ISAR Consensus Guidelines on Add-Ons Treatment in *In vitro* Fertilization

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ABSTRACT

Study Question: What are the good practices for the use of ADD-ON Treatments in IVF cycles in INDIA? **What is Already Known:** Add on treatments in IVF are procedures and technologies which are offered to patients in hope of improving the success rates. A lot of add on treatments exist; most of them have limited evidence and data for the Indian patient population is miniscule. These interventions may have limited effects, so it is imperative that any new technology that is offered is evaluated properly and has enough evidence to suggest that it is safe and effective. **Study Design, Size, Duration:** This is the report of a 2-day consensus meeting where two moderators were assigned to a group of experts to collate information on Add on treatments in IVF in INDIA. This meeting utilised surveys, available scientific evidence and personal laboratory experience into various presentations by experts on pre-decided specific topics. **Participants/Materials, Setting, Methods:** Expert professionals from ISAR representing clinical and embryology fields. **Main Results and the Role of Chance:** The report is divided in various components including the health of the Offspring, the various ADD ons available to an ART center, consensus points for each technology & qualifications and trainings for embryologists, the report and recommendations of the expert panel reflect the discussion on each of the topics and try to lay down good practice points for labs to follow. **Limitations, Reasons for Caution:** The recommendations are solely based on expert opinion. Future availability of data may warrant an update of the same. **Wider Implications of the Findings:** These guidelines can help labs across the country to standardise their ART services and improve clinical outcomes, it will also motivate clinics to collect data and report the use of Add ons to the national registry. **Study Funding/Competing Interest(S):** The consensus meeting and writing of the paper was supported by funds from CooperSurgical India.

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Introduction to Add-Ons Treatment in In vitro Fertilization

Add-ons are classified as optional treatments that can be offered with standard fertility treatments at an additional cost. Some techniques may have shown promising results in the initial studies, or they may have been around for several years but have not yet proven to improve pregnancy or birth rates.[1]
These guidelines are designed primarily as an educational resource for centers offering add-on treatments to help them in providing evidence-based medical services.[1]

The Health of the Offspring

Background

Over the years, there have been concerns about the short-term and long-term risks for children conceived through assisted reproductive technology (ART) compared to naturally conceived children. Children conceived through ART have been thought to be phenotypically and biochemically different from those who conceived naturally, but the mechanisms underlying these differences and the subsequent health implications are unclear.[2] Health parameters that need to be monitored are namely birth defects, growth and developmental delays, blood pressure and blood glucose levels, predisposition to asthma, neurological dysfunction, autism, mental disorders, cancers, retinoblastoma, retinopathy, *de novo* imprinting disorders, and microdeletions.[3,4]

Supporting evidence

	Abstract of evidence	Conclusion
Birth defects	Systematic review analysis was performed by Lacamara *et al*.[5] to indicate that the use of ICSI increases the risk of congenital malformation in children born from singleton pregnancies versus naturally conceived children Twenty-one of the 104 publications listed in the literature search were included in the analysis Observational studies reported mostly an increased risk for congenital malformation; the risk of congenital defects is 7.1% in ICSI and 4.0% in the general population (OR 1.99, 95% CI [1.87-2.11]). However, attributing higher risk solely to ICSI might seem far-fetched, as *in vitro* and simulation procedures, patient diseases, and ICSI indication may also be associated with a higher risk of malformation	Marginal increase in birth defects has been seen in ART children, cannot be directly correlated to ART
Developmental delays	The outcome of the pregnancy and the developmental well-being of children conceived from 12,866 consecutive ICSI cycles were assessed. A total of 3277 couples delivered 5891 neonates. There was a higher than normal incidence of *de novo* chromosomal abnormalities in a small sample of ICSI offspring. Controlling for maternal age showed that the frequency of low-birth weight and gestational length were comparable with the naturally conceived counterpart. Rates of malformation in ICSI offspring ranged from 3.5% to 6.2%. At 3 years of age (n=811), the proportion of children at risk for developmental delays was 10.4% in ICSI and	No conclusive data on Indian ART babies and their developmental milestones. There is a need to monitor and setup registry for ART babies

	Abstract of evidence	Conclusion
	10.7% in IVF singletons. However, high-order gestations were characterized by 19.4% of the children having compromised development. Epigenetic analysis of assisted reproductive technique concept uses found minor imprinted gene expression imbalances. ICSI offspring presented with genetic defects that were inherited or arose *de novo*. Obstetric and neonatal outcomes of singleton pregnancies appeared to be dependent upon maternal age. ICSI and IVF seemed to exert a negative effect on the well-being of offspring mainly because of the association with multiple gestations. All assisted reproduction procedures should be monitored for the eventual effect of environmental aggressors on offspring epigenesis[6]	
Bodyweight	A multicenter, double-blind RCT comparing the use of embryo culture media in IVF. Between July 2010 and May 2012, 836 couples (419 in the HTF group and 417 in the G5 group) were included. The allocated medium (1:1 allocation) was used in all treatment cycles a couple received within 1 year after randomization, including possible transfers with frozen-thawed embryos[4]	Culture media could affect birth weight; and needs to be monitored
	In this trial, birth weight data from 380 children: 300 singletons (G5: 163, HTF: 137) and 80 twin children (G5: 38, HTF: 42) were retrieved[4]	
	Birth weight was significantly lower in the G5 group compared with the HTF group, with a mean difference of 158 g (P=0.008). More singletons were born preterm in the G5 group (8.6% [14/163] vs. 2.2% [3/137]), but singleton birth weight adjusted for gestational age and gender (Z-score) was also lower in the G5 than in the HTF group (−0.13±0.08 vs. 0.17±0.08; *P*=0.008)[4]	
Asthma, blood pressure, blood sugar	A significantly increased risk for asthma, albeit small, was found in children conceived by IVF (aOR 1.28, 95% CI 1.23-1.34), increasing the absolute risk from 4.4% to 5.6%[7]	No conclusive data on Indian ART babies to establish a direct correlation with ART
	The risk increase for asthma was the same in boys and girls, in singletons and twins, and after caesarean section and vaginal delivery. The risk was higher for preterm than term singletons. For children with a low Apgar score, respiratory diagnoses, mechanical ventilation, continuous positive airway pressure or neonatal sepsis, the effect of IVF on asthma risk was low and statistically nonsignificant[7]	
	Adjustment for the length of involuntary childlessness eliminated the effect, and removal of infants whose mothers had used anti-asthmatics in early pregnancy reduced the risk[7]	
	This study verifies an association between IVF and asthma in children. This can be partly explained by neonatal morbidity and by maternal asthma acting as mediators, but the leading risk factor is parental subfertility[7]	
Neurological dysfunction	Research on cognitive and behavioral development of children born after assisted conception is inconsistent	No direct association to ART

	Abstract of evidence	Conclusion
Cancers	Recently, the report of an increased risk of childhood cancers after ART (IVF/ICSI) has generated considerable concerns	No direct association to ART
De novo mutations	A significantly higher rate of *de novo*, noninherited chromosomal abnormalities in children born after ICSI was observed compared with the rate in the general population (1.6 vs. 0.5%), especially with the associated male factor. This finding was related to sperm concentration and motility. The significantly higher rate of observed inherited anomalies (1.4 vs. 0.3%-0.4% in prenatal tests in the general population; $P<0.001$) was related to a higher rate of constitutional chromosomal abnormalities, mainly in the fathers[8]	Babies born through ICSI need to be monitored

ICSI=Intracytoplasmic sperm injection, ART=Assisted reproductive technology, OR=Odds ratio, CI=Confidence interval, IVF=*In-vitro* fertilization, RCT=Randomized controlled trials, aOR=Adjusted OR, HTF=Human tubal fluid

Consensus on the health of children conceived by assisted reproductive technology

Factors	Group consensus
Advanced age	ARA is a risk factor for female infertility and subsequent conception. The cautionary approach is needed for this group
ICSI	Should be used judiciously in indicated cases only
Male factor	Semen quality of young adult ICSI offspring: These first results in a small group of ICSI men indicate a lower semen quantity and quality in young adults born after ICSI for male infertility in their fathers

ARA=Advanced reproductive age, ICSI=Intracytoplasmic sperm injection

Recommendations

- Counsel the patients for the associated risks[9]
- Take informed consent whenever necessary.

Parameters	Group consensus
Endpoints to assess - antenatal complications, CPR, LBR, birth weight, developmental milestones, semen parameters in ART conceived men, ovarian reserve in ART conceived women	Need to assess and monitor regularly as suggested by the Indian Academy of Pediatrics
Creation of a national registry	Yes, it is recommended

CPR=Clinical pregnancy rate, LBR=Live birth rate, ART=Assisted reproductive technology

DNA Fragmentation Index, Computer-Assisted Semen Analysis, and Sperm Quality Analyzer

Background

DNA fragmentation index (DFI) can be tested using the laboratory investigations such as terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), COMET, sperm chromatin structure assay (SCSA), and sperm chromatin dispersion tests [Table 1]. However, some of these tests have limitations such as interlaboratory and interobserver variability. The cutoffs for many tests are not described clearly. Of the mentioned tests, SCSA and TUNEL are used by most of the clinicians as they are worldwide standardized and validated tests for all users.[10-15]

Table 1: Advantages and disadvantages of the technologies		
Technology	Advantages	Disadvantages
SCSA	Flow cytometry test evaluates 10,000 cells rapidly	The assay requires a flow cytometer and dedicated software
	Inter-lab variation is minimal	
	The clinical threshold of 30% DFI has been already established and HDS levels >25% are associated with a negative pregnancy outcome	The specificity of SCSA is lower than the alkaline comet assay
	High reproducibility	
TUNEL	Standardized and validated test	It does not include the lysis step, the accessibility of TdT to all the 3'oh ends in tightly packed sperm genome is limited
	Sensitive test	
		The clinical threshold is low
Comet	The comet assay is a versatile and sensitive method for measuring single- and double-strand breaks in DNA	Specificity is not absolute
		Not able to detect small DNA fragments
SCD	Simple to perform	Inter-observer subjectivity
	Displays higher sensitivity for detecting sperm DNA fragmentation	Operator dependency and low efficiency
		SCD also does not give information on HDS
	Minimal requirement of laboratory equipment	
	Cost-efficient	
HBA assay	Quick and reliable sperm screening	Standardization and optimization are required
	Provides the information needed to make the right decision in minutes	
	A reliable alternative to expensive DNA integrity assays	
	HBA score positively correlates with fertilization, pregnancy and cleavage rates	
	Reliable and reproducible	
CASA	CASA is an advanced system that meets WHO standards for semen analysis and gives complete data compared to SQA	High acquisition cost
		Regular maintenance is necessary
	Fast analysis	Different settings may dramatically change results
	Highly reproducible with same settings	Can overestimate or underestimate sperm count
	Detailed analysis including sperm motility and morphology	Sperm count should be between 20-50 million/ml for accurate analysis
	Increased objectivity and consistency of measurement	
	High accuracy and precision of analysis	Expensive, inaccessible for most laboratories
SQA	Rapid, easy and low-cost quantitative evaluation of semen quality	Standardization and optimization are required

HDS=High-DNA stainability, HBA=Hyaluronan binding assay, CASA=Computer-assisted semen analysis, SCSA=Sperm chromatin structure assay, DFI=DNA fragmentation index, TUNEL=Transferase dUTP nick end labeling, SCD=Sperm chromatin dispersion, SQA=Sperm quality analyzer

Supporting evidence on factors affecting DNA fragmentation index

Age and DNA fragmentation index

Increasing male age is significantly associated with higher DFI. The impact of increasing male age on DFI revealed that males >45 years of age had the highest DFI and the lowest high DNA stainability compared to all other age groups ($P < 0.001$). When intrauterine insemination (IUI)–artificial insemination (AIH) pregnancy rates were compared between patients with DFI <30 vs. >30, the pregnancy rates achieved were 14% and 3%, respectively. Serial ejaculation every 24 h for 4 days showed a reduction in DNA fragmentation.
Performing a DFI test in unexplained infertility before planning an IUI is recommended because there is a strong negative correlation between DFI and time to pregnancy with IUI. An early intracytoplasmic sperm injection (ICSI) based on the DFI test prevents the loss of valuable biological time, increases cost of failed IUI cycles of repeated unsuccessful IUI, and decreases the time to pregnancy.

Lifestyle factors and DNA fragmentation index

Lifestyle factors such as smoking, obesity, drugs, diabetes, seminal infection, environmental/occupational exposures, and clinical varicocele can have a negative impact on sperm quality. Sperm DNA testing can help reinforce the importance of lifestyle modification and antioxidant therapy and monitor the patient's response to an intervention.
Several studies have been conducted to observe the impact of DFI and its effect on ICSI outcomes which have been further correlated with lifestyle factors (age, body mass index [BMI], and smoking). In summary, the results of this study indicated that 14% of men had high DNA fragmentation. BMI and smoking showed a positive correlation with increased DFI.[16]

Iatrogenic factors and DNA fragmentation index

Table 2: Iatrogenic causes of DNA fragmentation index

Factor	Effect	Reference
Incubation at 37°C (2 h)	Increased vacuolated nuclei	Peer *et al*., (2007)
Sperm centrifugation	Increased DNA denaturation in samples of infertile men	Zini *et al*., (2000)
Swim-up processed sperms	Increase in DNA fragmentation after long incubation	Muratori *et al*., (2003)
Density gradient processed samples (normo and astheno)	Significantly decreased DFI	Donnelly *et al*., (2000)
Overnight shipping simulated by storage in 2-4°C for 24 h	DNA integrity remained unchanged	Huszar *et al*., (2004)

DFI=DNA fragmentation index

Sperm processing and DNA fragmentation index

The mean DFI of the sample after processing (swim-up, density gradient centrifugation, or density gradient followed by swim-up) was significantly lower when compared with fresh and simple wash samples.

Conclusion

- The current semen analysis testing is incomplete to assess male fertility
- Sperm DNA fragmentation testing has strong associations with every fertility checkpoint in ART
- Sperm DNA fragmentation has strong associations with miscarriage rate
- Sperm DNA fragmentation testing can explain "unexplained" infertility.

Consensus

Indications	Consensus
Routine test	Insufficient evidence to suggest DFI as a routine test for all patients
Multiple IUI failure	Possible indication for DFI testing
IVF failure	Possible indication for DFI testing
ICSI failure	Recommended indication for DFI testing
Lifestyle history	Possible indication for DFI testing
Male age >40	Recommended indication for DFI testing
Poor embryo quality	Direct correlation cannot be ascertained, clinicians discretion advised
Oligoasthenoteratozoospermia	Direct correlation cannot be ascertained, clinicians discretion advised
Unexplained infertility	Direct correlation cannot be ascertained, clinicians discretion advised

IVF=*In-vitro* fertilization, IUI=Intrauterine insemination, ICSI=Intracytoplasmic sperm injection, DFI=DNA fragmentation index

Consensus recommendations

	Increased DFI
Lifestyle modifications	Recommended
Antioxidants (6 months)	Recommended
TESA	Can be utilized
PICSI (count >10 million)	Need robust RCTs to recommend use
Low-quality evidence that HA-ICSI decreases miscarriage rates. Cochrane 2018*	though miscarriage rates are lower in recent meta-analysis*
No effect on live birth, HA-ICSI decreases miscarriage rates: viz., Miller *et al*., Lancet 2019*	
IMSI	Need robust RCTs to suggest use
Very low-quality evidence in support of use	
Data collection is recommended	
Varicocele repair	Recommended when symptomatic
Microfluidics/MACS Data collection is recommended	Need robust data to suggest use

TESA=Testicular sperm aspiration, PICSI=Physiological intracytoplasmic sperm injection, IMSI=Intracytoplasmic morphologically selected sperm injection, MACS=Magnetic cell sorting, RCT=Randomized controlled trials, HA-ICSI=Hyaluronic acid-intracytoplasmic sperm injection, DFI=DNA fragmentation index, RCT=Randomized controlled trials

Sperm Selection Techniques

Background

In natural conception, a few sperm cells reach the ampulla or the site of fertilization. This population is a selected group of cells since only motile cells can pass through cervical mucus and gain initial entry into the female reproductive tract.

Natural sperm selection in the humans is a rigorous process, resulting in the highest quality sperm reaching and having an opportunity to fertilize the oocyte. Relative to other mammalian species, the human ejaculate consists of a heterogeneous pool of sperm, varying in characteristics such as shape, size, and motility. The sperm selection techniques provide highly motile sperm populations or try to replicate the complex selection processes as seen in nature. Several methods have now been developed to mimic some of the natural selection processes that exist in the female reproductive tract.[17]

Advanced sperm selection techniques have developed as means of improving ART outcomes in specific clinical scenarios. Any selection mechanisms taking place *in vitro* must not harm the spermatozoa or modulate their interaction with the female reproductive tract if fertility is to be maintained. Biomimetic

selection of the best quality spermatozoa for artificial insemination (AI) or for cryopreservation could improve pregnancy rates and may help reverse the decline in fertility.[18]

New technologies are being developed to aid in the diagnosis, preparation, and selection of spermatozoa in ART. There are different techniques involved in the sperm selection process, namely direct swim-up, pellet swim-up, density gradient, physiological intracytoplasmic sperm injection (PICSI), intracytoplasmic morphologically selected sperm injection (IMSI), MACS, microfluidics, sperm birefringence, and others, advantages and disadvantages of which are discussed below:

Table 3: Sperm selection techniques: Advantages and disadvantages

Techniques	Indications in literature	Advantages	Disadvantages
Direct swim-up method	ICSI	Simple and easy to execute Not much skill required Reduces risk of ROS generation Inexpensive method	Not a very clean method of sperm separation Applicable mostly to normozoospermic samples
Pellet swim-up	IUI, ICSI	Simple and easy to execute Not much skill required Inexpensive method	Not preferable for IVF Applicable mostly to normozoospermic samples
Density gradient	IUI, ICSI, IVF, TESA	Much cleaner and morphologically normal sample obtained	DNA integrity could be affected, need more data
PICSI	Poor sperm parameters like abnormal morphology or poor motility Previous IVF Treatment failures The previous cycle had poor grade embryo High DFI of sperm Patient with a history of repeated miscarriages Previous ICSI cycle had poor fertilization or when no embryos were formed	Any ICSI trained embryologist can perform PICSI	Not suitable for men with poor sperm count but in such cases use can be substituted with sperm slow which follows the same principles
IMSI	Repeated ICSI failures Those suffering from repeated miscarriages Poor sperm morphology (teratozoospermia) The high rate of sperm aneuploidy High levels of DNA fragmentation Marked alterations of seminal parameters due to severe testiculopathy	An ICSI trained embryologist can perform IMSI with training	Installation cost is high which translates to cost of treatment for the patient Conflicting reports No proper consensus on indications Extensive randomized studies and meta-analysis studies are required

Contd...

Table 3: Contd...

Techniques	Indications in literature	Advantages	Disadvantages
MACS	Patients undergoing IUI Infertile patients with a high level of sperm DNA fragmentation Low sperm quality. Patients who have had repeated miscarriages with an unidentified cause Patients with previous implantation failure attributed to poor quality embryos when oocyte morphology was normal	A safe and efficient method to select functional sperm with consistently good results. It improves pregnancy rates when used to complement standard sperm selection methods in ART	Setup cost and long term safety needs to be ascertained
Microfluidics	--	Microfluidic systems can work with small volume samples. Consequently, the operational cost is reduced Microfluidic systems have high sensitivity and low response times Lower rate of sperm DNA fragmentation observed because reactive oxygen species generated during centrifugation was avoided by this method Sperm selection happens naturally No special skills required Inexpensive and quick Microfluidics is a superior technique to isolate sperm with high motility, enhanced percent of normal morphology and significantly reduced percentage of sperms with DNA fragmentation	Some operators have found a difficulty to achieve good sperm separation in oligo and/or aestheno-zoospermic samples
Sperm birefringence	-	By the addition of a polarizing and analyzing lens in the inverted microscope of the micromanipulation system, normal sperm can be selected for ICSI real-time, without compromising their vitality	Standardization required

PICSI=Physiological intracytoplasmic sperm injection, IMSI=Intracytoplasmic morphologically selected sperm injection, ICSI=Intracytoplasmic sperm injection, ART=Assisted reproductive technology, TESA=Testicular sperm aspiration, IVF=*In-vitro* fertilization, IUI=Intrauterine insemination, MACS=Magnetic cell sorting, DFI=DNA fragmentation index, ROS=Reduction of oxidative stress

Importance of sperm selection

We need to assume that treatments are still far from being 100% effective, as many patients fail to achieve pregnancy and others frequently need several attempts to achieve parenthood. Even when using the best gametes, obtained from young, healthy, and previously fertile donors, it can take several embryo transfers to achieve a pregnancy in certain patients. In relation to the egg, it has been reported that <7% of the retrieved oocytes lead to live birth. From the male viewpoint, the improvements needed are closely linked with the need to establish robust sperm quality indicators, to use them as a diagnostic tool to increase the success of ART, and to design appropriate strategies for sperm selection. Implementing such sperm diagnostic and/or selection techniques could significantly improve live birth rates, still acknowledging that outcomes will also depend heavily on oocyte and endometrium quality.[10]

Surgical Sperm Retrieval Laboratory Requirements and Protocols

Background

Different surgical methods such as percutaneous epididymal sperm aspiration (PESA), MESA, testicular sperm aspiration (TESA), testicular sperm extraction (TESE), and micro-TESE have been developed to retrieve spermatozoa from either the epididymis or the testis according to the type of azoospermia [Table 4], i.e., obstructive or nonobstructive.[19] Laboratory techniques are used to remove contaminants, cellular debris, and red blood cells following collection of the epididymal fluid or testicular tissue. Surgically-retrieved spermatozoa may be used for ICSI and/or cryopreservation.

Table 4: Advantages and disadvantages of sperm retrieval techniques for assisted reproduction[20]

Procedures	Advantages	Disadvantages
PESA	Fast and low-cost method	Variable sperm retrieval
	Minimal distress, repeatable	Risk of fibrosis and obstruction at aspiration site
	No microsurgical expertise required	
	Few instruments and materials required	Risk of hematoma at aspiration site
	No surgical exploration	DNA integrity is questionable
MESA	Sufficient number of sperm retrieved	Surgical exploration required
	Good chance of sperm cryopreservation	Increased cost and time-demanding
	Reduced risk of hematoma	Microsurgical instruments and expertise required
		Postoperative discomfort
TESA	Fast and low cost	The relatively low success rate in NOA
	Reproducible	Insufficient sperm retrieved in NOA
	No microsurgical expertise required	Cryopreservation limited
	Few instruments and materials are required	Risk of fibrosis, hematoma or testicular atrophy
	No surgical exploration	
micro-TESE	Patients with NOA can benefit using this technique	The relatively low success rate
		Relatively few sperm retrieved
		Risk of testicular damage
		Postoperative discomfort
		Microsurgical expertise needed

PESA=Percutaneous epididymal sperm aspiration, MESA=Microsurgical epididymal sperm aspiration, TESA=Testicular sperm aspiration, micro-TESE: Micro surgical testicular sperm extraction, NOA=Nonobstructive azoospermia

Conclusion

ICSI provides lower fertilization rates per injected oocyte as well as clinical pregnancy and delivery rates when testicular spermatozoa from men with NOA are used in comparison to ejaculated sperm or epididymal/testicular sperm from men with OA. Testicular spermatozoa from men with severely impaired spermatogenesis could have a higher tendency to carry deficiencies such as the ones related to the centrioles and genetic material, which could ultimately affect the capability of the male gamete to activate the egg and trigger the formation and development of a normal zygote and a viable embryo. The risks of congenital malformations, infertility, and other diseases in children conceived are still poorly determined. The laboratory plays a crucial role in finding sperms and selection of the best-quality sperm for ICSI. Laboratory personnel should adhere to strict procedures for better clinical outcomes, which are listed below:

- Receive the best-quality surgically retrieved specimen possible, with minimal or no contaminants such as red blood cells and microorganisms
- Minimize iatrogenic cellular damage during sperm processing by mastering the technical skills and controlling factors such as centrifugation force, duration, temperature variation, laboratory air quality, dilution and washing steps, quality of reagents, culture media, and disposable materials
- Improve sperm fertilizing potential, if possible by selecting viable sperm for ICSI when only immotile spermatozoa are available.

In vitro maturation: Background

Oocyte *in vitro* maturation (IVM) is a process in which oocytes are retrieved from the antral follicles of unstimulated or minimally stimulated ovaries and matured *in vitro*. IVM of human oocytes has emerged as a promising procedure. This technology has advantages over controlled ovarian stimulation, such as reduction of costs, simplicity, and elimination of ovarian hyperstimulation syndrome (OHSS). By elimination or reduction of gonadotropin stimulation, IVM offers eligible infertile couples a safe and convenient form of treatment, and IVM outcomes are currently comparable in safety to those of conventional IVF. IVM has been applied mainly in patients with polycystic ovary syndrome or ultrasound-only polycystic ovaries.[15]

In vitro maturation of oocytes: Indications

Marked improvements in the clinical and laboratory aspects of IVM treatment have led to better clinical outcomes. The recent development of fertility preservation has extended the indications of IVM beyond patients at risk of OHSS. IVM may represent a viable option in patients suffering from ovarian resistance to FSH, whose ovaries are endowed with an average number of antral follicles. Because IVM may be performed without exogenous gonadotropin administration, therefore avoiding supraphysiologic serum estradiol (E2) levels, it is applicable in emergency situations as well as in patients suffering from E2-sensitive diseases. Given the simplicity and the safety of the IVM procedure and the possibility to combine immature oocyte retrieval with ovarian cortex cryopreservation and given the risk of disease recurrences after grafting in some cases, we consider that IVM should systematically be offered to candidates undergoing ovarian tissue freezing. In leukemia patients, IVM currently represents the only viable option of fertility preservation as transplantation of cryopreserved ovarian tissue is still contraindicated.[21]

Various reported indications of IVM: Group consensus	Yes	More data needed	No
PCOS	Recommended		-
PCO-like ovaries	-	✓	-
Normo-ovulatory patients	-		-
Previous failed IVF attempts	-		✓
History of OHSS	-	✓	-
Oocyte maturation problems	-	✓	-
Emergency oocyte retrieval due to malignancies (estrogen-sensitive tumors)	-	✓	-
Oocyte retrieval from ovarian tissue after vitrification	Recommended	-	-
Poor responders	-	✓	-
IVM can be a new source of oocyte donation	-	-	✓
Women with a history of empty follicle syndrome	-	-	-

IVM=*In vitro* maturation, IVF=*In vitro* fertilization, OHSS=Ovarian hyperstimulation syndrome, PCOS=Polycystic ovary syndrome

Traditional *in vitro* fertilization versus *in vitro* maturation

Traditional IVF	IVM®
Relatively more oocytes/embryos	Fewer oocytes and embryos
"Higher" pregnancy rate/OPU	Lower pregnancy rate/OPU
Daily hormone injections	Minimal hormone injections - or
hCG injection	No hCG injection
Emotional stress	Reduced psychological impact
Long treatment time 4-6 weeks	Reduced treatment time - 2 weeks
Potential side effects (e.g., OHSS)	Reduced interference with daily life
	Long term effects unknown

OPU=Ovum pick-up, OHSS=Ovarian hyperstimulation syndrome, hCG=Human chorionic gonadotropin, IVM=*In vitro* maturation, IVF=*In vitro* fertilization

In vitro maturation: Advantages and disadvantages

Advantages	Disadvantages
A gentle way to stimulate a woman's ovaries	The retrieval of fewer eggs because less fertility medication is used
Provides a way for women who are likely to develop OHSS to undergo IVF safely	Not all immature eggs will mature in the lab, resulting in a smaller number of viable eggs for IVF treatment
A viable option for couples who have male factor infertility	The process of IVM is relatively new, so historical success rates are not available
The ability to do IVF with less discomfort due to side effects from fertility medication	Because IVM is a cutting-edge procedure, not all fertility centers will offer this option

IVM=*In vitro* maturation, IVF=*In vitro* fertilization, OHSS=Ovarian hyperstimulation syndrome

Recommendation

In accordance to the Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, the following recommendations are suggested:

Evidence on *in vitro* maturation
Presently, no robust data for comparison
The retrieval of *in vivo* matured oocytes, implantation and pregnancy rates of IVM are less than expected in comparison to conventional ART
Priming with hCG and/or FSH appears to improve implantation and pregnancy rates compared to no priming[20]
IVM should only be performed as an experimental procedure in specialized centers for carefully selected patients evaluating both efficacy and safety. Informed consent must include information regarding pregnancy rates of IVM in comparison to conventional ART and alternative options if any[22]

IVM=*In vitro* maturation, ART=Assisted reproductive technologies, hCG=Human chorionic gonadotropin, FSH=Follicle-stimulating hormone

Artificial oocyte activation

Indications of artificial oocyte activation

Artificial oocyte activation (AOA) is mainly used in case of oocyte-related activation deficiency, IVM oocytes, low numbers of oocytes at retrieval, severe teratozoospermia, severe oligoasthenoteratozoospermia (OAT), globozoospermia, surgical sperm collection either for azoospermia or high sperm DNA fragmentation index, previous fertilization failure or poor fertilization, unexplained infertility, and frozen-thawed oocytes.

Assisted oocyte activation is not beneficial for all patients with a suspected oocyte-related activation deficiency

Despite the success of ICSI, total fertilization failure (TFF) still occurs in 1%–3% of all ICSI cycles. ICSI followed by AOA (ICSI-AOA) can restore fertilization, most efficiently in cases of sperm-related fertilization deficiency. The indication for ICSI-AOA is less apparent when the capacity of the sperm to activate oocytes is considered normal, as proved by a heterologous ICSI model, such as the mouse OAT.
According to Vanden Meerschaut *et al*., for patients with a suspected oocyte-related activation deficiency, as diagnosed by a heterologous ICSI model, the indication for ICSI-AOA remains debatable.[23]
The study data showed that ICSI-AOA is very efficient in patients with a suspected oocyte-related activation deficiency and previous TFF after conventional ICSI. In contrast, when there was a history of low fertilization (LF) in another center, one should be careful and test the efficiency of ICSI-AOA on half of the sibling oocytes, because ICSI-AOA is not always beneficial for patients with previous LF and a suspected oocyte-related activation deficiency. For these patients, a split ICSI-AOA cycle using sibling oocytes can help distinguish between a molecular oocyte-related activation deficiency and a previous technical or other biological failure. Moreover, this split ICSI-AOA strategy enables us to set the appropriate plan for future treatment cycles.

Calcium ionophore method for artificial oocyte activation

Calcium ionophore method for AOA has the most reports, and the resulting children have no reports of anomalies. Clinical use of these agents in assisted reproduction is limited by insufficient knowledge about their potential cytotoxic, teratogenic, epigenetic, and mutagenic effects on oocytes and embryos. It should be noted that issues of genetic safety and abnormal imprinting have not been addressed for the combined use of these oocyte activation methods. However, some studies revealed no physical or mental developmental disorders associated with babies born through these procedures, even 12 months after birth.

Culture Media Additives: Embryo

Background

For a successful pregnancy to occur, embryos that can implant are essential. Several studies have suggested that the composition of the media that embryos are cultured in may have an impact on the quality of embryos generated in IVF/ICSI cycles, thereby influencing implantation and pregnancy rates.[24]

In the past, the culture of human gametes and embryos has been performed with surprisingly little standardization and a lot of experimentation. Today, commercially available culture media range from simple 8–10 different salts and sugars to media containing nearly 80 various components including amino acids, lipids, vitamins, trace ions, and bioactive molecules, such as hormones and expression modulators. Today, culture media for embryos can be used either as a sequential system, with different compositions for days 0–3 and 3–6, or as a single medium used for the whole culture period.[25]

Various culture media have different effects on the epigenome of the developing pre-embryo, and data suggest that the *in vitro* culture of gametes and embryos may influence the phenotype of the offspring.

Table 5 summarizes the currently available knowledge on the main components of culture media and their possible effects on embryo and perinatal development and long-term outcomes.[26]

Table 5: Evidence-based review on culture media

Culture media additives	Evidence-based review
Energy substrate	Zygotes and subsequent cleavage stages prefer pyruvate as the primary source of energy, while the post eight-cell-stage embryo uses glucose[22]
	Glucose-free media are still advertised for the early stages of embryo culture even though the inhibitory role of glucose is no longer a dogma and the content of phosphate during days 1-3 varies from zero to high concentration (Quinn, 2004)[27]
EDTA	Still a matter of debate[26]
Amino acids	Experiments in mouse embryos demonstrated that nonessential amino acids are essential during early embryo development while essential and nonessential amino acids should be included in the medium after the 8-16-cell stage[28]
Antibiotic supplementation	Most primary or normal human cells show reduced growth rates in the presence of antibiotics, which is why their role in embryo culture is debated by many who feel that keeping the cells free from microorganism contamination can be accomplished with proper knowledge of good laboratory practice[29]
Protein supplementation	Zhu *et al*. suggest that protein source/HSA has a significant effect on birthweights of singleton newborns[30]
Growth factors and cytokines	Study comprising of around 14 fertility units and >1300 women (Ziebe *et al*., 2013).[31] For supplementation of culture media with GM-CSF showed a significant increase in LBR in a subgroup of patients with at least one previous miscarriage but only in low HSA media
	GM-CSF supplementation appeared beneficial for LBR in a retrospective study for patients with previous miscarriages by Renzini *et al*. (2013)[32]
	A pilot study by Sfontouris *et al*., 2013[33] long-term follow-up of the offspring are needed to determine the benefit and safety of GM--CSF-supplemented media for the general IVF population and these patient groups (Siristatidis *et al*., 2013)[34]
Hyaluronan rich media	A recent update on this Cochrane review including 16 RCTs concluded that the addition of hyaluronic acid to embryo transfer medium yielded improved LBR. However, only six trials reported on LBR and the obtained evidence was of moderate quality (Bontekoe *et al*., 2014)
	Balaban *et al*. (2011) found significantly increased LBR for the hyaluronan group

HSA=Human serum albumin, Gm-CSF=Granulocyte-macrophage colony-stimulating factor, LBR=Live birth rate, IVF=*In-vitro* fertilization, RCT=Randomized controlled trials, EDTA=Ethylene diamine tetra-acetic

Implantation-promoting medium

Hyaluronan-rich media

Hyaluronan-enriched embryo transfer (ET) medium aids in implantation of embryos and hence improves pregnancy rates in IVF-ET cycles (IVF-ET). In patients with recurrent implantation failure, it may be considered as a useful transfer medium.[35]

Effect of granulocyte-macrophage colony-stimulating factor in embryo culture medium

Ziebe *et al*. established that the addition of granulocyte-macrophage colony-stimulating factor (GM-CSF) to embryo culture medium elicits a significant increase in the survival of transferred embryos to week 12 and live birth. The study results were consistent with a protective effect of GM-CSF on culture-induced embryonic stress. GM-CSF may be particularly efficient in women with previous miscarriage.[31]

Common medium versus advanced in vitro fertilization medium for cryopreserved oocytes in heterologous cycles

GM-CSF plays a crucial role during embryo implantation and subsequent development. Poverini *et al*. evaluated the effects of embryo cell culture medium, with the inclusion of GM-CSF on embryo development and pregnancy rate. The study results showed that the rate of fertilization and the pregnancy rate were increased using medium added with this cytokine (GM-CSF).[36]

Recommendations

- For IVF laboratories, the use of devices and equipment, including culture media, is a constant decision-making process, which must be made based on several criteria such as availability, user-friendliness, and clinical results, as well as cost/benefit ratio
- End-users must be aware of when changes are being made. It is, therefore, essential that culture media manufacturers notify clinics of all changes being made to their products
- In parallel, the clinics should have a system to validate the products they use in their own laboratory. The results obtained with new media or other methodological changes must be carefully monitored and assessed
- Results regarding the fertilization rate and embryo quality as well as pregnancy, implantation, and live-born baby rates must be carefully recorded and analyzed. Each IVF unit should develop a system for following up also the health of the offspring and this should be one of the crucial quality control indicators.

Regulatory Aspects of ART Culture Media

- European Union (EU) would regulate ART culture media as medical devices (Class III) (Medical Device Directive 93/42/EEC, 1993; MEDDEV2.2/4 January 2012)
- Conformité Européenne marking is a regulatory requirement to show a product is safe, effective, fit for purpose and meets EU regulations
- If the medium contains a medicinal product or human-derived blood product, such as HSA or antibiotics, the Notified Body also must undertake consultation with a Medicines Competent Authority and/or European Medicines Agency.

Vitrification

Background

Vitrification is now the dominant approach for cryopreservation of human oocytes and embryos. The application of vitrification considerably improved the *in vitro* and *in vivo* development of cryopreserved

blastocysts and oocytes and opened new perspectives for extended embryo culture, single blastocyst transfer, blastocyst biopsy, and alternative ways for fertility preservation or oocyte donation, respectively. Cryopreservation protocols have improved the efficiency of ART outcomes in the last few years. However, it remains important to always seek amelioration on cryopreservation protocols and devices to ensure a significant benefit and patients' safety during procedures. We need to find a method that combines a high cooling and warming rate, high survival, and function of cells and tissues and is made in a way that ensures patient safety.[37]

Supporting evidence

Cryodevices

- These are the devices used to store biological cells at low temperature
- In ART, they are used for cryopreserving sperm, testicular tissue, oocyte, embryo, blastocyst, and ovarian cortex.

Types

For most embryologists, the two categories are easily distinguishable. Open systems allow, and closed systems eliminate direct contact between the sample-containing medium and liquid nitrogen. Consequently, from a disease transmission point of view, open systems are hypothetically unsafe, and closed systems are safe. There is also a semiclosed type in practice.

Open system[38]

- Open devices are devices that allow direct contact of the biological sample to be frozen with the cooling solution
- Generally, using open devices, the achieved cooling rates are approximately 20,000°C–24,000°C/min, which favor good vitrification of the sample
- The problem is that being in direct contact with the cooling solution there is a risk of pathogen transmission to the biological sample at the time of freezing and a high-risk of cross-contamination in the common cooling containers.

Closed system[34]

- In such systems, the biological sample is not in connection with the cooling solution at the time of freezing or at the time of storage in the conventional containers
- This prevents contamination by contact and cross-contamination from shared containers
- The main feature is that the cooling rate is much lower with these closed devices
- The closed devices can be locked or sealed in many ways, but most importantly a hermetic seal must be made, preventing entry to the inside and leakage of pathogens to the outside
- Thermo seal, radiofrequency seal, and ultrasound seal are some of the most used systems that ensure that the sample remains free of contamination.

Factors affecting the vitrification process

The principle of vitrification in cryobiology is to eliminate ice formation totally in the medium that contains the sample, in all phases (cooling, storage, and warming) of the procedure. It can be achieved either by increased cooling and warming rates, or increasing concentration of cryoprotectants; in practical situations, both approaches are applied. The higher the cryoprotectant concentration, the lower the cooling rate required and vice versa. As highly concentrated cryoprotectants may cause

toxic and osmotic injury, the preferred strategy is to use the highest possible cooling and warming rates, then to apply the lowest concentration of cryoprotectants that ensures safe ice-free solidification under these circumstances.

Cooling and warming rate

The easiest way to achieve high cooling and warming rates is to use the smallest solution volume and the highest temperature conductivity between the sample-containing medium and the cooling or warming agent, preferably liquid nitrogen for the former purpose.
The requirements and relationships for conditions to achieve satisfactory vitrification in the area of mammalian ART are well displayed in the equation of *Yavin* and *Arav*.

Cooling and warming rate of the vitrification devices			
	Open	Closed	Semi-closed
Cooling rate	High	Comparatively lower	High
Warming rate	High	High	High

Slush nitrogen

- As a cooling agent, this technique uses slush nitrogen, much colder than LN2 (−196°C vs. −210°C) and with the property of avoiding the Leidenfrost effect. When something is submerged in LN2, bubbles rise to the surface through the device, varying the thermal conductivity from the outside into the inside of the device. This does not happen with slush nitrogen. Slush nitrogen is achieved with a vacuum pump in 5–10 min, and it remains slush for a further 5–10 min before returning to liquid. It was shown for oocytes and embryos that increasing the cooling rate could improve survival rates by up to 37%.

Cross-contamination

In reproductive biology, including mammalian and human-assisted reproduction, no disease transmission caused by liquid nitrogen-mediated cross-contamination, or another cryopreservation-related source, has been reported.

	Open	Closed	Semi-closed
Hypothetical risk of cross-contamination	High	Low	High

- No single infection after ART with processed reproductive samples has been reported to date
- Suitability of closed systems for vitrification is scarce compared with the vast amount of published and unpublished excellent results with open systems.

Automated vitrification of embryos

In IVF clinics, the vitrification is conducted manually by highly skilled embryologists. Processing one oocyte/embryo takes the embryologist 15–20 min, depending on the protocols chosen. Due to poor reproducibility and inconsistency across operators, the success and survival rates also vary significantly.[39]
The process of realizing robotic vitrification (RoboVitri) is ongoing and ultimately aims to standardize clinical vitrification from manual operation to fully automated robotic operation.[35]
Tests on mouse embryos demonstrated that the system could perform vitrification with a throughput at least three times that of manual process and a high survival (88.9%) and development rate (93.8%).[35]
Novel automatic vitrification devices are capable of producing high survival rates of oocytes and embryos. Researchers anticipate that as the demand for vitrification of gametes, embryos, and reproductive tissues increases worldwide, the availability of an automated vitrification device will become indispensable for standardization, simplification, and reproducibility of the entire process.[40]

Table 6: Embryo vitrification experimental results			
Method	Success rate (%)	Survival rate (%)	Development rate (%)
Control	N/A	15/15 (100)	14/15 (93.3)
Manual	83.3	11/15 (73.3)	10/11 (90.9)
Robotic	18/20 (90)	16/18 (88.9)	15/16 (93.86)

N/A=Not applicable

Consensus

- Open devices can be used to vitrify embryos as no incidence of cross-contamination is reported so far
- The aim should be to use a system with no chances of cross-contamination
- Closed devices are hermetic, can be used, but need more data to support the use in the laboratory
- Separate storage containers for infective samples should be used
- Automation is experimental but could be the future for vitrification.

Assisted Hatching

Background

Assisted hatching (AH) involves the artificial thinning or breaching of the zona pellucida (ZP) and has been proposed as one technique to improve implantation and pregnancy rates following IVF. Several methods have been employed over the past few years to improve implantation rates in patients undergoing IVF/ICSI. Hatching of the zona was performed mechanically or chemically or by using a laser. All these methods aim to create a hole in the ZP or to thin a part of it, for assisting the embryo in hatching out at the time when the blastocyst expands, and it is ready to implant.[41]

Supporting evidence

Few studies in the literature compared laser with other techniques of AH. In 2002, Hsieh *et al*. compared 85 women aged ≥38 years, whose embryos had laser-AH, with 56 women of the same age group whose embryos had chemical AH. They concluded that laser hatching is more effective than the chemical method in enhancing the implantation and pregnancy rates of women with advanced age. The implantation rate of transferred embryos was significantly improved in the laser group, whereas pregnancy rates showed a tendency to increase, even though the difference did not reach statistical significance.[41]

Recommendation on assisted hatching-LASER

Indication	Consensus
Frozen embryos Frozen blastocysts Thick ZP Advanced maternal age >38 years RIF Poor-quality embryos	No robust evidence to suggest increase in live birth rate following AH. Data collection and evaluation for Indian subpopulation is needed

RIF=Repeated implantation failure, ZP=Zona pellucida, AH=Assisted hatching

Time-Lapse Imaging

Background

Time-lapse is defined as an intervention that comprises undisturbed embryo culture and the prospective use of visual information obtained by time-lapse monitoring (TLM) for embryo evaluation. Time-lapse recording introduces several dynamic morphological parameters for embryo evaluation. However, the data

analysis and comparison between studies are complicated due to diverging nomenclature and definitions of events and their timing. Consensus on how to collect and report data is therefore desirable.[42]

Table 7: Potential advantages and disadvantages of the time-lapse imaging method

Advantages	Disadvantages
Uninterrupted embryo culture	No robust evidence for improvement of primary outcome measures (CPR and LBR), and secondary outcome measures (IR, MR)[43]
Possibility of obtaining developmental data to select or deselect embryos	Standardization of terminology and annotation needed.
Excellent quality control and research tool	No definite concordance with ploidy status of selected embryos
Off-site data analysis	
Patient counseling tool	
Possible increase in IR, CPR and LBR; decrease in MR and TTP	

IR=Implantation rate, CPR=Clinical pregnancy rate, LBR=Live birth rate, MR=Miscarriage rate, TTP=Time to pregnancy

Supporting evidence

Noninferiority as compared to standard embryo assessment

Application of TLM together with embryo-evaluating algorithm was associated with a significantly higher ongoing pregnancy rate, a significantly lower early pregnancy loss and a significantly higher live birth rate. Extremely close agreement (ICC = 0.99) was found for dynamic parameters including the timing of the following: pronuclei breakdown, completion of blastocyst hatching and the appearance and disappearance of the first nucleus after the first division. Observations of cleavage divisions were strongly correlated (ICC >0.8), indicating close agreement. Measurements of the static morphologic parameters, i.e., multi-nucleation and evenness of blastomeres at the 2-cell stage showed fair-to-moderate understanding (ICC ≤0.5).
Review of the literature on morphokinetics shows that the range of timing of events in embryo development may provide additional clues to embryonic potential without the need for unnecessary exposure. There are several retrospective studies evaluating specific cell cycle kinetic parameters evaluated with time-lapse imaging associated with blastocyst formation and/or pregnancy outcomes. Research has suggested that embryos cultured in TLM systems have a higher implantation potential. Recently, a study has used time-lapse imaging to assess embryo quality and evaluation of known implantation data scores and concluded that quality assessment of the embryos was better with TLM than with the conventional method.[44]

Proposed morphokinetic variables

Definitions for the dynamic monitoring of human preimplantation embryo development are given in Figure 1.[45]

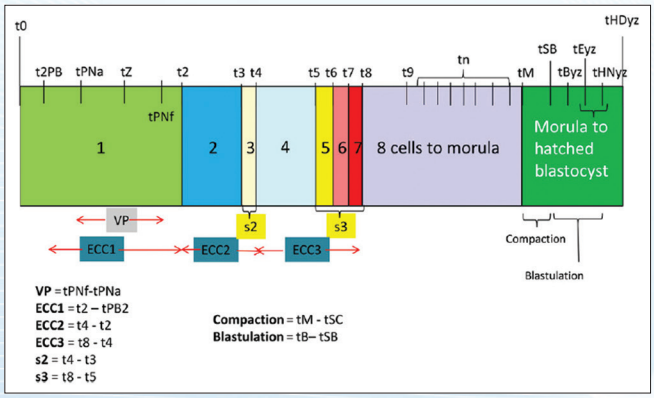

Figure 1: Definitions for the dynamic monitoring of human preimplantation embryo development

Ploidy concordance

Campbell *et al.* showed that embryos with multiple aneuploidies showed delay at the initiation of compaction (tSC; median 85.1 h postinsemination [hpi]; $P = 0.02$) and the time to reach full blastocyst stage (tB; median 110.9 hpi, $P = 0.01$) compared with euploid embryos (tSC median 79.7 hpi, tB median 105.9 hpi).[46] Embryos having single or multiple aneuploidies (median 103.4 hpi, $P = 0.004$ and 101.9 hpi, $P = 0.006$, respectively) had delayed initiation of blastulation compared with euploid embryos (median 95.1 hpi). Morphokinetic parameters should not be used yet as a surrogate for PGT to determine embryo ploidy *in vitro* [Table 5].

Currently, there are numerous examples where adjunct treatments are used in the absence of evidence-based medicine and often at an additional fee. Robust studies are needed to confirm the safety and efficacy of any adjunct treatment or test before they are offered routinely to IVF patients.

Consensus on indications and outcomes

Indication	Consensus
Unexplained infertility	No robust evidence to suggest an increase in LBR though time to pregnancy may decrease
Older age	
Previous failures	
Repeated implantation failure	
Previous poor-quality embryos	
As a marker for embryo ploidy	Not recommended
Outcomes	**Consensus**
Reduced time to pregnancy	Low quality evidence
Improved cumulative pregnancy rate	No robust evidence
Safe to use	Agree
Help in deprioritizing embryos	Agree

LBR=Live birth rate

Consensus on the benefits of time-lapse imaging

- It improves the flexibility of assessment
- It enables documentation and traceability of the laboratory's handling and selection of embryos
- Training of laboratory personnel is recommended
- Validation of the laboratory's embryo assessment is recommended.

Spindle View

Background

The meiotic spindle (MS) of human oocytes in metaphase II (MII) is a temporary dynamic structure consisting of microtubules that are associated with the oocyte cortex and its network of subcortical microfilaments. The MS microtubules are linked to the kinetochores of the chromosomes and participate in segregation during meiosis. It is known that the oocyte MS integrity is essential for chromosome segregation and that it is susceptible to various factors such as ageing, thermal changes, insufficient oxygen supply during culture, and oocyte manipulation. The MS plays a vital role in the oocyte during chromosome alignment and separation at meiosis.

Summary of points	Description
Factors affecting MS	Highly sensitive to physical and chemical change
	Slightest temperature fluctuations
	In-vitro ageing of an egg
	Increased maternal age

Summary of points	Description
Importance of location of MS	The first polar body displacement could lead to potential damage to the MS during oocyte micromanipulation
	Rienzi *et al*. demonstrated that oocytes with spindle deviation of >90° from IPB had lower fertilization rates
	MS dislocation affects embryo development as its position indicates the first cleavage plane
	Spindle view allows the correct orientation of MS with respect to injection needles during ICSI
Advantages of spindle view	No oocyte fixation and staining are required so the spindle can be observed in a noninvasive fashion
	It preserves oocyte viability
	It provides information about oocyte maturation and developmental potential
	Thought to improve implantation rates minimizing multiple pregnancies
	Removing spindles under the polscope can achieve a higher enucleation efficiency rate
Limitations of spindle view	During oocyte handling the influence of temperature and pH on the microtubules of MS can interfere with visualization
	It cannot be used as a noninvasive marker to predict IVF outcome

ICSI=Intra-cytoplasmic sperm injection, MS=Meiotic spindle, IVF=*In-vitro* fertilization, IPB=Irregular polar bodies

Consensus for indications

Indications	Consensus
Frozen oocytes	Could assist in timing of ICSI. Need more evidence
PCOS	Could assist in improving ICSI. Need more evidence
Advanced maternal age	No robust evidence to suggest use improves outcomes
Poor responders	No robust evidence to suggest use improves outcomes

PCOS=Polycystic ovary syndrome, ICSI=Intra-cytoplasmic sperm injection

Training for Embryologist

Why is training for add-ons necessary?

- IThere is growing evidence to suggest that add-ons are being offered to patients, without conclusive evidence to date
- ISpecific add-on treatments are readily available and can be provided in any laboratory scenario
- ITo maintain the transparency about the add-on treatments, they offer, including the potential costs, to ensure their chance of having a baby
- ITo ensure that the validation of the laboratory and staff.

The recommendation for training and process validation: An expert panel survey analysis

The expert panel survey was conducted on 151 respondents; 74.3% of the survey participants recommended imparting the training to embryologists before using the following methods or procedures [Figure 2].

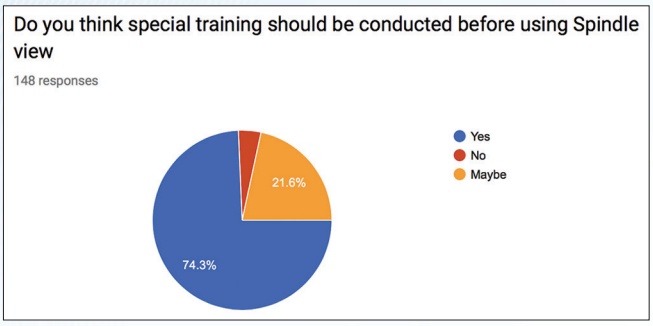

Figure 2: Survey responders in support of training and process validation

Sperm DNA fragmentation

- IAbout 91.4% of respondents believed that embryologists should be trained for sperm DNA fragmentation test
- About 67.7% (100 responded out of 150) respondents believed hands-on training would be beneficial followed by on the job training (33.3%, 50 responded out of 150), workshop/observer course (22.7%, 34 responded out of 150), and then outsource dependency [Figure 3].

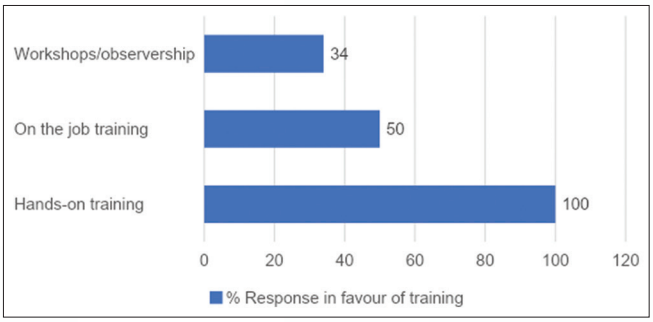

Figure 3: Survey analysis of the recommended training types

Whether training is recommended for sperm selection modalities?

Q.1. Which of the sperm selection modalities requires training?
Based on the survey 79.9 % felt IMSI required training before use, 64.4% felt PICSI required training, 55 and 54.4 % felt microfluidics and. MACS required additional training

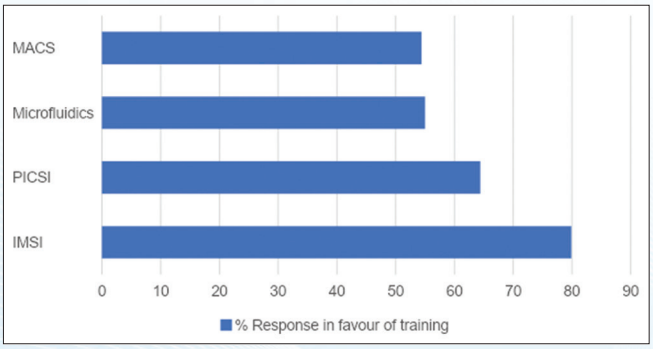

Figure 4: Survey responders in support of training for sperm selection modalities

Q.2a. Which type of training is recommended for sperm selection modalities?
IMSI: Hands-on (61.6%), on the job (23.2%) and workshop/observership (15.2%) training.

Q.2b. Which type of training is recommended for PICSI?
Hands-on course (55.3%), on the job (27.3%), and workshop/observership (17.3%).

Q.2c. Which type of training is recommended for microfluidics/MACS?
Hands-on course (39.2%), workshop/observership (31.1%), and on the job (29.7%)

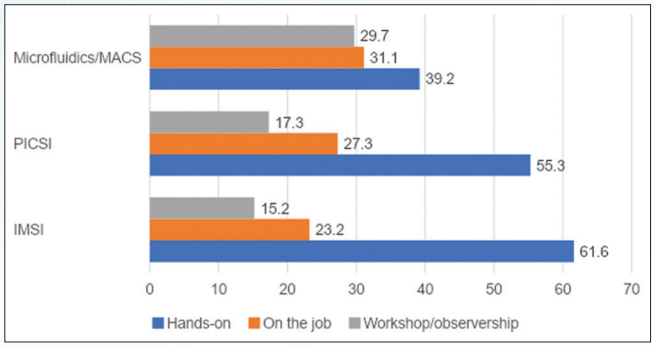

Figure 5: Survey responders in support of training for sperm selection modalities

Whether training is recommended for sperm selection and preparation for surgically retrieved sperm?

Q.3 Which of the following procedures requires training for sperm selection and preparation?
TESE (86.4%), TESA (75.5%), and PESA (49.7%).

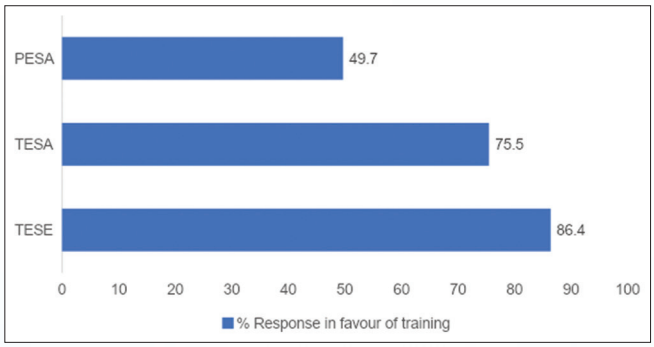

Figure 6a: Survey responders in support of training for surgically retrieved sperm procedures

Q.4 What type of training is recommended?
Hands-on job (75%), hands-on course (50.7%) and workshop/observership (29.1%).

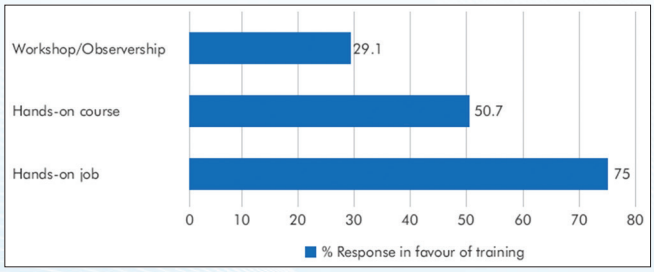

Figure 6b: Survey responders in support of the type of training for surgically retrieved sperm procedures

Q.5a. Do you think a theoretical understanding should be imparted to embryologists before they use an advanced culture media?
Calcium ionophore (73.5%), embryo glue (51.7%), theophylline (51%), and embryogen (47.6%).

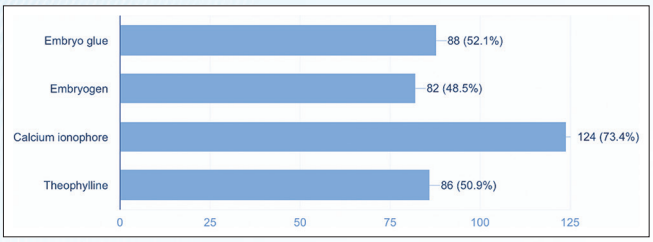

Figure 7: Survey responders in support of training to embryologists before they use an advanced culture media

Q.6a. Do you think specialized hands-on training should be conducted before opting for oocyte vitrification?
Yes (85.4%), maybe (9.9%), and no (negligible response).

Q.6b. Do you think specialized hands-on training should be conducted before opting for blastocyst vitrification?
Yes (77.7%), maybe (14.9%), and no (negligible response).

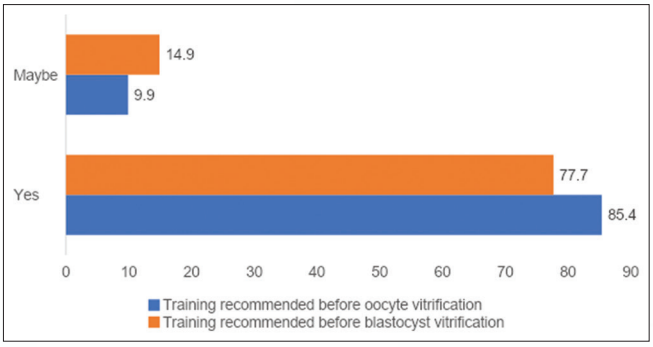

Figure 8: Survey responders in favor of training to embryologists for oocyte and blastocyst vitrification

Assisted hatching

Q.7. Do you think specialized training should be conducted before opting for AH?
Yes (74.7%), maybe (14.7%), and no (10.7%).

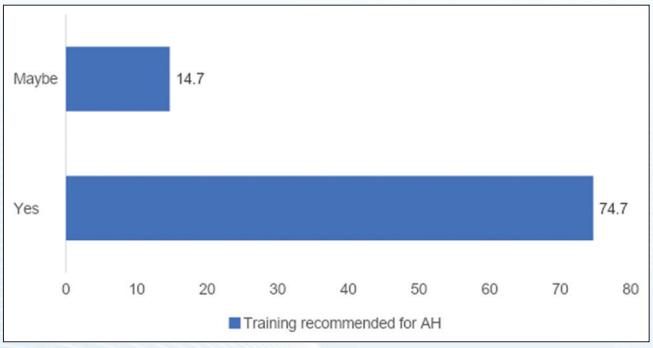

Figure 9: Survey responders in favor of training to embryologists for assisted hatching

Q.8 What type of training do you recommend for AH?
Hands-on (66.7%), on the job (33.3%), and workshop/observership (22.7%).

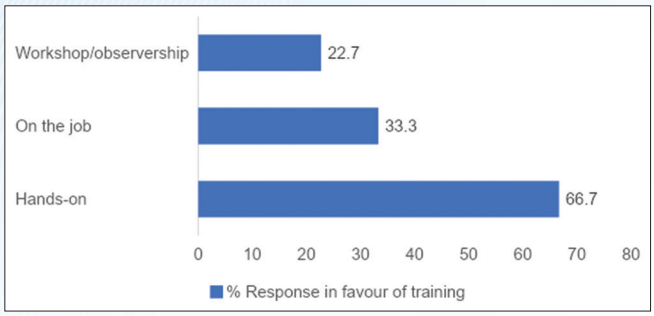

Figure 10: Survey responders in favor of the type of training for assisted hatching

Time-lapse and electronic witnessing

Q.9. Do you think specialized training should be conducted before using time-lapse and electronic witnessing?
Yes, 75.5%, maybe 21.2% and no negligible response.

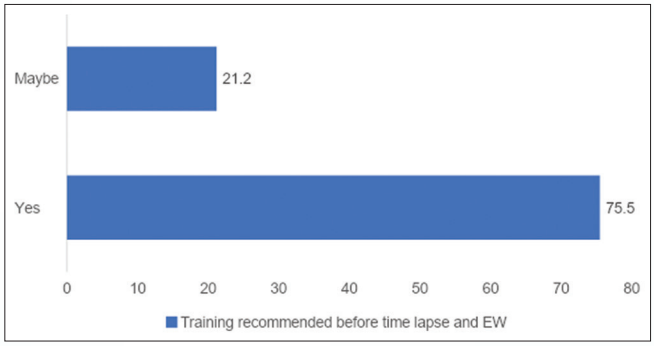

Figure 11: Survey responders in favor of imparting training before using time-lapse and electronic witnessing systems

Q.10. Do you see any harm in utilizing these procedures without training? (multiple choices)
Yes, identification error 30.1%, poor embryo selection 26.7%, more exposure on embryo 24.7%, and unable to utilize the technology 87.7%.

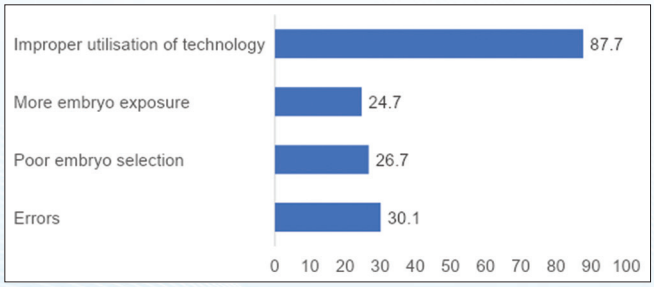

Figure 12: Negative consequences of lack of training

Q.11 Do you think specialized training should be conducted before using spindle view?
Yes, 74.3%, maybe 21.6%, and no (negligible response).

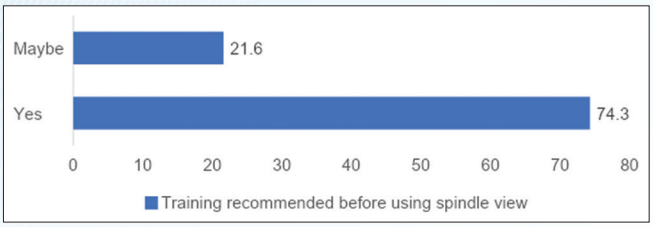

Figure 13: Survey responders in favor of imparting training before using spindle view

Q.12. What are the benefits of additional training and validation? (multiple choices)
Utilize technology to fullest 63.1%, better identification of intended patients 36.2%, better outcomes with the selected technology 73.2%, more confidence in using technology 63.8%, and no benefit 1.3%.

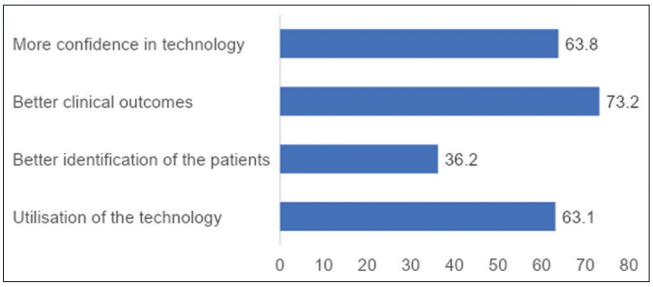

Figure 14: Overall benefits of the training and process validation

Consensus

Group consensus	
Type of training	Hands-on for all add-on's recommended. Duration of training May vary
Who should train	Organizational certified embryologists, clinics or companies
Validation required	Required for specific add-ons (IMSI, oocyte activation, spindle view, time-lapse, assisted hatching)
Number of cases to be observed	Interventional procedure-20 Diagnostic procedures-10
	Regular up gradation of knowledge through CMEs and workshops

IMSI=Intracytoplasmic morphologically selected sperm injection, CME=Continuing medical education

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