afsoon Aghlmandi and Reza Safaralizadeh wrote the initial draft of the manuscript and prepared figures. Leili Aghebati_Maleki, Majid Ebrahimi Warkiani and Farhad Motavalli Khiavi reviewed and edited the final version of the manuscript. Mehdi Yousefi supervised the study.

Declaration of competing interest

Authors declare no conflict of interest.

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