Review paper

Videosurgery

Improving the safety of the embryo and the patient during *in vitro* fertilization procedures

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Abstract

In vitro fertilization (IVF) is a method of treatment for infertility in selected indications. Recent years have brought dynamic development of technologies related to IVF. This article presents problems pertaining to the safety of technology with respect to the patient, as well as the embryo, based on an analysis of scientific reports and our own experience. Invasiveness of the IVF procedure for the woman and the embryo varies on an individual basis. Minimization of the invasiveness of IVF requires experience of the staff performing the procedure, especially with respect to the assessment of risk for an individual patient. Technologies related to IVF are constantly being improved, and the effectiveness of the selected individual treatment methods is not always scientifically confirmed.

Key words: *embryo, in vitro fertilization, safety.*

Introduction

The problem of the lack of offspring is becoming an increasingly serious challenge for medicine, because nearly every fifth couple at reproductive age has problems with conception. Evidence suggests that the scale of this phenomenon will still increase [1]. Fertility and the health state of the baby depends on genetic, environmental, social factors and healthcare both [2]. For many couples the only chance for possessing offspring will be the procedure of *in vitro* fertilization (IVF). *In vitro* fertilization procedures are the most effective method of infertility treatment, and its chance for success depends on many factors [3]. The procedure of this method of treatment is multi-stage and requires the engagement of specialists in various domains. The provision of safety to the embryo is directly translated into its quality and, consequently, the probability of achieving pregnancy and, to a great extent, depends on the cooperation between the physician and the embryologist [4]. At present, the minimization of invasiveness of the procedure of IVF is the main factor which can contribute to the achievement of pregnancy and delivering a healthy baby after this procedure.

The procedure of IVF consists of several stages: stimulation of ovulation, retrieval of oocytes (puncture), fertilization of the ova, culture of embryos, and subsequently, the transfer of embryos to the uterine cavity. Unused oocytes or embryos are frozen in order to use them in the future. The principles of managing IVF in Poland are regulated by the Act of 25 June 2015 in the matter of infertility treatment [5]. The most important indications for performing the IVF procedure are: bilateral obstruction of fallopian tubes, considerable deterioration of the quality of sperm parameters, advanced endometriosis, as

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well as the situation when other treatment methods have failed, such as intrauterine insemination [4, 6, 7]. The IVF procedure is also recommended to women who are at risk of exhaustion of the ovarian reserve. *In vitro* fertilization procedures may also secure the fertility of patients after radiotherapy and chemotherapy.

Stimulation of ovulation

In order to obtain good quality egg cells during IVF, stimulation of ovulation is performed using gonadotropins in combination with the co-administration of a gonadotropin-releasing hormone-antagonist (GnRH) or GnRH agonist. Selection of the proper dose of gonadotropins, on the one hand, is the precondition for obtaining a proper number and good quality ova, and on the other hand, allows one to avoid the risk of occurrence of the ovarian hyperstimulation syndrome (OHSS), which creates a life threat for patients subjected to IVF [8].

The ovarian hyperstimulation syndrome is the complication of stimulation of ovulation taking place with the enlargement of the ovaries. The basic feature of the ovarian hyperstimulation syndrome is increased permeability of capillaries, resulting in the movement of fluid from the vascular space to the third space. The consequence of this may be embolism, renal, hepatic and respiratory failure. Mortality in OHSS is caused by electrolyte disorders, multi-organ failure, and thrombosis of the cerebral vessels. According to the latest European Society of Human Reproduction and Embryology report, the incidence of OHSS ranges from 0.18% to 1.40% in European countries [9]. The risk factors of this syndrome include age under 35, polycystic ovary syndrome (PCOS) with a high level of anti-Müllerian hormone (AMH), asthenic silhouette, concentration of estradiol over 3,000 pg/ml, the number of ovarian follicles exceeding 20, and application of GnRH protocols. The occurrence of this syndrome exerts a very negative effect on the quality of the embryos obtained [10].

The opinion in the matter of OHSS was specified by a group of experts in 2015. The group considered that there is a need for standardization of the definition and classification of the clinical syndrome of OHSS to allow further conclusive research. Interventions with evidence of effect in reducing OHSS include the use of metformin in women with PCOS, use of a GnRH antagonist rather than a GnRH agonist, and use of GnRH agonist triggers in GnRH antagonist stimulation cycles. The consensus view was that reducing the dose of follicle-stimulating hormone (FSH), freezing all embryos and transferring a single embryo were appropriate interventions to reduce OHSS. Agreement could not be reached on coasting, the lowest number of oocytes to consider freezing all embryos and management after cancellation of oocyte retrieval [11].

Knowledge of the risk factors of OHSS is the basic method to avoid its occurrence. Early threats resulting from the course of OHSS concern primarily the period of 3–7 days after puncture and depend on the results of stimulation, whereas late risks occur 12–17 days after the procedure and depend on pregnancy. Puncture performed in these patients, due to the considerable enlargement of the ovary, is a more invasive procedure than in the case of an ovary of a normal size.

Ovarian puncture

Ovarian puncture is the procedure of collecting the oocyte from the follicles in the ovary, which is performed 35–36 h after the induction of ovulation induced by the administration of human chorionic gonadotropin (hCG). This procedure is performed using a needle introduced through the vaginal fornix into the ovary under the control of a transvaginal ultrasound probe. The aspirated follicular fluid is taken to the laboratory, where the oocytes are isolated. The procedure is most often performed under short-term intravenous anaesthesia. The invasiveness of this procedure depends on the anatomical location of the ovary, the risk of introduction of infection, and the possibility of inducing an internal and external hemorrhage, as well as the experience of the person performing the puncture [12].

The thickness of the puncture needle is of great importance for the safety of the procedure. A smaller diameter of the needle, in case of tearing of the blood vessel, decreases the risk of occurrence of hemorrhage and reduces the trauma of tissue which is punctured in order to reach the ovary. At the same time, fine needles are less stiff, which in the case of puncture of an ovary with a thickened shell located at a considerable distance from the ultrasound probe may cause technical difficulties with puncturing the ovary wall. In such a situation, the needle may easily bend and slip from the ovary, traumatizing adjacent tissues.

The thickness of the needle and speed of aspiration of the follicular fluid also affect the pressure within the needle, which may exert an effect on the quality of the oocyte, and consequently the embryo. This problems was analyzed by Rose, who published human and animal studies, which together with topics from mathematics and mechanics were used to try to understand the importance of different choices that could be made in structuring a transvaginal oocyte retrieval procedure in humans. The published literature suggests that the highest oocyte recovery rate occurs using higher pressures and thicker needles, but this comes at the cost of damaging the cumulus oocyte complex. It is likely that this damage is caused by the sheer stress forces exerted on the cumulus oocyte complex, due to parabolic forces associated with laminar flow within the needle, and is likely worsened by irregular forces during intervals of turbulent flow occurring with entry into the needle. Larger needles also cause more pain and may be associated with more blood loss. Higher velocity entry into the follicle, needle rotation to prevent premature blockage of the lumen, and carefully timed applications of aspiration pressure theoretically optimize the oocyte retrieval technique [13].

The location of the ovaries in the smaller pelvis varies individually, and also depends on the past inflammatory states or procedures which, by generating adhesions, may contribute to the hindered access to them with the puncture needle. Such a situation increases the invasiveness of the procedure of oocyte retrieval, and sometimes requires puncturing the urinary bladder or the uterus. Reduction of the number of insertions of the needle contributes to minimization of the occurrence of hemorrhage or introduction of infection. It is the more difficult the larger the ovary, with which we are confronted, among other situations, during the OHSS [14].

Embryo culture

After obtaining the egg cells and preparation of the sperm, oocytes are fertilized by the classic IVF method or intracytoplasmic sperm injection (ICSI), according to the quality of ejaculate. The selection of the proper sperm and appropriate preparation of sperm exert an important effect on the outcome in the form of pregnancy, and determine the state of health of the conceived child. This results from the fact that the sperm does not possess mechanisms which would be capable of repairing its DNA. This damage is intensified within the time after sperm donation, and only the egg cell is able to repair it, which depends on the capacity of the oocyte to repair damage, as well as the character of this damage [15]. Considering the above factor, it is extremely important to maximally reduce the time which should elapse between sperm donation and its use for fertilization.

The selection of sperm for the ICSI procedure seems to be of key importance for obtaining an optimum embryo and a healthy child. Analysis performed by Sakkas *et al.*, concerning the usefulness of hyaluronic acid (HA) binding capacity and the motile sperm organelle morphology examination (MSOME) in the selection of sperm, neither unequivocally confirms the usefulness of these methods in clinical practice nor shows the superiority of one method over the other. Studies by Sakkas *et al.* showed that not many studies have evaluated the results obtained by the use of sperm selected by their ability to bind HA in clinical settings. Currently, there are no HA threshold values established and widely accepted in order to predict the outcome of ART. This limits the value of estimating the proportion of HA-bound sperm in predicting IVF outcome. The clinical diagnostic value for HA binding theoretically attempts to mimic which sperm will bind to the cumulus cells. As sperm binding to the cumulus–oocyte complex is one of the final steps in natural selection, the utility of this test would appear to be promising; however, larger studies are urgently needed [16].

Intracytoplasmic morphologically selected sperm injection with motile sperm organelle morphology examination (IMSI-MSOME) consists in the introduction into the oocyte of the sperm with comprehensively assessed morphology. Assessment of the morphology of the oocyte is performed using modern microscopic equipment. Prior to its introduction into the oocyte, the sperm is assessed in detail in terms of the number, quality and distribution of vacuoles on its surface, as well as the shape, which allows selection of the best sperm based on visual evaluation.

In the case of this method, Sakkas *et al.* refer to the most relevant information available from a Cochrane review which concluded that the results from RCTs do not support the clinical use of IMSI, while the evidence of the effect on live birth or miscarriage and the evidence that IMSI improves clinical

pregnancy is of very low quality. Perhaps this lack of evidence could have arisen because of questions relating to the ultimate resolution of the light microscope. The maximum useful magnification of an image is usually set at 1,000×. Magnifications higher than this will yield no further useful information or finer resolution of image detail, and will usually lead to image degradation. The numerous studies reporting IMSI must report whether adequate instrumentation was used, as any aberrations seen at 5,000× or 6,600× may represent artefacts and not real structures [16].

The environment in which the embryos grow has a direct effect on their quality and the achievement of the outcome which is pregnancy after IVF. The most important goal of an optimally functioning laboratory is the provision of constant physical and chemical conditions in which embryos are grown, as well as the elimination of a potentially unfavorable effect of various physical and chemical agents (substances used for maintaining cleanness, environmental physical factors). Obtaining a constant temperature, humidity and concentration of gases during culture is possible due to the application of good quality incubators. Their structure also provides protection against exposure to electromagnetic waves. In order to eliminate potential contamination which may be present in gases delivered to the incubators, special protective filters are applied, placed between the gas bottle and the incubator. It is also recommended to purify the air in the laboratory using specially designed systems. It is important that the staff working in the laboratory do not use an excess of perfumes and antiperspirants, and only the persons indispensable for performing these procedures should have access to the rooms where the embryos are cultured. Agents used for disinfection of the laboratory should be certified and specially designed for this purpose. In the laboratory, artificial light is preferred, because daylight unfavorably affects the gametes. The provision of constant conditions in laboratory incubators, refrigerators and containers with liquid nitrogen for biological material should be constantly monitored and registered by the systems specially designed for this purpose.

However, the stabilization of these conditions is disturbed while opening the incubator in order to assess embryos during culture. This harmful effect may be eliminated using time-lapse monitoring technology, which enables observation of the whole process of the development of embryos with high precision, without the necessity for taking them out of the incubator. This system allows the tracing of the *in vitro* process from the fertilization of the egg cell to the selection of proper embryos for embryo transfer [17].

The time-lapse monitoring technology was tested for the first time in April 2011. In February 2012, the first child was born with the aid of an IVF Embryoscope® incubator. Eventually, in July 2012, the system was presented at European Society of Human Reproduction and Embryology (ESHRE) and in November at the American Society for Reproductive Medicine (ASRM) [18]. At present, the system is distributed by several producers: Embryoscope (Fertilitech), Primo Vision (Vitrolife), Eeva (Auxogyn, Inc.), Miri TL (Esco) and Geri (Genea Biomedix).

Embryoscopes allow the simultaneous observation and photographic documentation of maximally 72 embryos. A high class camera with Hoffman modulation contrast may take photographs at 9 levels of sharpness, which enables the precise morphological analysis of the embryo. By using LED lighting with red light of the wavelength of 635 nm, the exposure of the embryo to light is considerably decreased to less than 50 s per day. Lack of invasiveness of the observation considerably reduces the possibility of occurrence of infection, in relation to a minimum number of manipulations (system of closed quality assessment). The structure of the Embryoscope and sectorial plates allows the supply of warmth to individual wells containing the medium with the embryo, and high stabilization of temperature even when the Embryoscope is opened. High quality filters retain nearly 100% of particles larger than 0.3 µm. Embryoscope software from some producers enables observation of the embryos in real time, as well as a retrospective 4D analysis of individual embryos of each patient. Additionally, the software can also create personalized reports for the doctor on the development path of transferred embryos. Furthermore, the analyzer software allows sharing of results with colleagues and patients in a video format on portable devices and mobile phones.

The use of this technology popularized the assessment of the quality of the embryo by measuring the time of reaching individual developmental stages. Embryos which more quickly reach individual developmental stages are classified as better class embryos. The dynamics of embryo development as-

sessed using time lapse monitoring also combines the pace of achieving individual stages with the embryo's gender, and the occurrence of aneuploidy [19, 20]. A Cochrane review comparing the effectiveness of IVF procedures using time lapse technology and classic microscopic assessment of embryos showed that there is insufficient evidence of differences in live birth, miscarriage, stillbirth or clinical pregnancy to choose between the time-lapse system and conventional incubation. Further data explicitly comparing the incubation environment, the algorithm for embryo selection, or both, are required before recommendations for a change of routine practice can be justified [21].

In order to avoid traumatization of the reproductive cells and embryos, the distance is minimized between the surgical room where the punctures and transfers are performed and the laboratory. This, however, creates the risk of penetration of various substances applied for disinfection of the surgical room, which may be avoided by using air circulation directed from the laboratory to the surgical room.

Embryo transfer

Insertion of the embryo into the uterine cavity consists in the introduction of the embryo placed in a special catheter into the uterine cavity and injecting it into the endometrium. This procedure is traumatizing for both the embryo and uterine tissues. In order to reduce the invasiveness of this procedure, the physician should comprehensively assess the individual anatomical conditions occurring in individual patients. This consists in ultrasound assessment of the angle between the endometrium line and the canal of the cervix, and obtaining information concerning possible difficulties while introducing the catheter which had occurred during previously performed insemination procedures or IVF. This is to minimize damage which may be caused by the catheter introduced into the uterine cavity, such as the occurrence of bleeding into the uterine cavity or, in extreme cases, perforation of the uterus muscle.

Both strong anteflexion and retroversion of the uterus may contribute to the technical difficulties during the transfer of the embryo. This type of structure of the uterus may favor the situation when the introduced catheter will scratch the internal orifice of the uterus and endometrium. In cases of anteflexion of the uterus, this problem may be avoided by the strong filling of the urinary bladder with urine which, by exerting pressure on the uterus, will increase the angle between the endometrium line and the canal of the cervix. As the last resort, during difficulties with the introduction of the catheter, the cervix may be pulled with forceps. Ruggedness and stenoses occurring in the cervix, which hinder free introduction of the catheter, may also cause great difficulties during its introduction. In such a situation, the transfer must be preceded by the procedure of dilatation of the uterus (Hegar dilator), and the interval between these two activities should be as long as possible.

According to the degree of difficulty of transfer, there is a possibility to select the most appropriate type of catheter for the performance of this procedure. The differences between catheters consist in their stiffness, possibilities for stiffening, diameters and visibility during ultrasound. The decision concerning the selection of a catheter is individual. It is important that the IVF laboratory has at its disposal a wide choice of these instruments. Ruhlmann's results suggest that a softer catheter may help with difficult embryo transfers. Softer catheters, as also reported by other authors, resulted in better implantation rates [22].

The lack of invasiveness of embryo transfer is possible due to the preview of catheter insertion into the uterine cavity by ultrasound. The visibility of the catheter allows the positioning of the embryo in an optimum place, and also allows avoidance of perforation of the wall of the uterus. Visualization of the catheter depends of the type of the catheter and individual structure of a patient. The introduction of the catheter may be controlled trans-abdominally with a full urinary bladder; however, in obese patients or those with strong retroversion of the uterus, this method is of limited usefulness. If the trans-abdominal assessment is ineffective, the catheter may be traced using a transvaginal probe (tv).

The site of insertion of the embryo may be selected without insertion of the catheter under ultrasound guidance, but performing previous uterine length measurement (ULMb-ET). Revelli *et al.*, in a prospective, randomized, non-inferiority trial, compared the embryo transfer technique based on previous uterine length measurement with trans-abdominal ultrasound-guided embryo transfer (UGET) in a large population of patients submitted

to IVF. The researchers confirmed that ULMb-ET technique leads to IVF results comparable to those obtained with UGET, but is better tolerated than UGET and is technically easier to perform for a single operator [23]. Kwon *et al.* undertook an attempt to evaluate at what distance from the fundus of the uterus (1 or 2 cm) it is best to place the embryo, and did not find any differences in the number of achieved pregnancies according to the placement of the embryo [24].

The use of the two-piece catheter in case of possible difficulties with the introduction of the catheter into the uterine cavity shortens the time of the embryo staying outside the incubator. In addition, the first catheter inserted without the embryo is placed relatively shallowly in order not to irritate the walls of the uterus, while the second catheter – thinner and softer – which is introduced into its lumen has less possibilities to damage the tissues of the uterus.

In order to minimize the damage to the embryo during its transfer, it is very important to maintain a relatively low pressure while introducing it into the uterine cavity. Experiments by Grygoruk *et al.* on animal models showed that fast ejection of the transferred load can trigger both morphologic changes and apoptosis in mouse blastocysts. A reduction of the ejection speed of the transferred load minimizes injury to the embryos [25, 26]. Recently, a report was published concerning the effectiveness of using a pump-regulated embryo transfer (PRET) device to minimize the pressure during transfer. Caanen *et al.* confirmed that the PRET device generates significantly smaller variance of the positioning of the embryo(s) into the uterine cavity. This resulted in an ongoing pregnancy rate of 21% in the PRET vs. 17% in the manual ($p = 0.22$) transfer group; frozen-thawed embryo transfers resulted in 17.5 vs. 10.9% ($p = 0.097$), respectively [27]. It seems that the availability of this device in Poland may contribute to the reduction in invasiveness of embryo transfer.

The final stage involves checking whether the embryo became stuck in the catheter after the procedure. If it happens, this allows its re-introduction into the uterine cavity; however, in such cases, our experience suggests an unfavorable prognosis. The cause of the occurrence of this phenomenon is unknown. An analysis performed by Craciunas and Tsampras concerning the recommendations for patients after transfer concerning bed rest showed that bed rest following ET did not improve clinical pregnancy and live birth rates, but reduced the implantation rate [28].

Conclusions

Invasiveness of the IVF procedure for the woman and the embryo varies on an individual basis. Minimization of the invasiveness of IVF requires experience of the staff performing the procedure, especially with respect to the assessment of risk for an individual patient. Technologies related to IVF are constantly being improved, and the effectiveness of the selected individual treatment methods is not always scientifically confirmed.

Conflict of interest

The authors report no conflict of interest.

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