Review Article

Intermediary step – a double-blind sword

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Abstract

To improve the clinical pregnancy rate, the *in vitro* fertilization clinics worldwide have ignored the "ethical and cardinal" restrain by offering scientifically unproven safe treatments for their patients and possibly compromising the unborn child's health. Human fertilization and embryology authority requires all their licensed clinics to take into account the welfare of the unborn child and guides clinics and patients. They have instituted a traffic light monitoring system for various intermediary steps currently offered by the clinics. This review discusses the value of the scientific evidence available against the safety of the patients and the unborn child against the extra cost implications for utilizing these intermediary steps. Those in the red category should not be used as there is insufficient good-quality evidence for these steps. Those in the yellow, while having good quality evidence, require additional evidence before they are considered safe. The steps in the green category only have proven their safety by establishing good quality evidence. The intermediary steps discussed include: oocyte activation, use of time-lapse systems, need for hatching, use of hyaluronic acid, routine assessment of sperm DNA fragmentation, and use of advanced sperm selection techniques. The author offers his interpretation of the evidence and concludes by questioning the acceptability of using these intermediary steps routinely.

Keywords: Hatching, hyaluronic acid, ooctye activation, sperm DNA fragmentation, sperm selection technique, time-lapse system

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BACKGROUND

The birth of Louise Brown four decades ago has been a blessing in disguise for millions of couples worldwide who have difficulty conceiving naturally. Standard ovarian stimulation and laboratory protocols have evolved in the last four decades, which have resulted in more than 5 million live births worldwide. In a quest to achieve higher pregnancies and live birth rates, clinicians and scientists have started using procedures that lack good scientific evidence supporting the safety of the unborn child.[1,2] Therefore, it is mandatory that both clinicians and the laboratory scientist adequately evaluate the benefits against safety and cost implications of any intermediary steps (ISs) used for patient's treatment. This aspect is more pertinent in countries where the fertility sectors are not regulated.

All the fertility clinics in the United Kingdom are regulated by the human fertilization and embryology authority (HFEA). The authority believes any IS, used as an optional treatment, is likely to add to the total cost of a treatment cycle and should be discussed with the patients. However, the HFEA also recognizes that some of these steps effectively improve the chances of

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having a live birth.[3] HFEA segregates each IS into a traffic light category based on available scientific evidence to avoid confusion for clinicians, embryologists, and patients. IS assigned to the green category have sufficient good quality evidence, and techniques can be included routinely to enhance the outcome. Methods in the yellow category do not have enough quality evidence, and laboratories should not offer them as part of the treatment. IS, that fall in the red category, lack evidence and should never be used. Although these are broad categories, licensed clinics in the United Kingdom must provide sufficient evidence before offering any IS in their treatments.

This review discusses the benefits of the IS in laboratory techniques routinely used in *in vitro* fertilization (IVF) laboratories and considers the IS's safety and advantages.

Is oocyte activation necessary?

Data from worldwide registers reported no fertilization in approximately 3% of intracytoplasmic sperm injection (ICSI) cycles.[4] However, there could be many reasons for failed fertilization, and sperm quality contributes to one cause.[5] A morphologic disorder found in globozoospermia is classified as: type 1–total globozoospermia and type 2–partial globozoospermia. Patients with type 1 globozoospermia report no fertilization, and this is due to defects around the nucleus and the absence of acrosome.[6] Typically, entry of spermatozoon into the oocyte initiates oocyte activation, a physiologic process leading to the release of phospholipase C zeta. This release induces an oscillatory rise in calcium, leading to downstream events of fertilization and embryo development. However, any disruption in the oscillatory increase of calcium and prevention of sequences that follow other molecular events may lead to fertilization failure and possibly delayed embryo development.[7]

The protocols currently used to artificially activate oocytes after ICSI procedures include chemical, mechanical, and electrical stimulations. Although the activation process leads to an influx of calcium into the ooplasm restoring downstream events of fertilization, embryo development, epigenetic imprinting, and live births, there remains a concern that this stimulation can potentially result in abnormal pregnancy obstetrics neonatal outcomes.[4]

Based on limited good-quality evidence in systemic reviews of randomized control trials (RCTs) and the absence of safety data,[8,9] artificial activation is not recommended for routine use. Although HFEA has placed oocyte activation in a yellow category in the United Kingdom, requiring further evidence of normal live births, the unregulated fertility sectors worldwide continue to use this technique routinely.

Importance of time-lapse imaging

After insemination of oocytes, the sequence in the IVF laboratory includes checking for the fertilization, moving the zygote to a fresh drop of cleavage medium, and scoring the developing blastocyst on day 5 for an embryo transfer.[10] A time-lapse system (TLS) allows monitoring embryos in real time, facilitating the best blastocysts to be selected based on the cumulative information recorded on the developmental kinetics and morphogenetic parameters without compromising the culture environment.[11] TLS achieves this by analyzing detailed recorded digital images of the developing embryo in a stable embryo culture environment of correct temperatures, pH, humidity, and gas composition.[12] TLS has built-in algorithms that utilize morphogenetic parameters and allow the selection of an optimal blastocyst for transfer.[12-14]

A recent Cochrane review comparing conventional incubation and TLS demonstrated no clear advantage in live birthrates using the built-in algorithms for selecting the best embryo for transfer.[15] This study of nine RCTs concluded that in the absence of good quality evidence in live birth rates, ongoing pregnancies, miscarriages, and stillbirths, selecting embryos using TLS software algorithms did not justify the additional cost per cycle charged by some clinics.[15] Because some TLS systems lack humidification, there is concern about increased osmolality within the culture drops.[16] In the absence of conclusive evidence that TLS images improve birth rates, the clinics should decide after discussion with their patients if TLS use is warranted. Lack of regulatory guidance may find clinics using TLS without consulting their patients.

Is there a need to hatch?

The hatching of trophectoderm cells and the inner cell mass onto the endometrial bed are timed physiologic events. Before hatching, the zona pellucida (ZP) capsule facilitates fertilization and embryo development to blastocyst. The hatched cluster of cells interacts with the endometrial cells initiating the implantation process. Evidence suggests that ZP, a glycoprotein envelop, hardens during in vitro culture,[17] thereby delaying or preventing the hatching process,[18] leading to implantation failure. An acid tyrode solution, a laser or

mechanical technique, is used to breach the ZP and create an opening to avoid this failure. As acid tyrode digests the ZP, mechanical partial zona dissection requires the use of a micropipette. However, an accurately controlled opening in the ZP is only possible by laser photoablation. It is recognized that any one of these procedures can damage the embryo and potentially risk multiple pregnancies.[19]

Furthermore, patients whose embryos were artificially hatched ended up with monozygotic twining.[20] Thirty-one RCTs presented in a Cochrane review[19] demonstrated the benefits of assisted hatching in achieving higher clinical pregnancies without increasing the live birth rate.[19] When analyzing clinical pregnancy rates in trials that reported live birth, no differences were observed between the assisted hatching and the control group. Similarly, no difference in live birth rates was reported in a different systemic study; however, a slight increase in clinical pregnancies and multiple pregnancy rates were reported with assisted hatching.[20] Although the assisted hatching group demonstrated increased multiple pregnancies in both systemic reviews, it is not easy to interpret as more than one embryo was transferred in these studies.[19,20]

Assisted hatching resulted in 1.36% monozygotic splitting after elective single embryo transfers.[20] Blastocyst transfers could be an additional cause of monozygotic twinning.[21] The American Society for Reproductive Medicine (ASRM) concurs with the findings of these studies.[19-23] However, it has to be acknowledged that assisted hatching may improve clinical pregnancy rates in poor prognosis patients but not improve live birth rates. The HFEA, the ASRM, and the National Institute for Health and Care Excellence (NICE UK) recommend that patients undergoing IVF should not be offered assisted hatching as the benefits are not conclusive and more well-designed studies are needed.[3,22,23]

What purpose does hyaluronic acid (HA) serve?

Failed IVF cycles are due to failed implantation and included younger women with euploidy embryos.[24] The human implantation rate between 10% and 30% is considered low. Commercial media manufacturers recommend adding HA to the embryo transfer medium (ETM) to improve pregnancy rates and live birth.[25] HA added to the ETM may indirectly promote angiogenesis and improve cell-to-cell and cell-to-matrix adhesion. The highly viscous HA prevents accidental expulsion of the embryo from the uterine cavity and help in embryo apposition and attachment to the endometrial cells. CD44 is the primary receptor for HA, which gets expressed in the preimplantation embryo and the endometrium. The question is, can HA facilitate implantation? An analysis of a Cochrane review of 16 RCTs comparing results with HA added to the transfer medium and those without HA supplement reported a minimal improvement in live birth rates.[24] A similar RCT of 581 cycles found no evidence of improved live birth rates when the two groups were compared.[25]

In the absence of good quality conclusive evidence, HFEA recommends that more studies should be conducted to help patients, clinicians, and embryologists decide the effectiveness of HA. Furthermore, it is recommended to reduce multiple embryo transfers due to increased implantation rates, thereby reducing the number of multiple pregnancies.[26] However, it is worth noting that in these studies, the multiple pregnancy rates increased minimally. This observation is likely to be due to the transfer of multiple embryos using HA supplemented medium.[25] Therefore, clinics using HA supplemented ETM should closely monitor their multiple pregnancy rates by encouraging an elective single-embryo transfer policy.[24,25]

Should clinics routinely measure the percentage of sperm DNA fragmentation (SDF)?

A semen analysis reveals the sperm concentration, progressive motility, and percentage of normal morphology.[27-29] Any chemical changes in the DNA structure are referred to as SDF. The structural damage can lead to single- or double-strand breaks and influences the fertility outcome. Currently, there are four main "SDF tests," and they require an accredited laboratory equipped with expensive instruments and highly skilled technicians to carry them out. The four different tests include sperm chromatin structure assay, sperm chromatin dispersion test, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling, and single-cell gel electrophoresis assay.[30] All these methods measure different aspects of DNA damage.[31-38]

As a consequence, their utilization and clinical significance lack standardization. The new WHO guidelines[27] recommend that SDF assessment be part of routine semen analysis as SDF could be due to defective apoptosis, excessive reactive oxygen species production, and decreased seminal antioxidants. Cigarette smoking and pollution, toxic effects of drugs, high testicular temperature due to varicoceles, or systemic fever are also responsible for SDF. Patients who are obese or of advanced age had significant DNA damage.[30] Understanding the minimum clinical SDF index cutoff

values will allow clinicians and embryologists to decide the correct technique in sperm selection. The clinician should take caution to interpret the evidence that antioxidant supplements minimally improve live birth rates in subfertile men.[39] The analysis of the results confirms that this study reflects couples attending fertility clinics and is not restricted to men with high DNA fragmentation levels. The consensus is that the chances of spontaneous conception with an SDF index of more than 20% are low. However, patients with an SDF index between 30% and 40% have an almost no chance of pregnancies.[40,41] The British Fertility Society[42] and the ASRM[43] do not recommend routine clinical use in predicting treatment outcomes without standardizing DNA fragment results. It is worth noting that other multiple factors also influence the results, such as the quality of the oocytes and the patient's age. In older oocytes, repair of DNA damage in parental genomes after fertilization is considerably reduced.[44] As cutoff thresholds vary between studies, it is not easy to draw any conclusion on the predictive value of SDF tests and their usefulness in deciding treatment options.[30,44]

What advanced sperm selection techniques to use?

The use of healthy, euploidy (genetically balanced) mature sperm is needed to achieve a viable pregnancy and live birth. Although most laboratories use swim-up and discontinuous density gradient separation techniques to prepare sperm for insemination in IVF and ICSI, there is no guarantee that sperm prepared is free of any SDF abnormalities. Advanced sperm selection techniques have been recommended to minimize SDF abnormalities.

A multicenter trial on HA-bound sperm [physiologic ICSI (PICSI)] reported clinical pregnancy rates to be no different between the use of bound and unbound sperm.[45] Sperm expressing receptors bind HA to have lower rates of SDF with better chromatin structure. These sperms also have better morphology and better progressive motility. In addition, techniques of sperm selection based on zeta potential-cell surface charge, separation of apoptotic sperm using magnetic-activated cell sorting, and the use of microfluidic chips for processing neat semen samples have gained popularity.[46,47] Although the results of these techniques are encouraging, their effectiveness is unclear. A Cochrane review comparing results of eight studies did not find a significant increase in the live births for the first three techniques listed above.[46] However, more studies are required to establish the usefulness of microfluidics chips prepared sperm in achieving live births. Likely, a combination of these techniques with PICSI can identify a better sperm to use in ICSI treatment, influencing live birth rates and reducing miscarriages. Availability of limited data from magnetic-activated cell sorting and zeta potential sperm selection and cost implication do not permit recommending the use of these two techniques routinely in sperm preparation. The HFEA currently acknowledges that, given limited evidence, advanced sperm selection techniques cannot be considered safe and effective.[3]Furthermore, ASRM has not issued any directives on this topic. It is worth noting that there is limited data from RCTs on congenital abnormality in pregnancies utilizing advanced sperm selection techniques. Therefore, the availability of more data on congenital abnormalities will facilitate the acceptance of the newer methods.

Can intracytoplasmic morphologically select sperm injection (IMSI) achieve higher clinical pregnancy and live birth rates?

A new technique that allows the selection of the most potentially viable sperm to fertilize an oocyte was introduced in 2002.[48] The IMSI facilitates identifying sperm abnormalities using higher magnification (6000–13,000×). Most of the abnormalities are associated with the acrosome and post acrosome lamina neck. Mitochondrial, tail, and nucleus are also analyzed. As the identification requires more time to examine and select the spermatozoa[49] and the cost of the magnification lenses, most clinics have opted out of adopting the technique. However, this technique offered no more risks to the patient and embryos when compared with ICSI. A Cochrane review and three additional studies identified no improvement in clinical pregnancy, live birth rate, or miscarriage rate with IMSI.[50-53] No congenital abnormalities were reported in any of these studies. However, a retrospective analysis of babies born following IMSI demonstrates that babies born were small for gestational age (<2500 g) compared with ICSI babies. Furthermore, perinatal outcomes had no significant differences.[54]

CONCLUSION

Any IS added to the laboratory process requires an in-depth risk analysis to ensure that the IS is clinically proven to deliver a safe treatment. The quest to achieve better clinical results should not compromise the unborn child. Evidence favoring most of the IS discussed is limited. In the absence of conclusive evidence, the author believes that the routine use of any of the IS described here for better outcomes of clinical treatments is wrong. There is a need for greater collective understanding between the clinicians and the embryologists to support innovative techniques and avoid confusion.

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