



Review article

IVF add-ons: effectiveness, efficiency, safety, and additional costs – A position statement by the SIFES-MR

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ABSTRACT

Objective: To critically appraise IVF add-ons, based on current evidence, international recommendations, and expert consensus from SIFES-MR.

Study design: Consensus developed by a multidisciplinary panel of Italian reproductive medicine experts over two years, based on clinical expertise, revision of guidelines from ESHRE, HFEA, ISAR, and ASRM, and additional more recent references retrieved from the literature. A list of statements was produced and agreed upon the panel using a Delphi consensus workflow. The manuscript was circulated to SIFES-MR executive committee and society members for final approval. Add-ons were categorized by their putative impact on IVF efficacy, efficiency, or safety. Cost justification and patient communication were also considered.

Results: While some add-ons, such as preimplantation genetic testing for aneuploidies (PGT-A) in select patient populations, demonstrate potential clinical benefits, others lack sufficient evidence or pose safety concerns. Several interventions, including endometrial receptivity testing and mitochondrial DNA load measurement, are not currently recommended. Cost considerations and patient counseling are crucial.

Conclusions: The SIFES-MR panel underscores the importance of evidence-based practice in ART. Clear patient communication, fundamental research, and regulatory oversight are essential to ensure IVF add-ons provide real clinical benefits rather than unnecessary financial burdens.

Introduction

Reproductive medicine is not a foolproof science. Depending on maternal age, sperm factor and other relevant confounding variables,

the likelihood of achieving live birth per attempt ranging significantly, from over 50 % to less than 20 %, however, it is never 100 %. Implementing a multicycle strategy is the primary strategy to address the limited success of a single-cycle approach. This involves attempting to

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collect a cumulative number of oocytes tailored to each couple's characteristics, which can maximize their chances of delivering one or more healthy babies. However, this journey is often long and challenging. Our professionals are responsible for providing couples with all the necessary care while striving to achieve this goal with the least possible reproductive risks, using patient-centered, timely, and cost-effective strategies. The inherent nature of reproductive science requires that patient satisfaction and reproductive autonomy are respected. Given that each clinical setting has its strengths and limitations, and considering the variability in costs, local regulations, expertise, social needs, and patient populations, issuing universally applicable guidelines is challenging [1]. Therefore, scientific societies have developed good practice recommendations based on expert opinions, evolving to include randomized controlled trials (RCTs), large retrospective observational studies, registry data, and more recently, big data and real-world evidence. Yet, the non-exact nature of this field complicates counseling, as there are no one-size-fits-all solutions, and different couples may benefit from different strategies to improve their chances of live birth or reduce reproductive risks. The Italian Society of Fertility and Sterility and Reproductive Medicine (SIFES-MR) discussed the use of the "IVF add-ons" in daily practice. In this manuscript, we define IVF add-ons — with slight modifications to the recent ESHRE definition [2] — as *supplementary options available to patients in addition to standard fertility procedures or offered to patients without a clear indication according to national and/or international guidelines, and that may incur in an additional cost*. While many international societies have extensively discussed IVF add-ons, this manuscript aims to summarize the current perspectives on each, critically review them, and provide insights from Italian IVF professionals. The objective is to guide reproductive specialists in making informed decisions regarding the main DOs and DONTs during the couple's journey and to offer criteria for counseling patients about their reproductive choices in any setting, whether private or public.

Material and methods

This position statement on IVF add-ons was developed on behalf of SIFES-MR by a selected group of its members. The group was appointed by the Executive Committee of SIFES-MR to include Italian reproductive clinicians, embryologists, and researchers chosen among active members of the society, who had previously investigated the topics covered by this consensus. Participants represented IVF centers from the south, center and north of Italy, spanning both private institution and public/university-affiliated facilities. All panel members declared no conflict of interest or commercial ties related to the add-ons discussed in this document. The group met in person six times over a two-year period. Twenty-eight consensus statements were developed during the first four meetings, following a comprehensive review of the ESHRE [2], HFEA (<https://www.hfea.gov.uk/treatments/treatment-add-ons/#treatment-add-ons>), ISAR [3], and ASRM (<https://www.asrm.org/practice-guidance/practice-committee-documents/>) guidelines and good practice recommendations. Additional relevant literature published up to 2024 was incorporated when deemed pertinent, following a systematic search using specific keywords for each add-on. Each expert contributed to the add-ons corresponding to their field(s) of expertise. The 28 consensus statements were circulated via Google Forms, and panel members were asked to rate their agreement using a 10-point Likert Scale: 1 = absolutely disagree, 10 = absolutely agree. All statements received agreement scores of 7–10 from over 70 % of the group. The manuscript was drafted during the fifth in-person meeting and subsequently circulated to the SIFES-MR executive committee and members' mailing list for internal review over a two-months period. During the sixth in-person meeting the manuscript was finalized and then submitted for peer-review.

Among the add-ons discussed in some guidelines, we did not address the following:

1. Double stimulation in the same ovarian cycle (DuoStim), as it was considered an ovarian stimulation protocol compatible with a multicycle counseling approach for advanced maternal age (AMA) and/or poor prognosis couples.
2. Indomethacin and sildenafil, as they have never undergone clinical trials and should not be considered for clinical use outside of the research context.
3. Artificial Intelligence (AI)-based gamete/embryo selection tools, as these tools still require more clinical appraisals.
4. Complementary or alternative medicine and post-IVF add-ons (e.g., non-invasive prenatal testing), as they fall outside of this panel's expertise.
5. Elective freeze-all, because according to Italian regulations, this is permitted only with a clear indication, such as for PGT, risk of OHSS and/or for endometrial inadequacy.
6. Mitochondrial replacement therapy, as current evidence suggests it harms oocytes/embryos.
7. Clinical IVM, because it is regarded as a therapy for specific indications, such as fertility preservation.
8. Artificial sperm activation, because to our knowledge, it is not conducted in Italy.
9. Surgical retrieval of sperm, as this practice is considered an option solely for non-obstructive azoospermic men.
10. Technologies aimed at standardizing, automating, and/or enhancing safety in IVF clinics (e.g., electronic witnessing systems), because they were considered functional upgrades for clinics with no direct implications for individual couples.

Notably, PGT-A was evaluated by this group of experts solely for its purpose of reporting full chromosome uniform aneuploidies. The panel determined that segmental and/or mosaic aneuploidies are still influenced by technical and biological inconsistencies that currently jeopardize its clinical significance.

Add-ONS: short description, summary of the current recommendations, and SIFES-MR position

Table 1 provides a summary of the add-ons discussed by this panel throughout the IVF journey. It includes (i) the theoretical expected improvements in outcomes associated with their use, (ii) ESHRE, HFEA, ISAR, and ASRM general view on each add-on, (iii) SIFES-MR view based on the different patient populations that may benefit from each add-on, (iv) SIFES-MR opinion regarding the appropriateness of an additional cost to the patients associated with each add-on's utilization.

The outcomes were categorized as higher IVF efficacy, higher IVF efficiency, or reduced neonatal morbidity. Efficacy was defined by two main outcomes: improved embryological outcomes (e.g., higher fertilization rate), and higher cumulative live birth rate (CLBR) per oocyte pick-up (OPU). Efficiency was defined by two main outcomes: a higher live birth rate (LBR) per (first) embryo transfer (ET) and a lower miscarriage rate (MR) per clinical pregnancy.

Pre-IVF

Karyotype analysis

Constitutional aberrant karyotypes can negatively impact both female and male fertility. The prevalence of chromosomal abnormalities in a population of infertile Italian couples ranged from 0.9 % to 1.92 % in females and from 1.28 % to 2.02 % in males [4,5]. In couples who experienced multiple miscarriages, this rate largely increases of 5 to 10-fold [6]. A review of the literature reported that, among couples undergoing ART, the prevalence of chromosomal abnormalities ranges from 1.2 % to 2.1 % in females and from 1.1 % to 6.1 % in males [7]. Depending on the study, the prevalence of chromosomal abnormalities observed in couples undergoing IVF was comparable to or only slightly higher than that found in the general population. However, in specific

Table 1

Summary of the add-ons discussed by this panel clustered according to IVF stage of putative application, of the recommendations issued by ESHRE, ASRM, ISAR and SIFES-MR, of the populations of IVF patients that may benefit from these add-ons, and of the theoretical expected outcome(s) in terms of improved IVF efficacy and/or efficiency, or lower neonatal morbidity. √, positive opinion; ⊗, negative opinion; ±, more clinical data are worth being produced;?, to be tested in the research context; =, no opinion. CLBR, cumulative live birth rate; OPU, oocyte pick up; LBR, live birth rate; ET, embryo transfer; MR, miscarriage rate; DFI, DNA fragmentation index; OAT, oligoasthenoteratozoospermia; CS, carrier screening; OS, ovarian stimulation; GH, growth hormone; DHEA, dehydroepiandrosterone; POR, poor ovarian reserve; POI, premature ovarian insufficiency; MACS, Magnetic Activated Cell Sorting; SMF, severe male factor; NOA, non-obstructive azoospermia; IVM, in vitro maturation; IMSI, Intracytoplasmic injection of morphologically selected spermatozoa; PICSI, physiological ICSI; AOA, artificial oocyte activation; TFF, total fertilization failure; GFs, growth factors; PGT, preimplantation genetic testing (–A for aneuploidies; –M for monogenic conditions); AMA, advanced maternal age; RPL, recurrent pregnancy loss; mtDNA, mitochondrial DNA; niPGT, non-invasive PGT; PRP, platelet rich plasma; G-CSF, granulocyte colony stimulating factor; hCG, human chorionic gonadotrophin.

IVF stage	Add-on	Theoretical Expected outcome(s)				Scientific societies recommendations for all patients (unless specified)	SIFES-MR view	Additional cost to patients justified	
		IVF Efficacy		IVF Efficiency					Lower neonatal morbidity
		Better Embryological outcomes	Higher CLBR per OPU	Higher LBR per (first) ET	Lower MR per clinical pregnancy				
Pre-IVF	Karyotype analysis			x	x	x	ESHRE: = HFEA: = ISAR: = ASRM: = SIFES-MR: For all patients ⊗ For RPL and multiple IVF failures ± For NOA and severe oligozoospermia √	yes	
	(expanded)CS					x	ESHRE: = HFEA: = ISAR: = ASRM: = SIFES-MR: For all patients ±	yes	
IVF OS	GH, testosterone, or DHEA co-treatments		x				ESHRE: ⊗ HFEA: ⊗ ISAR: ⊗ ASRM: = SIFES-MR: For all patients ⊗ Testosterone in POR according to Bologna and/or Poseidon criteria ±	yes	
	Ovarian re-activation		x				ESHRE: ⊗ HFEA: = ISAR: = ASRM: = SIFES-MR: For all patients ⊗ <i>In POR according to Bologna and/or Poseidon criteria & POI</i> ?	—	
IVF Sperm preparation and selection	MACS, Microfluidics	x	x				ESHRE: = HFEA: = ISAR: = ASRM: = SIFES-MR: For all patients ⊗ In high sperm DFI, RPL ?	—	
IVF Oocyte retrieval	Rescue-IVM		x				ESHRE: ⊗ HFEA: = ISAR: = ASRM: = SIFES-MR: For all patients ⊗ In cancer patients √ Poor prognosis ?	no	
IVF Insemination	ICSI in non-SMF	x	x				ESHRE: ⊗ HFEA: ⊗ SIFES-MR: For all patients	no	

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Table 1 (continued)

IVF stage	Add-on	Theoretical Expected outcome(s)				Scientific societies recommendations for all patients (unless specified)	SIFES-MR view	Additional cost to patients justified	
		IVF Efficacy		IVF Efficiency					Lower neonatal morbidity
		Better Embryological outcomes	Higher CLBR per OPU	Higher LBR per (first) ET	Lower MR per clinical pregnancy				
						ISAR: = ASRM: ±	⊗ In PGT, IVM, cryopreserved oocytes, or previous low fertilization rates with conventional IVF √		
	IMSI	x	x			ESHRE: ± HFEA: ± ISAR: ± ASRM: ±	SIFES-MR: For all patients ⊗	—	
	PICSI	x	x			ESHRE: ⊗ HFEA: = ISAR: = ASRM: ±	SIFES-MR: For all patients ⊗	—	
	AOA	x	x			ESHRE: ± HFEA: = ISAR: = ASRM: ±	SIFES-MR: For all patients ⊗ For previous TFF ?	—	
	Rescue-ICSI	x	x			ESHRE: = HFEA: = ISAR: = ASRM: ±	SIFES-MR: For all patients ⊗ For TFF ?	—	
IVF Embryo culture	GFs-supplemented culture media	x	x			ESHRE: ⊗ HFEA: = ISAR: ± ASRM: =	SIFES-MR: For all patients ⊗ For all patients ?	—	
	Undisturbed culture in time lapse incubators	x	x			ESHRE: = HFEA: = ISAR: ⊗ ASRM: =	SIFES-MR: For all patients ±	no	
IVF Embryo assessment	Embryo morphodynamic assessment			x		ESHRE: ⊗ HFEA: = ISAR: ± ASRM: =	SIFES-MR: For all patients ±	no	
	PGT-A			x	x	x	ESHRE: ⊗ HFEA: <i>To increase the CLBR</i> ⊗ <i>To decrease the MR</i> √ ISAR: = ASRM: For all IVF patients, SMF-only or APA-only, oocyte donor	SIFES-MR: For all patients ± AMA, RPL √	yes

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Table 1 (continued)

IVF stage	Add-on	Theoretical Expected outcome(s)				Scientific societies recommendations for all patients (unless specified)	SIFES-MR view	Additional cost to patients justified	
		IVF Efficacy		IVF Efficiency					Lower neonatal morbidity
		Better Embryological outcomes	Higher CLBR per OPU	Higher LBR per (first) ET	Lower MR per clinical pregnancy				
							cycles ⊗ PGT-A in PGT-M cycles ± AMA, especially with good ovarian reserve ✓		
	mtDNA load measurement			x			ESHRE: ⊗ HFEA: = ISAR: = ASRM: =	SIFES-MR: For all PGT-A patients ⊗	—
	niPGT			x			ESHRE: ⊗ HFEA: = ISAR: = ASRM: =	SIFES-MR: For all patients ⊗ For all patients ?	—
IVF Preparation to ET	Hysteroscopy		x	x	x		ESHRE: Multiple implantation failures ± HFEA: = ISAR: ± ASRM: ✓	SIFES-MR: For all patients ⊗ Multiple implantation failures ± Uterine morphological abnormalities in TVS ✓	yes
	Endometritis testing + treatment		x	x	x		ESHRE: Multiple implantation failures ± HFEA: = ISAR: = ASRM: =	SIFES-MR: For all patients ⊗ Multiple implantation failures ±	yes
	Endometrial scratch		x	x			ESHRE: ⊗ HFEA: ± ISAR: = ASRM: =	SIFES-MR: For all patients ⊗	—
	Endometrial receptivity screening		x	x			ESHRE: ⊗ HFEA: ⊗ ISAR: = ASRM: =	SIFES-MR: For all patients ⊗	—
	Microbiome analysis		x	x	x		ESHRE: ⊗ HFEA: = ISAR: = ASRM: =	SIFES-MR: For all patients ⊗ For all patients ?	—
	Immunological tests + therapies		x	x	x		ESHRE: ⊗ HFEA: ±	SIFES-MR: For all patients	yes

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Table 1 (continued)

IVF stage	Add-on	Theoretical Expected outcome(s)				Scientific societies recommendations for all patients (unless specified)	SIFES-MR view	Additional cost to patients justified	
		IVF Efficacy		IVF Efficiency					Lower neonatal morbidity
		Better Embryological outcomes	Higher CLBR per OPU	Higher LBR per (first) ET	Lower MR per clinical pregnancy				
						ISAR: = ASRM: ±	⊗ Multiple implantation failures, RPL ±		
IVF ET	Assisted hatching		x	x		ESHRE: ⊗ HFEA: ± ISAR: ± ASRM: ⊗	SIFES-MR: For all patients ⊗	—	
	Hyaluronic acid enriched embryo transfer media		x	x		ESHRE: ✓ HFEA: ± ISAR: ✓ ASRM: =	SIFES-MR: For all patients ±	yes	
	Steroids adjuncts		x		x	ESHRE: ⊗ HFEA: ⊗ ISAR: = ASRM: ⊗	SIFES-MR: For all patients ⊗ Autoimmune conditions requiring steroid treatment ✓	yes	
	PRP intrauterine administration		x	x		ESHRE: ⊗ HFEA: = ISAR: = ASRM: =	SIFES-MR: For all patients ⊗ For multiple implantation failures, thin endometrium ±	yes	
	hCG intrauterine injection		x	x		ESHRE: ⊗ HFEA: = ISAR: = ASRM: =	SIFES-MR: For all patients ⊗ Multiple implantation failures ?	—	
	G-CSF intrauterine or subcutaneous administration		x	x		ESHRE: ⊗ HFEA: = ISAR: = ASRM: ⊗	SIFES-MR: For all patients ⊗ Multiple implantation failures ?	—	

patient categories, the prevalence was significantly higher compared to the general infertile population. For instance, chromosomal abnormalities were observed in 3.8 % to 18.4 % of males with severe oligozoospermia and in 14.7 % to 35 % of males with azoospermia [7]. Unfortunately, there are no studies evaluating the cost-effectiveness of routine karyotyping for infertile couples undergoing ART.

SIFES-MR Recommendation:

Universal karyotype screening is not recommended before IVF. However, karyotype screening may be considered in cases of recurrent pregnancy loss (RPL) and multiple IVF failures defined according to ESHRE [8,9]. For severe oligozoospermia or non-obstructive azoospermia, male karyotyping should be advised. An additional cost is justified.

(expanded) carrier screening

Expanded carrier screening (ECS) during the preconception period is currently considered the most effective way to identify 'at risk' couples (ARCs) of passing inheritable disorders to their offspring. This includes cases where both partners carry an autosomal recessive pathogenic variant on the same gene or where the female partner is a heterozygote for a recessive X-linked condition. ECS allows couples to make fully informed reproductive choices, such as: (i) undergoing IVF with pre-implantation genetic testing for monogenic conditions (PGT-M), (ii) arranging early prenatal diagnostic testing, (iii) opting for IVF with donor gametes, (iv) choosing adoption, (v) remaining childless, or (vi) taking no action.

A recent cost-effectiveness analysis compared three ECS panels—American College of Medical Genetics and Genomics (ACMG) Tier 1 screening, 'Focused Screening' for 15 severe and highly penetrant conditions, and ACMG Tier 3 screening—against no screening, the current healthcare model in Italy for couples seeking pregnancy without increased personal or familial genetic risk. The model-based analysis suggested that, from a healthcare system perspective, all three ECS panels would be more effective and less costly compared to no screening [10]. However, before implementing ECS in the diagnostic work-up for all couples selected for IVF, several structural and organizational changes in the Italian healthcare system would be necessary. Firstly, the shortage of professionals trained in clinical genetic counseling may create an operational bottleneck. Additionally, public health services would need to acquire advanced technologies and professionals skilled in variant interpretation and reporting for recessive disorders, at least until these processes can be automated.

Moreover, ethical considerations and proportionality are crucial issues that require careful examination. Ethical objections to preconception ECS include: the 'disability rights critique,' which argues that ECS may convey a discriminatory message towards individuals with certain disorders; the 'medicalization critique,' which suggests that ECS represents another form of reproductive medicalization; concerns about eugenics; and the potential for social stigmatization and discrimination against ARCs [11].

SIFES-MR Recommendation:

In line with the principle of reproductive autonomy, SIFES-MR suggests that ECS could be considered in couples indicated to IVF after adequate counseling about the test and its implications. An additional cost is justified.

IVF stage: ovarian stimulation

Growth hormone, testosterone, or dehydroepiandrosterone co-treatments

In recent years, the use of various adjunct therapies in IVF treatments, particularly for patients with poor reproductive prognosis, including those with diminished ovarian reserve, has garnered significant attention. Co-treatments with growth hormone (GH), testosterone, and dehydroepiandrosterone (DHEA) have been proposed as potential strategies to improve ovarian response and overall IVF outcomes. Although there is a biological rationale supporting the use of these adjunct therapies, the clinical evidence remains inconclusive, and it is still under debate [12–15]. To this regards GH may enhance follicular

development improving granulosa cell function and increasing the sensitivity of ovarian follicles to gonadotropins. Testosterone, administered transdermally or via injections, has been hypothesized to increase ovarian androgen levels and to augment FSH sensitivity thereby enhancing the recruitment of follicles and improving response to ovarian stimulation. However, a RCT showed that testosterone pre-treatment prior to IVF does not increase clinical pregnancy rates in poor ovarian responders [16]. Finally, DHEA, an endogenous precursor to androgens, has been proposed to enhance ovarian function by increasing intraovarian androgens, potentially improving follicular recruitment and oocyte quality.

All the scientific societies like ESHRE, HFEA and ISAR have provided guidance on the use of adjuncts in the context of fertility treatments showing that these strategies should not be considered standard practice due to the limited, mixed evidence [2,3] (<https://www.hfea.gov.uk/media/4gsj3ujt/2022-06-06-scaac-meeting-papers.pdf>). Indeed, no adjuncts were recommended for daily ovarian stimulation, especially due to inconclusive evidence of their benefits.

A recent Cochrane review supports the use of testosterone as an adjuvant to OS in poor responder patients [17], thus inciting future appraisals.

SIFES-MR Recommendation:

Further research and large-scale clinical trials are needed to determine the efficacy and safety of GH and DHEA in ovarian stimulation, until then, their use is not recommended. In poor ovarian reserve patients according to the Poseidon criteria (group 3 and 4) [18–20], testosterone use seems beneficial and deserves further clinical appraisal. In these cases, an additional cost might be justified.

Ovarian re-activation

Intraovarian injection of platelet-rich plasma (PRP) has been proposed for ovarian reactivation in women with diminished ovarian reserve (DOR) or premature ovarian insufficiency (POI), as platelets release several biologically active factors that are crucial for modulating folliculogenesis. Although mechanistic experimental studies are limited, preclinical trials were conducted. In these trials, PRP's effect on primordial follicles, isolated from healthy deceased women and embedded in a PRP-enriched 3D matrix (previously activated with thrombin), demonstrated follicle growth after a 10-day in vitro culture [21]. Following these results, this approach has been recently tested in DOR, POI and post- or peri-menopausal patients [22]. However, protocols vary across studies, with differences in applied volumes, activation techniques, and some studies omitting PRP activation altogether. This variation could explain the heterogeneity of results even among populations with similar characteristics. PRP injection has been reported to improve both the number and quality of retrieved oocytes, leading to a larger number of better-quality embryo cohorts [23], and different retrospective studies in POR women have shown promising results, particularly regarding improved ovarian reserve biomarkers, especially in the months following PRP infusion [24–26]. The largest prospective clinical trial to date, which included 510 POR women defined according to the Poseidon criteria, showed promising results [27]. Similar results were achieved in POI women [27], a patient population characterized by amenorrhea or oligomenorrhea, high FSH concentrations, and the absence of antral follicles that can be stimulated to grow with exogenous gonadotropins. The only RCT to date conducted in women younger than 38 years with history of two or more prior cycles and less than 3 oocytes retrieved, instead, reported no benefit of PRP ovarian injection [28]. In a murine model, the use of intraovarian PRP seems to improve the quality of the blastocyst, however these data are not still demonstrated in humans [29]. Recently, the infusion of human bone marrow-derived stem cells (BMDSC) has been proposed to promote follicular growth in both humans and mice by increasing ovarian vascularization, stimulating stromal cell proliferation, and reducing cell death in immunosuppressed animals with conditions mimicking POR and POI. This treatment facilitated fertility restoration and led to natural conception

[30]. Although few data are supporting this type of treatment for this reason it should not be used except for experimental purposes in research protocols.

ESHRE is the only scientific society commenting on ovarian reactivation techniques. The panel of experts did not support them due to the lack of clinical evidence from RCTs [2].

SIFES-MR Recommendation:

Despite preliminary promising data, there are significant concerns about the effectiveness of ovarian reactivation protocols. Therefore, PRP should not be recommended outside of strict research protocols for infertile women, particularly those with DOR or POI. Several RCTs are ongoing or in the pipeline, and it is advisable to await their results before making further recommendations. At present, an additional cost is not justified.

IVF Stage: IVF – Sperm preparation and selection

MACS, Microfluidics

Semen quality is crucial for achieving pregnancy, as it impacts both fertilization and subsequent embryo development. In case of high DNA Fragmentation Index (DFI), techniques such as Magnetic Activated Cell Sorting (MACS) or Microfluidics Sperm Sorting (MF) can help select spermatozoa with lower fragmentation. MACS is an immunomagnetic technique that should select more competent spermatozoa by removing those showing signs of cell damage or apoptosis. Damaged sperm are identified through membrane markers and isolated [31]. MF sperm selection techniques, instead, should isolate high-quality spermatozoa using laminar flow, mechanical factors, chemical factors, and temperature gradients. These systems leverage microscale fluid dynamic properties to efficiently manage the movement of motile spermatozoa [32].

SIFES-MR Recommendation:

MACS and MF have not been reported beneficial for all patients. They might reduce the DFI and improve IVF outcomes in RPL patients. Yet, this must be tested in the research context, as current evidence is too limited to support these techniques clinically. Therefore, an additional cost is not justified yet.

IVF Stage: IVF – Oocyte retrieval

Rescue-IVM

Approximately 10–20 % of oocytes retrieved after ovarian stimulation are immature, arrested at the germinal-vesicle (GV) or metaphase-I (MI) stages. The underlying causes of this immaturity are unclear but may include a lack of synchrony between follicular cells and oocytes, premature retrieval timing, aspiration of small follicles, or a poor response to ovarian stimulation. These immature oocytes are typically considered unsuitable for reproductive purposes and generally discarded. However, they can be kept in vitro for up to 24 h and inseminated if they mature to the metaphase-II stage, a strategy known as rescue in vitro maturation (rescue-IVM). Despite this potential, rescue-IVM is not routinely practiced in IVF clinics due to concerns based on historical data. These studies report meiotic defects, limited competence, and questionable safety associated with rescue-IVM [33]. Recently, though, several research groups worldwide have revisited the discussion on rescue-IVM. Immature oocytes that mature within 2–8 h after OPU demonstrate lower blastocyst development compared to sibling metaphase-II oocytes, but no additional impact on euploidy and implantation. Conversely, GV or MI-oocytes maturing overnight showed a significantly impaired competence [34–36]. Among international documents, only ESHRE included rescue-IVM as a potential add-on, acknowledging the lack of established effectiveness, procedural reliability, and long-term safety data for this practice [2]. More recently, ESHRE and Alpha updated the Istanbul consensus claiming that “due to their lower developmental potential, immature oocytes could be considered in case of poor prognosis individuals/couples and/ or when alternatives are not available” [37].

SIFES-MR Recommendation:

Rescue-IVM is not advisable as a routine IVF protocol. Conversely, it should be conducted in cancer patients undergoing emergency fertility preservation, without incurring an additional cost. This practice warrants more research in poor prognosis patients who collect none or a limited number of mature oocytes, particularly if this occurs across multiple attempts. However, statistically sound criteria should be developed to guide clinical decisions and outline clear protocols.

IVF Stage: IVF – insemination

ICSI in non-severe male factor

ICSI was introduced to improve fertilization and pregnancy rates in case of severe male factor (SMF) infertility [38]. Despite a stable incidence of male factor infertility, the use of ICSI has progressively increased accounting for over 70 % of all IVF cycles in 2019 [39]. While the routine use of ICSI despite normal sperm quality is required for PGT, IVM, and vitrified oocytes, and is advisable in case of previous low fertilization rate with conventional-IVF, its adoption for unexplained infertility, AMA, poor oocyte quality, and low oocyte yield remains controversial [40–43]. Moreover, ICSI remains operator-dependent, more expensive, and more time-consuming than conventional-IVF [43,44].

ESHRE and HFEA did not support the routine use of ICSI in non-SMF infertility [2]. Similarly, ASRM claims ICSI should be used only in selected cases [45].

SIFES-MR Recommendation:

ICSI is not superior to conventional-IVF in non-SMF infertility. However, ICSI is required for PGT-A, IVM, and cryopreserved oocytes, and it is advisable in cases of previous low fertilization with conventional-IVF. ICSI should not incur an additional cost.

Intracytoplasmic morphologic sperm injection (IMSI)

The selection of spermatozoa for microinjection during ICSI depends primarily on motility and morphological visualization, that is closely linked to the resolution of the available microscope [46]. Intracytoplasmic injection of morphologically selected spermatozoa (IMSI) enables a more detailed evaluation of sperm structure using a specialized lens with very high magnification. However, there is no consensus on the clinical value of IMSI, and various studies report contradictory results [46–50].

SIFES-MR Recommendation:

IMSI use and an additional cost are not justified as no improvement in clinical outcomes has been reported to date.

Physiological-ICSI (PICSI)

Physiological-ICSI (PICSI) involves selecting capacitated spermatozoa based on their maturity, which is indirectly indicated by their ability to bind with hyaluronic acid (HA), a component of the extracellular matrix surrounding the oocyte-cumulus complex [51]. Using HA binding for sperm selection can help reduce the genetic risks of chromosomal aberrations in the offspring. PICSI has been suggested for cases of SMF infertility or high sperm DFI [52]. To date, several studies have been conducted, with many indicating no statistically significant difference between PICSI and conventional ICSI [53].

SIFES-MR Recommendation:

The evidence to support PICSI in any patient population is scarce.

Artificial oocyte activation (AOA)

Total failure of fertilization (TFF) occurs in 1–3 % of IVF cycles. TFF is associated with difficulties in sperm penetration of the zona pellucida (ZP) and deficits in oocyte activation. Embryo development begins with and is regulated by a process known as oocyte activation, which is primarily controlled by intracellular calcium release. Mature oocytes remain arrested at the metaphase-II stage until sperm entry triggers the completion of meiosis, fusion of gametes, extrusion of the second polar body, and formation of the two-pronuclei (2PN). Increasing Ca^{2+} levels

during fertilization are essential for oocyte's meiosis resumption [54]. Artificial oocyte activation (AOA) is an effective method to prevent TFF in IVF cycles. This technique involves using a calcium ionophore to induce calcium oscillations in the oocytes, thereby initiating the fertilization process [55]. However, its safety remains a matter of concern as overexposure to ionophores may induce cytotoxic effects, disrupt cell homeostasis, and potentially cause epigenetic modifications with long-term adverse effects [56,57].

SIFES-MR Recommendation:

Despite some encouraging results, AOA cannot be considered an established protocol. Therefore, further appraisal in the research context is advisable in case of previous TFF.

Rescue-ICSI

Rescue-ICSI was first described 30 years ago [58] and entails an ICSI procedure performed 18–24 h after conventional-IVF on oocytes that show no sign of fertilization. Rescue-ICSI results in reduced clinical outcomes, but the earlier it is conducted the better the results, with pregnancy rates as high as 50 % in some cases [59]. Controversies, though, exist regarding its safety, especially regarding higher risks of chromosomal abnormalities in the embryos [60–62]. More evidence needs to be produced. Although most scientific societies were silent on the matter, a systematic review from ASRM concluded that rescue-ICSI in cases of TFF can lead to the birth of healthy babies despite the low pregnancy rates [63]. Evidence recently confirmed by an updated systematic review [64].

SIFES-MR Recommendation:

Rescue-ICSI cannot be considered an established protocol, and further appraisal in the research context is advisable in case of previous TFF.

IVF Stage: IVF – Embryo culture

Growth Factors-supplemented culture media

Growth factors (GFs)-supplemented embryo culture media have been explored in IVF to enhance embryo development and improve the chances of a live birth. Over the years, various GFs – natural proteins that regulate cell growth, differentiation, and function – have been employed. The goal is to replicate the complex microenvironment of the female reproductive tract by incorporating factors such as epidermal growth factor (EGF), insulin-like growth factor (IGF), and others. These GFs are designed to support essential cellular processes like proliferation, metabolism, and signaling, all of which are crucial for embryo development and maturation outside the womb. Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been the most extensively studied. Overall, although no adverse outcomes have been reported [65], the data to date do not unanimously support its supplementation to the culture media [65].

SIFES-MR Recommendation:

The supplementation of embryo culture media with GFs should be still studied in the research context.

Undisturbed culture in time lapse incubators

The use of time-lapse incubators to monitor preimplantation development without disrupting embryo culture may help minimize fluctuations in temperature, pH, and osmolarity, potentially improving embryological outcomes. However, the extent of this theoretical improvement may not be clinically significant for individual couples [66]. Furthermore, designing a study to investigate this would likely require a large sample size, with findings applicable only to the specific time-lapse and standard incubators tested, as well as the IVF laboratory management and practices in place during the study. Multiple variables that can impact the outcomes must also be considered, such as dry versus humidified environments, the type of standard incubator, the culture media used, the volume of culture drops, and the frequency of embryo assessments during standard culture. In 2020, the ESHRE working group provided a comprehensive overview of time-lapse

technology (TLT), detailing its advantages and disadvantages, along with well-balanced good practice recommendations for its use in IVF [67].

SIFES-MR Recommendation:

Currently, there is insufficient evidence to state that an undisturbed culture environment achieved through time-lapse incubators improves embryological and, consequently, clinical outcomes in IVF. More clinical studies are particularly needed in poor prognosis patients, such as couples with SMF infertility and/or few oocytes collected. If undisturbed culture is reported safer than standard incubation, though, the patients should incur in no additional costs for its utilization.

IVF Stage: Embryo assessment

Embryo morphodynamic assessment

Embryo morphodynamic assessment using TLT has expanded the available information for characterizing preimplantation development by combining timing data (e.g., time to reach specific stages such as n-cell, morula, and blastocyst formation) with qualitative observations (e.g., early embryo cleavage patterns, cell exclusion/extrusion, and blastocyst collapse and re-expansion) [67,68]. From a scientific perspective, a wide range of new features can be leveraged to complement static morphology and enhance non-invasive embryo assessments. AI-powered software have already been developed, commercialized, and implemented in some IVF clinics [69,70]. These tools automate annotations, reduce the technical workload of operators, make embryo assessment more objective and reproducible, and help standardize the process across clinics. However, there is still limited evidence to support that embryo selection based on morphodynamic parameters, with or without AI, improves outcomes [2,67,71,72].

The ASRM did not support nor discourage the use of embryo morphodynamic assessment. In contrast, ESHRE has advised against it for improving clinical outcomes [2]. ISAR advocated for more evidence [3]. HFEA declared that no clinical effect derives from its use (<https://www.hfea.gov.uk/treatments/treatment-add-ons/#treatment-add-ons>).

SIFES-MR Recommendation:

TLT implementation in IVF laboratories offers numerous advantages for research, education, training, innovation, standardization, workflow improvements, and quality control. However, these improvements primarily benefit the management of the IVF clinic and should not result in additional costs for couples whose embryos are cultured in time-lapse incubators, especially in the absence of clear clinical benefits.

Preimplantation genetic testing for aneuploidies (PGT-A)

PGT-A aims to distinguish aneuploid from euploid embryos within each cohort produced during an IVF cycle. Based on the diagnosis, aneuploid embryos are excluded from transfer, preventing their reproductive consequences. A common misconception is that PGT-A increases the chance of live birth per cycle, but PGT-A is not a therapeutic intervention. The main benefits of PGT-A are increasing the chance of live birth per transfer (if euploid blastocysts are obtained), reducing the risk of miscarriage, and minimizing the risk of chromosomal syndromes in newborns. Its initial implementation in the 1990 s was challenging, largely due to the use of a harmful biopsy protocol that involved removing a single blastomere from a cleavage-stage embryo, followed by FISH analysis, which assessed only 9 chromosomes [73]. This raised serious concerns about the true value of the technology. However, over the years, molecular techniques have significantly advanced. Currently, 24-chromosome testing technologies are used in daily practice and trophectoderm biopsy at the blastocyst stage has become the gold standard. This approach has been proven safe and reliable when performed by expert operators [73–75].

Following a 2015 paper [76], many centers started reporting putative 'mosaic' aneuploidies – aneuploidies present in some trophectoderm cells but absent in others due to mitotic post-zygotic missegregation – on a single trophectoderm biopsy. These reports were

based on intermediate chromosome copy numbers shown in plots, whose clinical significance though remains uncertain. This uncertainty arises from various sources of technical and biological variability, including, but not limited to DNA amplification bias and poor biopsy quality or handling [77–79]. In this context, a non-selection study clearly demonstrated that allegedly ‘mosaic’ blastocysts have the same reproductive outcomes as euploid blastocysts [80], an evidence that raised serious concerns about the practice of reporting diagnoses of uncertain significance, as it may lead to the exclusion of viable embryos from transfer due to the perception that they are abnormal [79]. The current recommendation is that ‘mosaicism’ should either not be reported, or that ‘mosaic’ embryos may be de-prioritized but should still be considered for transfer if no euploid embryos are available [79]. On the contrary, the report of uniform full-chromosome aneuploidies in non-selection studies showed a > 98 % embryo lethality rate (i.e., implantation failure or miscarriage). This highlights PGT-A as the most reliable predictor of embryo incompetence currently available in IVF [81]. When PGT-A was restricted to comprehensive karyotype testing for uniform aneuploidies, both RCTs and observational studies consistently showed higher live birth rates per transfer and lower miscarriage rates per clinical pregnancy compared to untested embryo transfers [82,83].

PGT-A should only be performed at IVF centers with sufficiently trained and closely monitored personnel to ensure embryo reproductive competence is not compromised.

ESHRE and HFEA took a critical position based on misleading RCTs (and related *meta*-analyses) that were often conducted with biopsy and analysis protocols that are not used anymore, in inappropriate patient populations, or with unrealistic endpoints. Nevertheless, HFEA recognized that PGT-A may reduce the risk of miscarriage. ISAR did not consider PGT-A as an add-on. ASRM highlighted the limitations of all RCTs and observational studies, as well as the controversies surrounding the invasiveness of the procedure, and the diagnostic uncertainties related to mosaicism and segmental aneuploidies. Therefore, they recommended a case-by-case assessment, discouraging routine use of PGT-A in all IVF patients, in oocyte donor cycles, for SMF infertility and/or advanced paternal age, but supporting its use in cases of AMA, especially when associated with good ovarian reserve. They also advocated for more research in PGT cases for monogenic conditions, as many unaffected embryos might still be aneuploid, and chromosomal testing can be conducted on the same biopsy without additional manipulations [84].

Following all these considerations, we agreed that there is no benefit in transferring aneuploid blastocysts to any patient, but as this protocol is invasive and expensive, it cannot be applied routinely to all couples. The patients that might benefit the most from PGT-A are the women more subject to producing aneuploid blastocysts, i.e., AMA (≥ 35 years). In case of RPL, euploid blastocyst transfer will allow excluding the main known embryonic cause of miscarriage. In case of previous multiple implantation failures, euploid blastocyst transfer will allow excluding the main known cause of implantation failure. Women younger than 35 are still subject to about 30 % aneuploidy rates in the embryos they produce [85–87], but the application of this technology should be cost-effective, and a cost-effectiveness analysis modeled in the context of a public Italian IVF center supported PGT-A in young women only if they produce ≥ 4 blastocysts [88].

In conclusion, we agreed that every patient should be fully informed about the risks of aneuploidies, as well as the benefits and drawbacks of PGT-A, and given the autonomy to decide whether they wish to be informed of the chromosomal status of their embryos before transfer. In the hands of expert centers adopting validated technologies, the use of PGT-A is in line with the principle of couples’ reproductive autonomy and may reduce the burdens associated with IVF failures and miscarriages.

SIFES-MR Recommendation:

PGT-A at the blastocyst stage using comprehensive chromosome testing

technologies may be performed in women at high risk of transferring aneuploid blastocysts (AMA and RPL) or in couples who wish to be informed about the health status of their embryos before transfer. Couples must receive thorough counseling on the benefits and risks of PGT-A before signing an informed consent to this procedure. This procedure should only be conducted at clinics with proven expertise and by highly skilled operators, as it involves invasive, time-consuming, and technically demanding procedures. Based on intermediate chromosome copy numbers mosaicism should not be reported, or mosaic embryos should not be considered comparable to aneuploid embryos. When PGT-A is conducted, an additional cost is justified.

Mitochondrial DNA (mtDNA) load measurement

MtDNA load measurement in trophoctoderm biopsies during PGT cycles has been proposed as additional test to prioritize euploid embryos for transfer. This test is based on the hypothesis that incompetent embryos may be more metabolically active and thus exhibit a higher mitochondrial load [89–91]. Nevertheless, hard data showed no benefit of mtDNA load measurement [92–95]. Indeed, ESHRE advised against its use [2].

SIFES-MR Recommendation:

MtDNA load measurement to improve embryo selection among euploid blastocysts is not supported by hard data. This should not be suggested as an add-on to PGT-A cycles.

Non-invasive PGT (niPGT)

Genomic DNA can be found in the spent culture media after IVF. Even though its quantity and quality is limited, several researchers developed protocols to analyze it aiming at establishing a non-invasive PGT (niPGT) approach [96]. Protocol adjustments improved the concordance between spent culture media and trophoctoderm biopsies analyses [97]. Nonetheless, amplification failure is still ≈ 10 %, full karyotype concordance is still ≈ 70 % or lower, and overall euploid-euploid or aneuploid-aneuploid concordance is still ≈ 80 % or lower [98,99]. NiPGT cannot be considered a diagnostic approach, and if conducted, embryos must not be discarded based on aneuploidies detected through this method. Furthermore, its clinical value for improving embryo prioritization for transfer has yet to be demonstrated. Indeed, ESHRE advised against the clinical use of this technology, yet encouraging further pre-clinical studies [2].

SIFES-MR Recommendation:

NiPGT should not be regarded diagnostic, but it may be considered a putative future strategy to rank embryos for transfer. Therefore, further studies in the research context are recommended.

IVF Stage: Preparation to embryo transfer (ET)

Hysteroscopy

Hysteroscopy is the sole tool available to gynecologists that provides direct visualization of the uterine and endocervical cavities [100,101]. With technological advancements [102,103] and the introduction of progressively smaller instruments with fluid distension [104], this technique has become increasingly integrated into routine practice [105]. It allows for a rapid and cost-effective evaluation of the uterine cavity without analgesia or hospitalization [106,107]. In infertile women, hysteroscopy enables a thorough assessment of various congenital and acquired uterine conditions [108–111] including cervical stenosis, endometrial and endocervical polyps, submucosal fibroids, synechiae, isthmocele, chronic endometritis, Müllerian anomalies, endometrial hyperplasia, adenomyosis, and cancer. Additionally, this technique allows both diagnosis and treatment of many conditions in a single step (“see and treat hysteroscopy”), such as endometrial and endocervical polyps, synechiae, and small fibroids [112].

While the utility of hysteroscopy in infertile patients with suspected pathology identified via transvaginal ultrasound (TVS) is well-established, current debate centers on its potential application as a screening investigation to all infertile women prior to IVF, even in the

absence of suspected lesions on TVS. A pragmatic multicenter RCT [113] involving patients with at least two failed IVFs did not demonstrate an improvement in live birth rate within one year in the hysteroscopy group compared to controls. Similarly, a large Dutch pragmatic multicenter RCT [114] focusing on naïve IVF patients did not observe benefits from hysteroscopy in terms of ongoing pregnancy rate within 18 months from randomization. Di Spiezio Sardo and colleagues [115], instead, in a meta-analysis of 9 RCTs reported that pre-IVF hysteroscopy improved clinical pregnancy in naïve patients and live birth rate in women with previous IVF failures. Their conclusion were corroborated by another meta-analysis of 12 studies [116]. The inconsistencies in the results across the studies may be primarily due to statistics' heterogeneity, different sample size, and missing data, making uncertain the overall assessment of hysteroscopy's benefits in women without suspected intrauterine pathology on TVS.

Of note, in a trial involving 678 asymptomatic naïve patients with normal TVS, Fatemi and colleagues [117] observed a 11 % prevalence of intrauterine abnormalities. Although this prevalence is lower than that reported by other studies (20–45 %), it remains potentially non-negligible. However, there is currently no evidence that removal of these minor intrauterine pathologies, not previously diagnosed on TVS, has a substantial effect on improving the likelihood of achieving a live birth after IVF.

SIFES-MR Recommendation:

Whenever TVS raises suspicion of intrauterine pathology, hysteroscopy should be suggested. In cases when TVS does not show uterine pathologies, offering hysteroscopy remains discretionary, as there is no data regarding benefit in these scenarios. Conversely, hysteroscopy should not be suggested to the general population before IVF. Regarding patients with multiple implantation failures, it is still worth investigating the clinical value of hysteroscopy.

Endometritis testing

Chronic endometritis (CE) is an inflammatory condition of the endometrium with an infectious origin, predominantly caused by enterobacteria [118,119]. It has been reported highly prevalent in idiopathic infertility [120] and patients with multiple previous implantation failures [121]. It has been hypothesized that its treatment would improve the chance of live birth. Nevertheless, there are several doubts on the scientific plausibility of the relationship between CE and a lower prognosis [122]. The main issue is the absence of a gold standard diagnostic approach. Generally, the disease is diagnosed through hysteroscopy, which identifies characteristic signs such as micro-polyps, stromal edema, and hyperemia [123], then confirmed through histological examination of endometrial biopsies, where plasma cells should be identified (using immunohistochemical staining such as CD-138 and MUM-1) [124]. However, hysteroscopic suspicion remains operator-dependent, and histological diagnosis lacks validated plasma cells' cutoff; indeed, the data on the clinical utility of CE testing and therapy are highly controversial.

SIFES-MR Recommendation:

Routine screening for CE is not supported by the current level of the evidence, therefore it is not generally recommended. Testing and treating the condition is worth being investigated clinically in patients who suffered multiple implantation failures.

Endometrial scratch

Endometrial scratch has been proposed to improve implantation rates in IVF. This endometrial injury was thought to trigger decidualization, activate wound-healing pathways, inflammatory response, expression of genes involved in implantation, and enhance blastocyst-endometrial communication [125]. A recent Cochrane review, though, concluded that endometrial scratch might have no impact on live birth or miscarriage rates [125], including naïve patients and patients with two previous implantation failures [126]. Conversely, another review suggested a benefit [127]. ESHRE does not recommend endometrial scratch [2]. Similarly, the HFEA has expressed doubts about its clinical effectiveness

(<https://www.hfea.gov.uk/treatments/treatment-add-ons/>).

SIFES-MR Recommendation:

Endometrial scratch is not recommended.

Endometrial receptivity screening

The mechanisms underlying the interaction between the blastocyst and endometrium are complex and not fully understood. Recently, several tests have been proposed to assess endometrial receptivity. The primary aim is to evaluate the endometrium to facilitate personalized embryo transfer (pET), adjusting the timing of embryo transfer based on progesterone administration according to each patient's unique window of implantation, namely the period of highest receptivity [128]. These tests have been suggested primarily to patients after multiple implantation failures but also to those undergoing oocyte donation, and even to good prognosis patients [129–135]. While the test has been able to detect altered gene expression in conditions like adenomyosis, adjusting progesterone timing has not improved IVF outcomes in most published studies and, in some cases, may even reduce success rates [136,137]. A RCT suggested benefits deriving from the endometrial receptivity test in terms of cumulative live birth rate considering all transfers conducted within 1 year [138], but it was heavily criticized for its design and conclusions [139]. Also, in the context of euploid blastocyst transfers, no benefit has been reported from conducting an endometrial receptivity test [140].

ESHRE and HFEA do not recommend endometrial receptivity tests in IVF [2] (<https://www.hfea.gov.uk/treatments/treatment-add-ons/>).

SIFES-MR Recommendation:

Although endometrial receptivity tests may show alterations in endometrial gene expression during the mid-secretory phase, adjustment of progesterone timing is ineffective. Endometrial receptivity tests should not be suggested to the general IVF population.

Microbiome analysis

Microbiome testing before or during IVF treatments is promising, as dysbiosis is a treatable condition. In fact, vaginal, follicular, endometrial and sperm microbiome have all been studied, with controversial results on their real impact in terms of live birth rate [141–144]. ESHRE states that vaginal and endometrial microbiome testing cannot yet be recommended clinically [9]; yet, vaginal and endometrial microbiome are worth being investigated in the context of embryo implantation as Lactobacilli colonization may improve IVF outcomes [144,145]. Unfortunately, though, the evidence regarding treatments with antibiotics or probiotics to improve maternal microbiota is much less consistent [146–148]. More studies are needed to demonstrate which interventions are suitable to modify the microbiome of the female reproductive tract, and whether these modifications may improve IVF outcomes.

SIFES-MR Recommendation:

Microbiome profiling is currently not recommended in all IVF couples. In the research context, it is worth investigating indications, protocols, reporting parameters, and putative therapies in the case of dysbiosis.

Immunological tests and therapies

Immunological tests in reproductive medicine include assessments of NK-cell levels and function in blood, Killer-cell immunoglobulin-like receptor (KIR) and human leukocyte antigen (HLA) genotyping, regulatory T cells (Tregs), Th1/Th2 ratios, and cytokine profiles. However, the representation of NK cells in the endometrium differs significantly from that in the blood. The proportion of NK cells in the blood varies widely among healthy individuals, yet an arbitrary cutoff of around 12 % is often used to define abnormal levels. Despite this, no therapeutic interventions have been proven effective for abnormal NK-cell levels or function, Th1/Th2 ratios, or other blood parameters. Similarly, endometrial biopsies for counting NK cells are difficult to interpret.

Embryo implantation is viewed as a natural allograft in the immunological context, where the maternal immune system must develop tolerance to fetal antigens to achieve a successful pregnancy.

Immunologically, pregnancy is comparable to allogeneic stem cell transplantation, where a defect in communication can lead to 'rejection,' disrupting the immunotolerance system established during pregnancy [149]. HLA class I (A, B, and C) molecules are expressed on nearly all nucleated cells and are crucial for self-recognition. Several studies have linked pregnancy disorders, such as pre-eclampsia, to specific combinations of maternal KIR and fetal HLA-C genetic variants [150]. A small retrospective analysis on the use of subcutaneous G-CSF in patients with a KIR/HLA-C mismatch undergoing IVF demonstrated reduced miscarriage rate and increased live birth rate [151]. Further research is certainly worth being run in this field.

Several treatments have been suggested to modulate the immune system aiming at improved implantation, such as steroids, lipid emulsion (Intralipid) infusion, Intravenous Immunoglobulin (IVIG), leukocyte immunization therapy, tacrolimus, anti-tumor necrosis factor (anti-TNF) agents, Granulocyte colony-stimulating factor (G-CSF). Due to the absence of solid studies supporting their use and uncertainties over which tests to use or how to interpret them, ESHRE does not recommend immune treatments in IVF [2]. Conversely, ASRM and HFEA claim the evidence is insufficient to make a recommendation in favor or against [152] (<https://www.hfea.gov.uk/treatments/treatment-add-ons/>).

SIFES-MR Recommendation:

Peripheral blood tests for immune parameters and uNK cell assessments cannot be recommended at present. For NK cell tests, the lack of established reference ranges may invalidate the results, and variations in NK expression could simply reflect normal differentiation of the secretory endometrium after ovulation. KIR and HLA typing cannot be recommended in the general IVF population. However, the mismatch between fetal HLA-C2 and maternal KIR AA in women with multiple implantation failures appears to be associated with poorer outcomes. In this regard, we encourage the collection of more clinical data.

IVF Stage: IVF – Embryo transfer (ET)

Assisted hatching

Assisted hatching (AH) is a procedure performed on embryos before ET, using a laser to create a small hole or to thin the zona pellucida (ZP). However, the procedure may involve risks, including damage to the embryo or individual blastomeres, which can reduce embryo viability, as well as an increase the prevalence of monozygotic twinning [153]. Several studies have been published on this subject, but none highlighted strong evidence of improvement in the live birth rate [154,155]. In 2021 the Cochrane review showed no association between AH and live birth rates [156].

ESHRE and ASRM discouraged the use of assisted hatching to increase the LBR, while HFEA and ISAR claimed that more clinical data are required.

SIFES-MR Recommendation:

A beneficial effect of AH cannot be supported, therefore – unless needed for PGT purposes – we do not recommend this additional embryonic manipulation.

Hyaluronic acid enriched transfer media

The supplementation of adherence compounds to ET medium has hypothesized to promote and support implantation, in turn optimizing the live birth rates. Hyaluronic Acid (HA) – a macromolecule highly present in the maternal reproductive tract [157] and whose local expression increases in the endometrium at implantation [158] – is one of the mostly studied compounds to this end. HA increases cell-to-cell adhesion and cell-to-matrix adhesion and produces a viscous solution [159]. HA also exerts autocrine and paracrine functions by acting on CD44 receptors, that are expressed both on the pre-implantation embryo and in the stroma of the human endometrium [160,161]. Notably, the concentrations of HA and CD44 receptor reached the maximum peak after the endometrium become receptive to embryo implantation [162]. In general, the most recent reviews and meta-analyses [163–165]

suggest that the addition of HA to ET medium might be clinically beneficial, although the overall quality of evidence was only low to moderate, mainly owing to imprecision and/or heterogeneity. According to ESHRE, HA addition to the ET media is recommended while monitoring for multiple pregnancy rate [2]. According to HFEA, it is not clear whether this add-on is effective to improve the treatment outcome because of conflicting evidence (<https://www.hfea.gov.uk/treatments/treatment-add-ons/>). According to ISAR, in patients with multiple implantation failure, it may be considered as a useful transfer medium [3].

SIFES-MR Recommendation:

Although the current level of the evidence supports HA supplementation to the ET media, stronger data are needed in all patients, but especially in patients with multiple implantation failures.

Steroid adjuncts

Steroids have been suggested to support the natural immune modifications that occur during pregnancy. However, the HFEA discourages their use in IVF due to insufficient evidence of efficacy and potential safety concerns, as highlighted in a review of 16 studies (<https://www.hfea.gov.uk/treatments/treatment-add-ons/>). Beyond the well-known adverse effects of steroid treatments, such as weight gain, sleep disturbances, and increased susceptibility to infections, there are also pregnancy-specific risks, including an increased risk of cryptorchidism, hypospadias, and talipes in the fetus [166]. According to ESHRE, while steroids may offer some benefits for women with autoimmune diseases, the available data are based on small studies with inconsistent results [2]. For these reasons, steroids are not typically prescribed to all IVF patients to improve pregnancy or reduce miscarriage rates.

SIFES-MR Recommendation:

Steroid treatment is not recommended for IVF patients without a clear medical indication due to its uncertain efficacy and safety. This does not apply to women diagnosed with autoimmune diseases, such as anti-phospholipid antibody syndrome, or to those taking steroids for other medical conditions.

PRP intrauterine administration

Platelet-Rich Plasma (PRP) is a concentration of autologous platelets, prepared by centrifuging fresh whole blood to remove red and white blood cells. It is characterized by a higher concentration of growth factors, adhesive proteins, and immune mediators [167] that in turn could promote molecular pathways involved in endometrial and ovarian regeneration such as mitosis, anti-inflammatory processes and neo-angiogenesis [168]. In the context of reproductive medicine, PRP application was experimented in the treatment of Asherman's syndrome [168], multiple implantation failure [169], premature ovarian failure [167], thin endometrium or unselected IVF patients [170]. PRP could be injected inside the uterus using an intrauterine catheter [171]. However, PRP preparation remains not standardized. Indeed, relevant heterogeneity was observed among trials regarding the amount of collected blood, the amount of anticoagulant solution, and the centrifugation process [170]. Even the amount of PRP injected, administration timing and catheter used greatly varies among trials [170]. Recent meta-analyses [169,170] concluded that PRP treatment could increase endometrial thickness and implantation, however none of the RCTs reported higher live birth rates [169]. Few studies reported data on cumulative live birth rates, making difficult to draw conclusions about the effect of PRP on IVF efficacy. In terms of IVF efficiency, low quality evidence sustains an effect of intrauterine PRP in women with history of multiple implantation failures. Despite initial promising findings, several critical issues must be addressed. First, most published studies, including RCTs, lack sufficient data on safety concerns, such as adverse events, allergic reactions, pain, and long-term infant health [2,170]. Therefore, it is unclear whether this procedure is safe to apply to all infertile patients without appropriate countermeasures or within a specific setting, such as a tertiary or hospital center. Additionally, PRP preparation methods vary widely across centers, and little is known about the optimal

preparation protocol. Lastly, the overall quality of the studies conducted thus far is low [2,170], hence the robustness of the findings so far collected is inevitably jeopardized.

SIFES-MR Recommendation:

Limited evidence supports the use of PRP Intrauterine injection/infusion in women with a history of multiple implantation failures and in those with thin endometrium. However, the lack of a standardized definition of these IVF populations among studies, the high risk of bias observed among trials and the lack of safety data incite the production of additional clinical data.

hCG or G-CSF intrauterine administration

In vitro and in vivo studies suggested that intrauterine injection of human chorionic gonadotropin (hCG) may modulate factors involved in embryo implantation, such as endometrial matrix metalloproteinases, growth factors, and cytokines, thereby enhancing endometrial receptivity [172]. Furthermore, hCG per se could play a pivotal paracrine role during embryo implantation [173]. G-CSF, instead, is a cytokine involved in endometrium-embryo crosstalk. Indeed, this molecule might promote embryo implantation process by modulating vascularization and immune processes [174]. The alternative route of hCG administration to increase IVF outcome is by intrauterine injection [175]. Conversely, G-CSF could be administered subcutaneously [176] or could be placed into the uterus through a catheter [177].

hCG administration was assessed by several RCTs summarized in a recent meta-analysis [175]. Promising effects were reported in cleavage-stage ET cases, while no significant effect was appreciated among blastocyst-stage ETs. More studies were then encouraged in women with multiple implantation failures [178].

Concerning G-CSF, two recent meta-analyses evaluated its effect in IVF [179,180], supporting improved clinical outcomes, while a recent RCT showed no benefit in terms of live birth rate or miscarriage rate [181]. Moreover, two routes of administration exist: intrauterine and subcutaneous, also being associated with controversial outcomes.

The main issues regarding both hCG and G-CSF administration is related to limited quality and sample size among trials, and the heterogeneity in terms of preparation and protocols adopted. Lastly, data regarding safety and/or long-term infant outcomes are still limited and should be carefully considered by clinicians.

SIFES-MR Recommendation:

Intrauterine hCG and subcutaneous G-CSF might improve IVF outcomes. Nevertheless, the data are still limited at present to fully assess its efficacy, efficiency and safety. This lack of information, along with the absence of a standardized procedure suggest that these practices should be still tested in the research context.

Discussion

The use of add-ons in IVF has increased over the years, often with the aim of improving treatment outcomes. However, many of the strategies classified as “add-ons” may offer benefits for secondary outcomes related to IVF safety or efficiency — such as miscarriage rates or treatment duration — or serve complementary roles, such as providing couples’ counseling before, during, and after each cycle. Assessing IVF success requires a comprehensive view that considers multiple aspects, and focusing solely on cumulative live birth rates per attempt as the primary metric is overly simplistic and limiting. Despite their growing availability, the clinical effectiveness and safety of many interventions proposed over the years remain controversial, and their widespread adoption is often backed by heterogeneous — and frequently insufficient — scientific support. Indeed, there is no consensus on the true effectiveness of many tools or strategies implemented to date. This variability is compounded by the diverse contexts in which IVF is practiced globally, influenced by distinct social needs, cultural settings, regulations, financial investments, levels of patient support, and the prevalence of either public or private healthcare systems in any given country. For some strategies and tools, such as PGT-A, hyaluronic acid-enriched

transfer media, and intrauterine PRP administration, potential benefits have been observed in specific subpopulations, although the quality of supporting evidence has been often heavily criticized. Other “add-ons,” like mtDNA quantitation or endometrial receptivity testing, generated substantial initial excitement but led to disappointing outcomes when widely implemented across clinics. This variability in clinical outcomes highlights the urgent need for robust clinical trials, greater education and training for both centers and practitioners, and, most importantly, a call for a more rigorous pre-clinical evaluation and stronger collaboration between technology providers and IVF practitioners. Such a collaborative approach is essential for the successful adoption of any “add-on,” ensuring that its integration reaches a stable level of clinical productivity once it becomes mainstream.

One of the main concerns highlighted in this SIFES-MR consensus document is that many add-ons are routinely offered in clinical settings despite lacking high-quality, consistent evidence. The panel is not particularly troubled by putative “add-ons” that, when accompanied by comprehensive counseling on their potential benefits and drawbacks, allow for an informed decision regarding their adoption and any associated costs. Rather, our primary concern lies with practices that may pose safety risks, which remain largely unclarified, such as the use of growth factors in embryo culture media. Moreover, the panel believes that each intervention — and the RCTs designed to test its effectiveness — should be tailored to the specific characteristics of the patient, such as age, reproductive history, and prognosis. PGT-A is a prime example. Over the years, it was theorized, tested, and embraced as a revolutionary tool. However, as limitations began to surface, it became clear that a reassessment was needed, leading to changes in how PGT-A is conducted, transitioning from blastomere biopsies analyzed through cytogenetic tests to trophectoderm biopsies assessed using comprehensive chromosome molecular tests. The primary misconception is that PGT-A should increase the CLBR per oocyte retrieval, while no embryo selection or diagnostic strategy can achieve this outcome. Nevertheless, it has proven beneficial in reducing miscarriage rates and improving embryo selection in patients with a high aneuploid blastocysts rate. Yet again, the reporting of “mosaicism” has introduced technical inconsistencies that have biased both biological and clinical interpretations. The issue is not in the technology itself or in its theoretical value, but rather in how these technologies have been implemented and practiced over the years, without effectively bridging the gap between theory and clinical practice, and between genetic laboratories and IVF clinics. Unfortunately, this has been the fate of many promising tools in recent decades of ART practice.

Given the uncertainty surrounding many add-ons, the primary consideration should always be providing the couples with a comprehensive, evidence-based counseling that will preserve their autonomy in making informed decisions as whether to use these additional interventions or not. The risk of over-promising outcomes or promoting treatments without adequate evidence must be avoided. Clear communication and transparency about the limitations of current evidence are fundamental to maintaining trust and respecting patient choice.

The recommendations provided in this manuscript reflect the unique regulatory and healthcare context in Italy. With significant variability in costs, local regulations, and accessibility, it is important for Italian IVF centers to implement these guidelines in a way that aligns with both patient needs and institutional capabilities. For example, while ECS is theoretically beneficial, structural and organizational challenges may limit its widespread use. Similarly, the regulatory restrictions around elective freeze-all strategies in Italy necessitate a case-by-case consideration.

Future directions and research priorities

The main challenge in adopting add-ons lies in the lack of high-quality evidence and the heterogeneity of the studies currently available. Future research should prioritize well-designed, multicenter RCTs

and/or big data and real-world evidence, while monitoring not only short-term but also long-term safety and effectiveness aspects. Particular attention should be given to: (i) defining the subpopulations most likely to benefit from specific add-ons, (ii) establishing standardized protocols for (re-)emerging technologies such as r-IVM and intrauterine PRP, (iii) conducting cost-effectiveness analyses to ensure that new interventions provide value, especially within the limits suitable for public healthcare systems. To this end, interdisciplinary collaboration and international harmonization of guidelines are essential to achieving a consensus on best practices. Given the dynamic nature of reproductive medicine, continuous updates based on new evidence will be necessary to refine recommendations and optimize patient care.

Conclusion

This document summarizes in Fig. 1 the position of SIFES-MR on the main IVF add-ons introduced to date. All 28 statements received over 90 % of scores ≥ 7 on the Likert scale from the panel of experts (Supplementary Fig. 1–28) and were subsequently approved by the

Executive Committee and circulated among all SIFES-MR members.

The non-exact nature of reproductive medicine necessitates a careful, evidence-based approach when considering the use of IVF add-ons. By aligning clinical practice with patient-centered care and prioritizing interventions with proven benefits, we can improve the efficiency and safety of ART treatments. Further research is needed to bridge the gaps in current knowledge, ensuring that IVF add-ons become a scientifically validated component of personalized reproductive care.

Ethical approval

Not applicable.

Availability of data and materials

Not applicable.



Fig. 1. Summary of SIFES-MR recommendations regarding the IVF add-ons discussed in this manuscript and clustered according to the stage of IVF during which they might be suggested. DFI, DNA fragmentation index; GH, growth hormone; DHEA, dehydroepiandrosterone; POR, poor ovarian reserve; POI, premature ovarian insufficiency; MACS, Magnetic Activated Cell Sorting; NOA, non-obstructive azoospermia; IVM, in vitro maturation; IMSI, Intracytoplasmic injection of morphologically selected spermatozoa; PICSI, physiological ICS; AOA, artificial oocyte activation; TFF, total fertilization failure; GFs, growth factors; PGT-A, preimplantation genetic testing for aneuploidies; AMA, advanced maternal age; RPL, recurrent pregnancy loss; mtDNA, mitochondrial DNA; niPGT, non-invasive PGT; PRP, platelet rich plasma; G-CSF, granulocyte colony stimulating factor; hCG, human chorionic gonadotrophin; TVS, transvaginal scan.

Authors' contribution

DC wrote the introduction. Each author wrote one or more paragraphs based on their own background and area of expertise. DC and MC wrote the discussion and conclusion. All authors reviewed and commented on the whole manuscript, which was then re-discussed to approve its final version. DC prepared the Table and Figures. DC, MC, and AB coordinated the working group.

CRedit authorship contribution statement

Danilo Cimadomo: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Conceptualization. **Mauro Cozzolino:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Andrea Busnelli:** Writing – review & editing, Writing – original draft, Conceptualization. **Andrea Carosso:** Writing – review & editing, Writing – original draft. **Alessandro Conforti:** Writing – review & editing, Writing – original draft. **Claudia Massarotti:** Writing – review & editing, Writing – original draft. **Valentina Spadoni:** Writing – review & editing, Writing – original draft. **Alberto Vaiarelli:** Writing – review & editing, Writing – original draft. **Roberta Venturella:** Writing – review & editing, Writing – original draft. **Amerigo Vitagliano:** Writing – review & editing, Writing – original draft. **Carlotta Zacà:** Writing – review & editing, Writing – original draft. **Andrea Borini:** Writing – review & editing, Writing – original draft, Funding acquisition.

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Appendix A. Supplementary data

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