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ESHRE Pages

Good practice recommendations on add-ons in reproductive medicine†

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ABSTRACT

STUDY QUESTION: Which add-ons are safe and effective to be used in ART treatment?

SUMMARY ANSWER: Forty-two recommendations were formulated on the use of add-ons in the diagnosis of fertility problems, the IVF laboratory and clinical management of IVF treatment.

WHAT IS KNOWN ALREADY: The innovative nature of ART combined with the extremely high motivation of the patients has opened the door to the wide application of what has become known as 'add-ons' in reproductive medicine. These supplementary options are available to patients in addition to standard fertility procedures, typically incurring an additional cost. A diverse array of supplementary options is made available, encompassing tests, drugs, equipment, complementary or alternative therapies, laboratory procedures, and surgical interventions. These options share the common aim of stating to enhance pregnancy or live birth rates, mitigate the risk of miscarriage, or expedite the time to achieving pregnancy.

STUDY DESIGN, SIZE, DURATION: ESHRE aimed to develop clinically relevant and evidence-based recommendations focusing on the safety and efficacy of add-ons currently used in fertility procedures in order to improve the quality of care for patients with infertility.

PARTICIPANTS/MATERIALS, SETTING, METHODS: ESHRE appointed a European multidisciplinary working group consisting of practising clinicians, embryologists, and researchers who have demonstrated leadership and expertise in the care and research of infertility. Patient representatives were included in the working group. To ensure that the guidelines are evidence-based, the literature identified from a systematic search was reviewed and critically appraised. In the absence of any clear scientific evidence, recommendations were based on the professional experience and consensus of the working group. The guidelines are thus based on the best available evidence and expert agreement. Prior to publication, the guidelines were reviewed by 46 independent international reviewers. A total of 272 comments were received and incorporated where relevant.

MAIN RESULTS AND THE ROLE OF CHANCE: The multidisciplinary working group formulated 42 recommendations in three sections; diagnosis and diagnostic tests, laboratory tests and interventions, and clinical management.

LIMITATIONS, REASONS FOR CAUTION: Of the 42 recommendations, none could be based on high-quality evidence and only four could be based on moderate-quality evidence, implicating that 95% of the recommendations are supported only by low-quality randomized controlled trials, observational data, professional experience, or consensus of the development group.

WIDER IMPLICATIONS OF THE FINDINGS: These guidelines offer valuable direction for healthcare professionals who are responsible for the care of patients undergoing ART treatment for infertility. Their purpose is to promote safe and effective ART treatment, enabling patients to make informed decisions based on realistic expectations. The guidelines aim to ensure that patients are fully

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informed about the various treatment options available to them and the likelihood of any additional treatment or test to improve the chance of achieving a live birth.

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DISCLAIMER: *This Good Practice Recommendations (GPRs) document represents the views of ESHRE, which are the result of consensus between the relevant ESHRE stakeholders and are based on the scientific evidence available at the time of preparation.*
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Keywords: add-on / good practice / guidelines / ART / IVF / ICSI / infertility / ESHRE

Introduction

In relatively new fields of medicine, innovation thrives and progress can be rapid. Reproductive medicine is an example of such a field with immense progress in treatments and outcomes since the first baby was born after the application of IVF treatment in 1978 (Steptoe and Edwards, 1978).

Despite this, no underlying cause of infertility is identified for many couples and even in patients with clear indications, the success of ART varies. The latest data from the European IVF Monitoring (EIM) Consortium reported that in the participating countries, pregnancy rates (PRs) per embryo transfer (ET) in fresh cycles were 34.1% for IVF and 32.1% for ICSI, and delivery rates per ET were 26.1% for IVF and 23.9% for ICSI (The European IVF-Monitoring Consortium for the European Society of Human Reproduction and Embryology *et al.*, 2022), while for frozen-thawed ET, pregnancy and delivery rates per ET were 34.3% and 24.9%, respectively.

Cumulative data on the chance of a couple who attend a fertility clinic achieving the birth of a healthy child are scarce. The EIM report mentions an estimated 'cumulative' delivery rate of 32.3%, not per patient, but calculated as the ratio of the total number of deliveries from fresh and frozen ET over the number of aspirations during the same year (The European IVF-Monitoring Consortium for the European Society of Human Reproduction and Embryology *et al.*, 2022). In a follow-up study of 557 couples 6 years after their initial fertility consultation, 54.2% achieved parenthood through either ART or spontaneous conception (Ferreira *et al.*, 2016). In a study based on The Swedish National Quality register for Assisted reproduction (Q-IVF), including mainly single embryo transfer cycles, it was shown that the cumulative live birth rate (LBR) per one oocyte pick-up (OPU) for 2019 was 36.3% when calculated for all patients that had OPU and 43.3% for the cohort of patients that achieved at least one ET (Saket *et al.*, 2021). Belgian registry data similarly showed a cumulative LBR of 33.2% per started cycle (De Neubourg *et al.*, 2021). A multicentre, multinational study reported a cumulative LBR of 43.9% after a single OPU including all fresh and frozen (Day 3 or Day 5/6) ETs performed within a 2-year period after OPU (Polyzos *et al.*, 2018).

The cumulative probability of LBR was analysed in 2002 by Olivius *et al.* (2002) showing that 63% of the couples were estimated to achieve childbirth after three completed conventional IVF or ICSI cycles, including all (Day 2) transfers. De Neubourg *et al.* (2021) also estimated the cumulative LBR for the total of six reimbursed OPU and ET cycles to be 55.4% or 76.8% (depending on the assumptions made for incomplete data). For many add-ons, the cumulative LBR would be the optimal outcome to evaluate their effectiveness; however, this is seldomly reported.

Owing to the still substantial risk of any ART cycle being unsuccessful, treatment remains a distressing event both for patients and their treating healthcare professionals. For some patients, this risk of failure combined with the financial aspects of ART may force them towards dropping out of treatment, while for others this fuels their desire for other, presumed better treatment options (Verberg *et al.*, 2008; De Neubourg *et al.*, 2021). Healthcare professionals may be driven by the wish to do the best for the patients, pressure from the patients and sometimes also by competitive and/or commercial motives to go beyond standard treatment (Iacoponi *et al.*, 2022).

The innovative nature of ART, coupled with the determination of patients, have paved the way for the extensive utilization of what is now commonly referred to as 'add-ons' in the field of reproductive medicine. These supplementary options are available to patients in addition to standard fertility procedures, typically incurring an additional cost. A diverse array of supplementary options is made available, encompassing tests, drugs, equipment, complementary or alternative therapies, laboratory procedures, and surgical interventions. These options share the common aim of stating to enhance pregnancy or LBRs, mitigate the risk of miscarriage, or expedite the time to achieving pregnancy. The availability of substantial evidence regarding the safety and efficacy of add-ons is frequently limited or lacking (Harper *et al.*, 2012, 2017; Lensen *et al.*, 2021a).

The context is an additional factor in the use of add-on tests and treatments. For example, in some settings ICSI is only performed when indicated, i.e. in couples with diagnosed male factor infertility or fertilization failure in the previous IVF cycle. In other countries or settings, ICSI is used in all couples, irrespective of the results of the fertility work-up and diagnostic interventions (The European IVF-Monitoring Consortium for the European Society of Human Reproduction and Embryology *et al.*, 2022). As such, ICSI is not an add-on in the first setting but should be considered so in the latter, particularly if extra costs are charged to the patients.

This article outlines a set of add-on tests and treatments, describes the biological rationale and—if available—the evidence of their efficacy and safety. This article further makes recommendations for clinical practice including under which conditions and precautions the procedures could be applied in clinical practice, or whether they should be further investigated in a research-context or at least monitored for safety and efficacy. Add-on tests and treatments are described in three subgroups: diagnosis and diagnostic tests, laboratory tests and interventions, and for clinical management.

Materials and methods

The current document was developed according to the manual for development of ESHRE good practice recommendations (Vermeulen *et al.*, 2019).

A working group was composed of experts in reproductive medicine ensuring variation in clinical and laboratory expertise, and geographical balance, supported by two methodological experts (N.V. and N.L.C.). Patient and consumer representation were also included. In the first meetings, the working group reached agreement on a list of add-ons being currently marketed that would be further evaluated. The progress was discussed in regular online meetings. During an in-person meeting, collected evidence was discussed and consensus was reached on recommendations for clinical practice.

For all the add-ons listed, a literature search of PUBMED was performed. Papers published up to 10 August 2022 were included. All titles and abstracts were screened to identify relevant papers, for which full-text papers were collected and summarized. The literature search was performed for the general infertility population, and if data were retrieved for a specific patient population this was specified in the text. In summarizing data for a specific add-on, priority was given to systematic reviews and randomized controlled trials (RCTs), where relevant data from observational studies were added as well. For each add-on, the current paper includes a short narrative summary of published data incorporated. For efficacy, (cumulative) LBR was considered the primary outcome, and only when not reported in the studies were PRs considered the primary outcome. Further, information on safety was summarized, as well as other technical and practical aspects of possible relevance for the clinic and the patient. Legislative aspects and CE marking were not discussed and are not considered to be within the scope of these recommendations. Four standard phrases were used to formulate recommendations with regard to the add-ons included in this good practice recommendations paper (Table 1).

Abbreviations used throughout this article are listed in Supplementary Data File S1. For the ease of reading, the acronyms of growth factors, kinases etc. are only explained in full in the list of abbreviations. An overview table with all recommendations formulated by the ESHRE working group on add-ons and discussed in this Recommendations for Good Practice paper can be found in Supplementary Data File S2.

The final draft was published on the ESHRE website between 1 November and 1 December 2022 for stakeholder review. A total of 272 comments from 46 reviewers were received and incorporated where relevant. The review report is available on www.eshre.eu/guidelines. The experts who participated in the stakeholder review are listed in Supplementary Data File S3.

Results

Diagnosis and diagnostic tests

Screening hysteroscopy

Screening hysteroscopy refers to the attempt for direct visualization of the endometrial cavity and endocervical canal in patients with infertility despite the lack of any apparent pathology using ultrasonography and/or hysterosalpingography. It has been evaluated in patients with unexplained infertility and prior to IUI or IVF treatment.

Efficacy

According to a Cochrane review, hysteroscopy before IVF treatment may increase LBR (relative risk (RR) 1.26; 95% CI 1.11 to 1.43; 6 RCTs; n = 2745; I^2 = 69%; low-quality evidence) when compared with patients that had not been screened with hysteroscopy (Kamath *et al.*, 2019). The participants were a mixture of unselected patients, first IVF cycles and patients with recurrent implantation failure (RIF), and significant results were primarily related to this last group. The main limitations in the quality of evidence were inadequate reporting of study methods and higher statistical heterogeneity. As such, sensitivity analysis performed by pooling results from trials at low risk of bias showed no increase in LBR following a screening hysteroscopy (RR 0.99; 95% CI 0.82 to 1.18; 2 RCTs; n = 1452; I^2 = 0%). There was little or no difference in miscarriage rate following screening hysteroscopy compared to no hysteroscopy (RR 1.01; 95% CI 0.67 to 1.50; 3 RCTs; n = 1669; I^2 = 0%; low-quality evidence) (Kamath *et al.*, 2019).

Similar to the two largest RCTs (El-Toukhy and El Tokhy, 2016; Smit *et al.*, 2016) included in the Cochrane review, a recent RCT confirmed a similar LBR when hysteroscopy was performed before IVF treatment or not (23.9% versus 19.3%; n = 171; P = 0.607) (Ben Abid *et al.*, 2021).

Table 1. Overview of the four standard phrases that were used for the formulation of the recommendations, and their implications.

Terminology	Implications
Recommended	The test/intervention can be applied to most patients or to those patient groups for whom it may be of relevance. The recommendation can be adopted as policy in most situations.
Can be considered	Can be applied after a thorough discussion of possible benefits and risks and with close monitoring, follow-up and evaluation.
Currently not recommended for routine clinical use	The test/intervention should not be applied routinely to patients at this stage, but this may change when more evidence on efficacy and safety becomes available. Optionally, the intervention can be applied to a specific patient group.
Not recommended	Based on safety concerns and/or lack of efficacy and/or lack of biological rationale, the test/intervention should not be applied to patients. Further evaluation of these tests/interventions can be done, but only in strict research settings.

A meta-analysis focusing on patients with RIF reported a significantly higher LBR after hysteroscopy compared to patients with RIF that did not have hysteroscopy (RR 1.29; 95% CI 1.03 to 1.62; 2 RCT and 2 cohort studies; n = 2247; $P = 0.046$) (Cao *et al.*, 2018). It should be noted that the meta-analysis was not restricted to RCTs and that the largest RCT included reported similar LBR regardless of whether or not a hysteroscopy was performed in patients with RIF (RR 1.01; 95% CI 0.80 to 1.49; 1 RCT; n = 702) (El-Toukhy and El Tokhy, 2016).

Time to pregnancy did not significantly differ when screening hysteroscopy was performed in women with a normal transvaginal ultrasound before a first IVF treatment (Smit *et al.*, 2016).

There are no data on the cost-effectiveness of screening hysteroscopy. Analysis of cost-effectiveness was part of the study by Smit *et al.* (2016), but because of the lack of benefit of hysteroscopy, the planned cost analysis was futile.

Safety

Four trials in the Cochrane review reported complications following hysteroscopy (odds ratio (OR) 7.47; 95% CI 0.15 to 376.42; 4 RCTs; n = 1872; I^2 N/A; very low-quality evidence); of these, three trials recorded no events in either group; in the fourth trial, one case of endometritis was reported (Kamath *et al.*, 2019).

Other aspects

In a recent study including 5151 women attending for outpatient hysteroscopy, pain was reported by most women (n = 4490; 87%) with 41% of them rating the pain as worse than 'slightly painful' (Mahmud *et al.*, 2021). In another study, an average pain score of 4.69 ± 2.892 on a 10 cm visual analogue scale (VAS) was reported despite all women receiving paracetamol/codeine before the procedure (Ben Abid *et al.*, 2021). However, the frequency and severity of pain might vary in relation to the diameter of the endoscope, the experience of the operator and the preference of distension medium or CO_2.

Recommendation

The results of three recent high-quality multicentre RCTs demonstrated no significant improvement in LBR following screening hysteroscopy prior to IVF treatment. However, in patients experiencing RIF, hysteroscopy may offer potential benefits, as indicated by the Good Practice Recommendations on RIF (ESHRE Working Group on Recurrent Implantation Failure, 2023).

Screening hysteroscopy is currently not recommended for routine clinical use.

Screening hysteroscopy can be considered in patients with recurrent implantation failure.

Endometrial receptivity tests

The mechanisms underlying human endometrium receptivity are complex and not well understood. Still, tests have emerged that investigate endometrial receptivity and classify the endometrium as being pre-receptive, receptive, or proliferative. The test results are then used to guide personalized ET (pET), in which the timing of the ET is set according to the receptiveness (Craciunas *et al.*, 2019). These tests have been mainly applied to patients presenting with RIF (Hashimoto *et al.*, 2017; Cohen *et al.*, 2020; Cozzolino *et al.*, 2020; Eisman *et al.*, 2021) but also to recipients of donated oocytes (Neves *et al.*, 2019) and good prognosis patients (Bassil *et al.*, 2018).

Efficacy

Several small retrospective studies failed to demonstrate a positive effect of endometrial receptivity tests in good prognosis patients (Bassil *et al.*, 2018), in oocyte donation cycles (Neves *et al.*, 2019) or RIF (Cohen *et al.*, 2020; Cozzolino *et al.*, 2020; Eisman *et al.*, 2021). For the RIF group, the study by Hashimoto *et al.* (2017) showed some benefit of endometrial receptivity tests and pET with regard to PR. With regards to receptivity testing and pET in frozen ET cycles, conflicting results have also been reported (Barrenetxea *et al.*, 2021; Bergin *et al.*, 2021).

Craciunas *et al.* (2019) summarized seven studies published up to 2019, but the authors were unable to perform a meta-analysis owing to clinical and methodological heterogeneity in patient populations (number of previously failed cycles), reported comparisons and unit of analysis (per couple or cycle). The studies evaluated a total of 1209 women and reported PRs of pET between 42% and 80%, but they did not compare the PRs with controls undergoing ET.

A recent RCT evaluated endometrial receptivity analysis and pET on LBR after the first ET. The intention-to-treat analysis showed no effect on clinical outcomes (Simón *et al.*, 2020). Cumulative LBR that considered both the first ET and cumulative rates after 1-year follow-up were also similar in both groups. This article received significant criticism both on the design of the RCT (Lensen *et al.*, 2021b) (with a rebuttal in Simón *et al.* (2021)) and on the fundamental utility of the endometrial receptivity test (Ben Rafael, 2021).

The most recent and largest RCT is a double-blind, randomized clinical trial at 30 sites in the USA, including 767 women who had at least one cryopreserved euploid blastocyst. Patients were randomized to an intervention group, undergoing receptivity-timed frozen ET, with an adjusted duration of progesterone exposure prior to transfer if indicated by receptivity testing, or to a control group undergoing ET at standard timing, regardless of receptivity test results. In the women with at least one cryopreserved euploid blastocyst, the use of endometrial receptivity testing to guide the timing of frozen ET did not significantly improve LBR as compared with standard ET (58.5% (223/381) versus 61.9% (239/386); RR 0.95; 95% CI 0.79 to 1.13) (DoyLe *et al.*, 2022).

Similarly, a large retrospective observational multicentre cohort study, including data from 5372 ETs in women with a previously failed transfer (both autologous and donated oocyte cycles), could not demonstrate a better outcome after endometrial receptivity analysis and pET (Cozzolino *et al.*, 2022). Both with autologous and donated oocytes, the LBR and cumulative LBR were significantly lower after receptivity testing and pET compared to fresh ET.

In prospective and retrospective observational studies, endometrial receptivity tests have also been investigated in combination with other add-ons such as quantification of natural killer (NK) cells (Hviid Saxtorph *et al.*, 2020; Jia *et al.*, 2021) and preimplantation genetic testing for aneuploidy (PGT-A) (Tan *et al.*, 2018; Neves *et al.*, 2019).

Safety

The endometrial biopsy procedure is considered safe and serious complications are rare (Williams and Gaddey, 2020). Since following an endometrial receptivity test the ET is performed in a subsequent cycle, the impact of the procedure on a subsequent pregnancy is considered minimal. Nevertheless, it needs to be acknowledged that endometrial biopsy has been associated with

significantly higher VAS pain scores as compared with sham procedures (Nastri et al., 2013).

Recommendation

Robust data on the efficacy of endometrial receptivity tests are lacking. Additionally, the existing tests do not account for the intricate interplay between the endometrium and the embryo, including the timing, location, and depth of the biopsy procedure.

The presently available endometrial receptivity tests are not recommended.

Reproductive immunology tests and treatments, including NK cells, killer-cell immunoglobulin-like receptor (KIR), and HLA

Immunological tests

This section does not relate to women with auto-immune diseases, including thyroid disease and anti-phospholipid antibody syndrome, or to women who are taking immune treatments, such as steroids, for other medical indications.

Based on the idea that the mother and her foetus are genetically different, a situation that has drawn parallels with transplantation of organs between different individuals (Medawar, 1953), a controversial view emerged that the 'foetus is rejected' unless there is a modification of the maternal immune response. More recently, a claim has been made that it is the dominant leucocytes in the endometrium, uterine NK cells (uNK), that can kill the foetus (Sharma, 2014). This is incorrect because the foetus is always separated from the maternal immune system by the placenta and uNK are only weakly cytolytic and cannot kill placental cells (Moffett and Shreeve, 2015; Moffett and Shreeve, 2023).

Immunological tests applied in reproductive medicine include measurement of NK cell levels and function in blood, typing for Killer-cell immunoglobulin-like receptors (KIR) and HLA genotypes, regulatory T cells (Tregs), Th1/Th2 ratios, and cytokines such as granulocyte colony-stimulating factor (G-CSF). There is no clear rationale for performing any of these tests (Moffett and Shreeve, 2015). Importantly, the local uterine immune populations are quite different from those in blood. NK cells are measured as either numbers, percentages, ratios or with functional assays. The proportion of blood mononuclear leucocytes that are NK cells varies widely in normal individuals (5–25%). Despite this, an arbitrary cut-off (usually ~12%) has been used by clinics to infer that levels above this cut-off are abnormal. Overall, there is no information to be gained to help direct treatment in measuring the number or function of NK cells, Th1/Th2 ratios, or any other parameters in peripheral blood before or during pregnancy.

Endometrial biopsies to count NK cells are difficult to interpret because NK cell numbers increase rapidly during the secretory phase and vary depending on histological features such as the amount of oedema present and the distance from the surface epithelium. How numbers might relate to their functions is also unclear as it is still unknown exactly what uNK cells do in normal or abnormal pregnancies. Indeed, NK cell functions depend in part on inherited highly variable NK receptors (KIR) that differ between individuals.

Efficacy

A recent meta-analysis summarized available studies investigating uNK cell testing in recurrent pregnancy loss (RPL) and RIF and found no significant difference in LBR in women with high uNK versus normal uNK (RR 1.00; 95% CI 0.77 to 1.28; 3 studies; n = 229; $I^2 = 11\%$; $P = 0.97$) (Woon et al., 2022). All studies included were judged as having moderate to serious risk of bias. No correlation between peripheral blood and uNK cells was confirmed (Woon et al., 2022). From measurements of uNK, the review did show a modest increase in the ratio of uNK/stromal cells in women with RIF. However, the confounding factors in these studies are considerable; age, hormonal therapy, the timing of biopsy, and the definition of RIF varied, and BMI was not considered.

Safety

Most of these parameters are evaluated through a blood test, apart from uNK-cell testing, which requires a uterine biopsy.

Killer-cell immunoglobulin-like receptor (KIR) and HLA genotyping

The reason that genotyping women for one family of NK receptors, namely KIRs, was introduced by some clinics is that they are highly polymorphic meaning that women have their own inherited repertoire of KIR genes. Some members of the KIR family bind to HLA-C ligands expressed by the invading placental trophoblast cells (Moffett and Colucci, 2015). Several studies of pregnancy disorders, such as pre-eclampsia, that occur late in gestation are associated with certain combinations of maternal KIR and foetal HLA-C genetic variants (Moffett and Colucci, 2015). This suggests that successful placentation depends in part on interactions between uNK cells and trophoblast but exactly how uNK functionally mediate this is still unknown. All the evidence so far points to the increased number of uNK cells in early pregnancy acting in a physiological process and there is no evidence that they are ever detrimental to pregnancy (Alecsandru and García-Velasco, 2017).

Efficacy

Although certain combinations of maternal KIR and foetal HLA-C genotypes are associated with some pregnancy disorders, particularly pre-eclampsia, they have not been studied in RIF (Moffett et al., 2016). One report has looked at oocyte donation pregnancies where the risk of pre-eclampsia is high (~25%) (Alecsandru and García-Velasco, 2017).

Recommendation

There is a lack of a clear biological rationale or clinical relevance for blood tests assessing various immune parameters and uncertainty regarding their selection and interpretation. For uNK cells, there is general ambiguity regarding their role in endometrial function and implantation, and no consensus on reliable normal reference ranges. Moreover, any observed changes in immune parameters and uNK-cell tests may be attributed to the effects of altered global differentiation of the secretory endometrium after ovulation in response to progesterone, rather than having a causative role. KIR and HLA genotyping require more studies in large clinically well-characterized cohorts of similar ethnic groups with appropriate controls. Detailed reasons for why these tests should not be introduced at present are outlined in the review by Moffett et al. (2016).

Peripheral blood tests for immune parameters and uNK-cell testing are not recommended.

KIR and HLA genotyping is currently not recommended for routine clinical use.

Immunomodulating treatments

Several treatments have been proposed to somehow modulate the immune system during the implantation process and thereby improve implantation and live birth. These treatments include steroids, lipid emulsion (intralipid) infusion, intravenous immunoglobulin (IVIG), leucocyte immunization therapy (LIT), tacrolimus, anti-tumour necrosis factor (anti-TNF) agents, G-CSF, and hydroxychloroquine. More recently, some of these treatments (e.g. LIT, G-CSF) have been infused into the uterus. The use of IVIG for recurrent miscarriage is covered in the ESHRE Recurrent Pregnancy Loss guideline (ESHRE Guideline Group on RPL *et al.*, 2023).

Efficacy

A recent systematic review and meta-analysis of interventional studies that were considered very low to low quality came to the conclusion not to recommend any of these immune treatments (Melo *et al.*, 2022). The use of intralipids was evaluated in two RCTs including 244 patients in which the pooled effect of intralipids on the LBR was uncertain (RR 1.78; 95% CI 0.95 to 3.34; $I^2 = 26\%$). The use of IVIG has mostly been investigated in cohort studies, pointing towards a higher LBR. However, only one RCT was identified, including 51 patients, and demonstrated no clear effect of IVIG on the LBR (RR 1.28; 95% CI 0.32 to 5.16; low-certainty evidence). Recombinant human Leukaemia inhibitory factor (LIF) was administered in one RCT, which showed a lower LBR in women receiving LIF compared to placebo (RR 0.47; 95% CI 0.24 to 0.91; n = 150; low-certainty evidence). Two RCTs, including 312 patients, were identified where intrauterine peripheral blood mononuclear cell (PBMC) treatment was compared with a placebo or no intervention. A pooled RR of 2.03 (95% CI 1.33 to 3.10; $I^2 = 0$) was found for LBR in favour of PBMC treatment; however, this was deemed very low-quality evidence (Melo *et al.*, 2022).

Details of intrauterine instillation of G-CSF and treatment with steroids can be found in the clinical management section.

Safety

Immunomodulation in ART has many known side-effects, some of which are serious (Moffett and Shreeve, 2015). Those for Intralipid therapy include hepatomegaly, jaundice, cholestasis, splenomegaly, thrombocytopenia, leucopenia, and fat overload syndrome; with IVIG treatment, aseptic meningitis, renal failure, thromboembolism, haemolytic reactions, anaphylactic reactions, lung disease, enteritis, dermatologic disorders, and infectious diseases have been reported; while with anti-TNF treatment, infection, lymphoma, demyelinating disease, autoantibody induction, congestive heart failure, injection site reactions, and lupus-like syndrome were found (Moffett and Shreeve, 2015; Sfakianoudis *et al.*, 2021). Tacrolimus has been shown to result in malformations in 4 out of 100 pregnancies in mothers using the agent after organ transplantation (Ali *et al.*, 2018).

Recommendation

Immunomodulating treatments, such as Intralipid, IVIG, rh-LIF, PBMCs, and anti-TNF, lack biological rationale, and evidence of clinical benefit. Additionally, potential serious side effects have been reported in other patient populations.

Immunomodulating treatments, such as Intralipid, IVIG, rh-LIF, PBMCs, and anti-TNF, are not recommended.

An overview of all recommendations on diagnosis and diagnostic tests with their level of evidence, benefit versus harm and other considerations that contributed to their formulation are available in Table 2.

Laboratory tests and interventions

Artificial oocyte activation

Physiological oocyte activation requires a sperm-derived enzyme called phospholipase C zeta (PLCζ) to induce the release of calcium (Ca^{2+}) in the form of oscillations from internal stores.

Oocyte activation occurs physiologically as a synergy between the sperm and oocyte. When there is a deficiency in the intracellular Ca^{2+} level, irrespective of whether the sperm or the oocyte is causative, this would negatively affect the process of activation, sometimes even precluding the use of ICSI to achieve fertilization. Nevertheless, human oocytes are tolerant to perturbations in Ca^{2+} balance as long as it is guaranteed that the total amount of Ca^{2+} available is uncompromised and passes a critical threshold. Consequently, Ca^{2+} levels can be brought up artificially—which is referred to as artificial oocyte activation (AOA)—by tapping into either of two potential Ca^{2+} sources: internal calcium stores and/or external culture medium.

There are several ways to perform AOA, none of which will result in physiological Ca^{2+} oscillations. Instead, mechanical, electrical, or chemical stimuli will generate a single Ca^{2+} peak (Kashir *et al.*, 2022). The least effective method to initiate AOA would be to modify the ICSI technique itself by making the injection process slightly more invasive (Tesarik *et al.*, 2002), which should cause the release of Ca^{2+} from internal stores owing to the additional mechanical manipulations with the injection pipette, or to accumulate metabolically active mitochondria at the site of fertilization (Ebner *et al.*, 2004). Alternatively, direct current voltage can create pores in the oolemma which would allow entry of extracellular calcium (Yanagida *et al.*, 1999). Since these AOA methods are associated with a high degeneration rate (Yanagida *et al.*, 1999) or require special equipment, the currently most common approach is using chemical compounds for AOA, mainly Ca^{2+}-ionophores such as calcimycin or ionomycin.

Calcimycin (also known as A23187) is an antibiotic that binds bivalent ions (mainly Mn^{2+}, Ca^{2+}, and Mg^{2+}) and allows their transport across biological membranes (Kashir *et al.*, 2022). Ionomycin is more widely used in ART because of its higher potency owing to its higher specificity for Ca^{2+}-ions, particularly if the ionomycin application was combined with the direct injection of 0.1 mol/l $CaCl_2$ during ICSI (Nikiforaki *et al.*, 2016).

The application of chemical AOA can be considered in cases of complete fertilization failure in a previous IVF/ICSI cycle, poor fertilization outcome (<30%), and cases of severe male factor infertility (Kashir *et al.*, 2022).The procedure is easy and based on the transfer of injected oocytes immediately after ICSI (0–60 min) to a pre-equilibrated ionophore solution for a 10–30 min culture followed by a series of washing steps.

Ionophores are also used to increase the mitotic cleavage rate of embryos in cases of previous embryonic arrest, developmental delay or low blastocyst formation (Ebner *et al.*, 2015b; Mateizel *et al.*, 2022; Shebl *et al.*, 2022). Although this may make sense as mitosis is also strongly Ca^{2+}-dependent, we have not included these applications as they are not considered classic AOA, even if they would be considered an add-on intervention.

Efficacy

A meta-analysis pooling results of 14 studies (4 RCTs, 4 prospective, 5 retrospective and one historical cohort study) showed that AOA with any kind of calcium ionophore increased LBR (OR 2.65;

Table 2. Overview of all recommendations on diagnosis and diagnostic tests with their level of evidence, benefit versus harm and other considerations that contributed to their formulation.

Intervention		Benefits versus harms (efficacy versus safety)	Level of evidence for efficacy (LBR/CPR)[1]	Level of evidence for safety[1]	Considerations	Recommendation
Screening hysteroscopy		Unselected patients: no benefit on LBR RIF: might be beneficial effect on LBR No evidence of an effect on miscarriage rate Complications are minimal	⊕⊕◯◯	⊕⊕◯◯	/	Screening hysteroscopy is **currently not recommended for routine clinical use**. Screening hysteroscopy **can be considered** in patients with recurrent implantation failure.
Endometrial receptivity tests		No effect on LBR, inconclusive effect on cLBR No data on safety, biopsy procedure can be painful	⊕⊕◯◯	No data	Clinical and methodological heterogeneity in patient populations (number of previously failed cycles), reported comparisons and unit of analysis (per couple or per cycle)	The presently available endometrial receptivity tests are **not recommended**.
Immunology tests and treatments	Immunology tests	Benefit on LBR or miscarriage rate is unclear due to lack of understanding of the mechanisms Harms: misinformation	⊕◯◯◯	No data	No rationale for these tests, no standardization	Peripheral blood tests for immune parameters and uNK-cell testing are **not recommended**. KIR and HLA genotyping is **currently not recommended for routine clinical use**.
	Immunology treatments	Benefit on LBR and miscarriage rate are unclear Significant safety concerns	⊕⊕◯◯	No data	No rationale for these treatments, no standardization	Immunomodulating treatments, such as Intralipid, IVIG, rh-LIF, PBMCs, and anti-TNF, are **not recommended**.

[1] Quality of Evidence Grades: ⊕⊕⊕⊕, body of evidence is of high quality (at least evidence from RCTs); ⊕⊕⊕◯, body of evidence is of moderate quality (evidence from RCTs or a number of observational studies showing a similar large effect); ⊕⊕◯◯, body of evidence is of low quality (mainly observational data); ⊕◯◯◯, body of evidence is of very low quality (few observational data).
cLBR: cumulative live birth rate; IVIG: intravenous immunoglobulin infusion; rhLIF: recombinant human leukaemia inhibitory factor; PBMC: peripheral blood mononuclear cell; PGT-A: preimplantation genetic testing for aneuploidy; RIF: repeated implantation failure; RCT: randomized controlled trial; KIR: killer-cell immunoglobulin-like receptor; uNK: uterine natural killer cells; TNF: tumour necrosis factor; PBMC: peripheral blood mononuclear cell.

95% CI 1.53 to 4.60; 14 studies; n = 3621; I^2 = 80%; P = 0.0005, publication bias detected) (Shan *et al.*, 2021). In a subgroup analysis, AOA with calcium ionophore was shown to significantly increase the LBR in patients with previous fertilization failure or low fertilization rate (OR 4.76; 95% CI 2.01 to 11.25; 7 studies; n = 1294; I^2 = 65%; P = 0.0004) and those with embryo developmental problems (embryonic development block, sperm factor or diminished ovarian reserve) (OR 4.59; 95% CI 1.35 to 15.65; 4 studies; n = 461; I^2 = 72%; P = 0.01). There was no significant effect on the miscarriage rate (OR 0.78; 95% CI 0.57 to 1.07; 13 studies; n = 1709; I^2 = 0%; P = 0.12).

Apart from complete fertilization failure, globozoospermia is the only indication that requires ionophore-based AOA to achieve fertilization. With sperm from a globozoospermic patient, AOA with ionomycin resulted in a higher amplitude of the intracellular Ca^{2+}-rise during ICSI compared to calcimycin and therefore could be the first-line option, even if the fertilization rate was not significantly different between compounds (30% versus 11.8%, respectively) (Nikiforaki *et al.*, 2016).

The use of one of the most promising AOA promoters, recombinant PLCζ, which was shown to induce repeated calcium oscillations in human oocytes (Yoon *et al.*, 2012) similar to those caused by sperm, is still in its experimental phase.

One obstacle when comparing studies dealing with ionophore-based AOA or interpreting meta-analyses is the variation in ionophore stimulus with respect to concentration, exposure time, and the number of exposures.

Safety

Ca^{2+}-ionophores can bind Ca^{2+}-cations and owing to their hydrophobic properties they form a complex at the lipid bilayer of the membrane. The conformation of their tertiary structure allows ionophores to then transport Ca^{2+}-molecules across the membrane and release them into the cytosol (Brasseur *et al.*, 1983). Thus, ionophores themselves do not necessarily enter the oocyte, which might explain the lack of detectable effect of ionophores on chromosomal segregation (Capalbo *et al.*, 2016), gene expression (compared to conventional IVF treatment), or morphokinetics (Shebl *et al.*, 2021). Furthermore, no increase in birth defects has been reported (Deemeh *et al.*, 2015; Miller *et al.*, 2016; Mateizel *et al.*, 2018; Li *et al.*, 2019a; Long *et al.*, 2020) and cognition, as well as language and motor skills, were normal in children aged 3–10 years born after AOA with ionophores (Vanden Meerschaut *et al.*, 2014). Congenital birth defects were reported in 13 out of 22 studies included in a recent meta-analysis on AOA. The reviewers observed no significant difference in birth defects

between the ICSI-AOA group and ICSI-only group (OR 1.33; 95% CI 0.70 to 2.53; 13 studies; n = 4320; $I^2 = 0\%$; P = 0.38), nor in the calcimycin or ionomycin subgroup (Shan *et al.*, 2021). However, because of the nature of the artificial Ca^{2+} signal (single Ca^{2+}-peak instead of oscillatory pattern), ionophores should only be used with proper indication.

Recently, changes in DNA methylation and gene expression have been observed using ionomycin in a mouse model (Yin *et al.*, 2021). Similarly, calcimycin was found to change the methylation level of the imprinted gene H19 in cleavage-stage embryos but not in blastocysts in a small-scale human study (Liang *et al.*, 2022).

Recommendation

There is evidence suggesting the effectiveness of AOA in certain situations such as complete activation failure (0% 2PN), very low fertilization (<30% fertilization), or globozoospermia. However, it is crucial to maintain continuous monitoring and assessment of the long-term effects and safety of children born through this procedure. Further research in this field is strongly encouraged and necessary.

Artificial oocyte activation is currently not recommended for routine clinical use.

Artificial oocyte activation is recommended for complete activation failure (0% 2PN), very low fertilization (<30% fertilization), or globozoospermia.

Mitochondrial replacement therapy

A clear distinction must be made between two very different aims of mitochondrial replacement therapy: the first aim is to avoid the transmission of mitochondrial DNA (mtDNA) diseases through the mtDNA present in the oocyte, while the second aim, which is considered an add-on, is to improve the quality of the oocytes in women with difficulties in conceiving linked to oocyte quality and/or fertilization failure. Nevertheless, the methodology of both strategies is the same, with the nuclear DNA of the prospective parents being transferred to enucleated donor oocytes. This has led to the term 'three-parent reproduction' because, besides the nuclear DNA provided by the parents, the ensuing embryo and child will carry mtDNA from the donor oocyte. The different techniques for mitochondrial replacement therapy, such as maternal spindle nuclear transfer (Tachibana *et al.*, 2013), pronuclear transfer (Hyslop *et al.*, 2016), and polar body nuclear transfer (Ma *et al.*, 2017), have been recently described and explained by Craven *et al.* (2017) and Siristatidis *et al.* (2022).

A variant technique whereby autologous mitochondria extracted from oocyte precursor cells, isolated from an ovarian cortex biopsy, are injected during ICSI into oocytes with diminished function was developed and is commercially available (Woods and Tilly, 2015). An RCT comparing autologous mitochondria transfer with regular ICSI was discontinued prematurely because of negative results (Labarta *et al.*, 2019). This technique is now suspended and not discussed further here.

Efficacy

Only a few papers have been published so far. Reports of healthy births after mitochondrial replacement therapy are only available in newspapers and websites. In a case report where the spindle transfer was applied in one patient carrying a mtDNA mutation and two patients with fertilization failure the authors demonstrated full replacement of the mitochondria in all cases. Still, the study was pre-clinical and all embryos obtained were used for further investigations (Tang *et al.*, 2022).

Safety

Given the limited clinical data, the complexity of the interventions and the considerable room for further basic research, the safety of nuclear transfer cannot be established (Siristatidis *et al.*, 2022). This is added to the significant concern regarding ethical questions (Craven *et al.*, 2017; Adashi and Cohen, 2018).

Kang *et al.* (2016) have shown that in some cases the acceptors' mtDNA haplotype takes over the donors' mtDNA.

Recommendation

Mitochondrial replacement therapy is considered experimental, and in many instances not allowed. Furthermore, there is insufficient evidence of a benefit on pregnancy outcomes or safety. Therefore, it should only be applied in strict research protocols, ensuring the safety of the patients and donors involved, as well as guaranteeing long-term follow-up of their offspring.

Mitochondrial replacement therapy to affect oocyte quality is not recommended.

In vitro *activation of dormant follicles*

In patients with premature ovarian insufficiency (POI), ART has limited efficacy which is attributed to inactive or dormant follicles that cannot be stimulated to produce mature oocytes. Growing evidence supports involvement of the TGFβ/SMAD, JAK/STAT, and MAPK cascades in this process (Grosbois *et al.*, 2020). *In vitro* activation (IVA) was proposed to activate dormant follicles. This procedure technically consists of activating the AKT pathway with phosphatase and tensin homolog (PTEN) enzyme inhibitors and phosphatidylinositol-3 kinase activators following ovarian fragmentation and prior to ovarian tissue transplantation (Wang *et al.*, 2021a). This can also be achieved by ovarian fragmentation only, i.e. drug-free IVA. Recently, the technique was also applied to patients with poor ovarian response (Díaz-García *et al.*, 2022).

Efficacy

Owing to the low chances of spontaneous conception in women with POI (Nelson, 2009), it is not surprising that there are no RCTs (or comparative studies) that compare IVA or drug-free IVA technique with expectant management. IVA has been evaluated in 51 women to whom a total of 3 babies were born, whereas drug-free IVA has been evaluated in 5 studies in which 15 babies were born to 126 women with POI (Wang *et al.*, 2021a).

A recent RCT in 34 women with poor ovarian response showed an increase in antral follicle count (AFC) in the ovary in which ovarian fragmentation for follicular activation was performed compared to the control ovary. An increased AFC was also reported in women after IVA compared to controls, but there was no effect on serum anti-Müllerian hormone and FSH levels or reproductive outcomes (LBR 6.7% versus 18.7% in the IVA and control groups, respectively) (Díaz-García *et al.*, 2022).

There are no established data for the cost per live birth in patients treated with either classical or drug-free IVA when the activation solutions, required surgical interventions and hospitalization are considered.

Safety

There are no data on the safety, adverse side-effects or long-term effects of the exposure of the oocyte, subsequent embryo and,

hence, on the health of the offspring. There are also no reports of adverse events from the procedure, even if it carries risks inherent to any surgical intervention.

Recommendation

Considering the limited efficacy, potential high cost, and safety concerns, IVA of dormant follicles is considered experimental and can only be applied within strict research protocols.

In vitro activation of dormant follicles is not recommended.

IVM

IVM is applied to obtain mature oocytes from immature cumulus–oocyte complexes retrieved from antral follicles (De Vos *et al.*, 2021). The technique is mainly used for women with polycystic ovary syndrome (PCOS) to avoid the risk of ovarian hyperstimulation syndrome (OHSS), and in the context of fertility preservation when conventional ovarian stimulation is contraindicated, or when the time available before the start of gonadotoxic treatment is short and cannot be delayed for ovarian stimulation treatment (ESHRE Guideline Group on Female Fertility Preservation *et al.*, 2020). When indicated in patients with PCOS, high responders or for fertility preservation, IVM is not considered an add-on.

IVM has been used in women with regular cycles and normal ovaries (Chang *et al.*, 2014), for infertile patients preferring a shorter, less hormonally taxing treatment. IVM can be considered an add-on in these situations.

Clinical IVM

Clinical IVM is performed in a natural cycle with minimal or no ovarian stimulation, and OPU is performed when the leading follicle measures between 9 and 12 mm (Fadini *et al.*, 2011; Wiser *et al.*, 2011).

Efficacy

A study included 536 women in their first IVM cycles and excluded repeated cycles and fertility preservation cycles (Wiser *et al.*, 2011). The ongoing PRs in women aged 20–25 years were 36.8%, 26–35 years were 30.0%, and those in 36–39 years were 31.9%. No clinical pregnancy was detected in women older than 40 years.

In a study including 177 normo-ovulatory women, 991 oocytes were recovered for IVM and microinjected. Twenty-eight biochemical pregnancies were reported, 25 of which developed into clinical pregnancies (14.1%/OPU or 16.6%/ET) involving 30 gestational sacs with a foetal heartbeat (Fadini *et al.*, 2011).

Gulekli *et al.* described two cases of women with oocyte maturation arrest undergoing IVM (GulekLi *et al.*, 2011). In the first woman, all immature oocytes were arrested in stage MI while in the second woman, one oocyte reached maturity but the ICSI procedure resulted in abnormal fertilization. Hourvitz *et al.* (2010) described a case series of IVM in seven women with abnormal follicular development. Three women had an ET of three to four embryos and two achieved a live birth. IVM has also been successfully applied to a woman with antral follicles that were unresponsive to endogenous and exogenous FSH (Grynberg *et al.*, 2013).

Performing IVM requires modified procedures and appropriate expertise. IVM requires no or minimal ovarian stimulation and consequently less time, monitoring and medication and fewer injections. It has been suggested that this results in a lower financial and emotional burden as compared to standard IVF/ICSI treatment (ASRM, 2021). A recent cost-effectiveness study showed IVM is less expensive than IVF treatment in women with a high AFC (Braam *et al.*, 2021). Additionally, performing IVM requires modified procedures and appropriate expertise.

Safety

Currently available data do not indicate an increase in imprinting errors after IVM, or a difference in the neonatal health and developmental outcome of children conceived with the technique as compared to those conceived through IVF/ICSI treatment (ASRM, 2021; Nguyen *et al.*, 2022; Vuong *et al.*, 2022). Aneuploidy rates also seem not to be increased (Li *et al.*, 2021b). However, these conclusions are based on limited data and need further exploration.

Rescue IVM or natural cycle IVF/M treatment

Rescue IVM has been used in poor responders or poor prognosis patients to increase the number of embryos available for transfer by combining the mature oocytes with IVM of the immature oocytes (prophase I (PI) or metaphase I (MI)) that were collected at OPU (Braga *et al.*, 2010).

Efficacy

There are a few small studies describing IVM performed in various groups of patients and heterogenous settings using different stimulation protocols (Liu *et al.*, 2003; Reichman *et al.*, 2010; Xu *et al.*, 2010; Li *et al.*, 2011; Álvarez *et al.*, 2013; Shin *et al.*, 2013; Lee *et al.*, 2016; Hatırnaz *et al.*, 2018; Al-Hussaini *et al.*, 2019; Chansel-Debordeaux *et al.*, 2021; Hatirnaz *et al.*, 2021; Yang *et al.*, 2012a), and hence, it is impossible to draw conclusions on the efficacy of the technique.

In a prospective cohort study, 77 regularly cycling women underwent a combination of IVF and IVM treatment (Tang-Pedersen *et al.*, 2012). When cycles with immature oocytes (both germinal vesicle (GV) and MI, and cultured in maturation medium with hormones) versus mature oocytes at retrieval were compared, LBRs of 6.7% and 10.7% were obtained, respectively.

In a prospective cohort study, 146 poor prognosis patients received rescue IVM (Group 1, n = 50; GV and MI oocytes) or double ovarian stimulation (Group 2, n = 96) (Liu *et al.*, 2020b). Immature oocytes were matured in culture medium without the addition of hormones. There was no significant difference seen in LBR (10% versus 16.9%) when the IVM part in Group 1 was compared to the luteal phase stimulation part in Group 2.

In a large cohort study, 440 poor-responder patients with <5 mature and at least one immature oocyte undergoing ICSI were divided in two groups (Braga *et al.*, 2010). Immature oocytes were matured in culture medium without the addition of hormones. In Group 1, only mature oocytes were injected, and in Group 2, cycles were included where at least one immature oocyte remained in culture for spontaneous maturation and injected for ICSI. No significant differences were found between mature and rescue IVM groups for clinical PR (CPR: 16.7% versus 16.5%, respectively) or miscarriage rate (25.5% versus 29.4%). However, the number of transferred embryos was higher in the rescue IVM group (1.87 ± 1.24 versus 2.35 ± 1.22). In 17 cycles, only embryos derived from rescue spontaneous maturation oocytes were available for transfer and two pregnancies were achieved (Braga *et al.*, 2010).

Safety

The safety of rescue IVM is questionable since these oocytes commonly have meiotic defects and are of poor quality (De Vos *et al.*, 2021).

Recommendation

There is a lack of established effectiveness, procedural reliability and long-term safety data for both clinical and rescue IVM in infertile patients.

Clinical IVM and rescue IVM or natural cycle IVF/M are currently not recommended for routine clinical use.

Sperm DNA damage testing/treatment and sperm oxidative stress measurement

It is suggested that sperm chromatin damage, indicated by sperm DNA fragmentation (SDF), plays a role in male infertility and reproductive outcome (Agarwal *et al.*, 2020). Various methods have been developed to evaluate SDF. The most commonly used tests are terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling (TUNEL), *in situ* nick translation assay (ISNT), sperm chromatin structure assay (SCSA), sperm chromatin dispersion test (SCD), and the Comet assay (Esteves *et al.*, 2021). Each test may have different clinical thresholds owing to the different DNA damage sites detected and the different technical aspects of each assay (Agarwal *et al.*, 2020).

Increased SDF levels have been observed in various conditions such as varicocele, accessory gland infection, advanced paternal age, cancer, chronic illness, exposure to environmental toxins, and lifestyle factors (Esteves *et al.*, 2020). DNA fragmentation is characterized by single-strand breaks (SSBs) and double-strand breaks (DSBs). Both SSBs and DSBs can affect male fertility but DSBs have more pronounced effects, negatively affecting embryo kinetics and implantation rates, and increasing the rate of recurrent miscarriages, while SSBs do not seem to significantly affect embryo development or implantation rates (Casanovas *et al.*, 2019; Agarwal *et al.*, 2020).

SDF can be caused by intrinsic and extrinsic factors, with the major contributor being oxidative stress (OS) (Aitken, 2020). Hence, the measurement of OS has also been proposed as a surrogate marker of SDF. A moderate association between OS and SDF has been previously reported (Henkel *et al.*, 2005; Mahfouz *et al.*, 2010; Homa *et al.*, 2019). It was reported that an oxidation reduction potential (ORP) cut-off value of 1.36 mV/106 sperm/mL could predict fertilization (Morris *et al.*, 2019). However, other studies reported little (Majzoub *et al.*, 2018; Arafa *et al.*, 2019) or no correlation between ORP and SDF (Homa *et al.*, 2019).

Efficacy

A systematic review and meta-analysis, including 20 prospective observational studies and eight case-control studies, showed that infertile men had higher SDF compared to fertile counterparts (mean difference (MD) −1.67; 95% CI −2.12 to −1.21; 28 controlled studies; n = 4177; $I^2 = 97\%$), and the SDF threshold level to discriminate infertile from fertile men was set to 20% (AUC 0.844, $P < 0.001$) (Santi *et al.*, 2018).

It has been proposed that SDF is associated with the fertilizing potential of the sperm and subsequent medically assisted reproduction (MAR) outcomes. However, the predictive value of SDF on pregnancy, live birth or miscarriage is still inconclusive as the quality of evidence is low and there is significant heterogeneity between different studies included in systematic reviews and meta-analyses (Zini, 2011; Zhao *et al.*, 2014; Osman *et al.*, 2015; Simon *et al.*, 2017; Ribas-Maynou *et al.*, 2021).

There seems to be weak evidence for the predictive value of SDF testing in patients with varicocele and RPL (Robinson *et al.*, 2012; Wang *et al.*, 2012; Zhao *et al.*, 2014; McQueen *et al.*, 2019; Tan *et al.*, 2019b; Yifu *et al.*, 2020; ESHRE Guideline Group on RPL *et al.*, 2023) suggesting that SDF testing may have a limited value in these patients (Cho and Agarwal, 2018; Dai *et al.*, 2021).

As the test for DNA fragmentation index (DFI) renders the tested sperm unusable for ICSI, advanced sperm selection techniques might be valuable to detect the appropriate sperm for injection. In an RCT, 302 men with abnormal SDF were randomized to density gradient centrifugation (n = 72), physiological ICSI (PICSI; n = 78) or magnetic-activated cell sorting (MACS; n = 79). Applying advanced sperm selection techniques (PICSI or MACS), rather than standard density gradient centrifugation, resulted in higher CPRs (69.2%, 67.1%, and 51.4%, respectively; $P = 0.025$) (Hozyen *et al.*, 2022). In contrast, in a prospective cohort study, including 80 males with DFI ≥30%, no difference in CPR was found with the use of MACS (Mei *et al.*, 2022).

As the passage of sperm through the seminiferous tubules and the epididymis might be a potential trigger to OS, leading to high SDF (Xie *et al.*, 2020), testicular sperm extraction (TESE) has been preferred in selected groups of patients. The most recent meta-analysis, including six cohort studies involving 578 male infertility patients with cryptozoospermia (761 ICSI cycles), reported significantly higher PRs with the utilization of sperm retrieved via TESE (RR 1.74; 95% CI 1.20 to 2.52) (Kang *et al.*, 2018). A meta-analysis, including four observational studies involving 507 ICSI cycles from male infertility patients with high SDF also presented a higher CPR with testicular sperm than with ejaculated sperm (50% versus 29.4%; OR 2.42; 95% CI 1.57 to 3.73) (Esteves *et al.*, 2017). Interpretation of the results is hindered primarily by the moderate quality of the available evidence and lack of matching for confounding factors (e.g. lifestyle factors, empiric treatments), making it necessary to conduct prospective large-scale RCTs for a clearer understanding.

A meta-analysis indicated a fair discriminatory capacity of the TUNEL and Comet assays in predicting pregnancy after IVF and ICSI treatment, but poor predictive capacity for pregnancy with MAR for SCSA and SCD. For SCSA, a meta-regression analysis indicated a difference in predictive value for pregnancy for IVF and ICSI (Cissen *et al.*, 2016). Laboratory conditions, such as incubation time, centrifugation and cryopreservation (Zini, 2011; Agarwal *et al.*, 2020), as well as the source of the sperm (ejaculated or processed (Liu and Liu, 2013; Aboulmaouahib *et al.*, 2017) or testicular (Agarwal *et al.*, 2020)), can significantly influence the results of SDF tests. Furthermore, there is no guarantee that the individual sperm one uses for ICSI is free of strand breaks.

Safety

No safety issues have been reported.

Recommendation

There is insufficient evidence for the relevance of SDF tests to predict pregnancy or guide treatment decisions. Further research in this field is strongly recommended to enhance our understanding and knowledge.

Sperm DNA damage testing is currently not recommended for routine clinical use.

Artificial sperm activation

Immotile sperm is one of the key problems in severe male factor infertility because embryologists face the problem of distinguishing between immotile but viable sperm and non-viable sperm.

Typically, aids such as manipulation with ICSI needles, hypo-osmotic solutions or laser pulses are used to identify viable spermatozoa with functional membranes, however, only pharmacological activation using chemical compounds would allow partial restoration of sperm motility in immotile but viable sperm.

cAMP is the key molecule driving sperm motility and any deficiency in its level would cause distinct asthenozoospermia, if not immobility.

The prevalent method of artificial sperm activation is using phosphodiesterase (PDE) inhibitors to increase cAMP levels. The two PDE inhibitors routinely used are pentoxifylline (PTX) and theophylline. Any effect on sperm motility is expected within 3–5 min and lasts for 1–2 h. In clinical use, a small volume of the PDE inhibitors is added to the sperm sample or the suspension containing, for example, testicular tissue. Usually, incubation with PDE inhibitors is carried out in an ICSI dish to facilitate the identification and catching of the sperm considered for the ICSI procedure. Before injection, spermatozoa are washed in culture medium and/or polyvinylpyrrolidone to avoid carryover of PTX or theophylline to the oocyte.

Efficacy

An RCT on 120 patients with mild to moderate asthenozoospermia revealed that use of spermatozoa artificially stimulated with PTX resulted in a significantly higher CPR (73.3% versus 60%, respectively, $P = 0.04$) (Amer *et al.*, 2013).

In a sibling oocyte approach (n = 842 oocytes), ICSI with frozen-thawed sperm, activated with ready-to-use theophylline, resulted in significantly higher rates of fertilization (79.9% versus 63.3%), blastocyst formation (63.9% versus 46.8%), clinical pregnancy (53.9% versus 23.8%), and LBR (53.9% versus 19.1%) as compared to ICSI with frozen-thawed unstimulated testicular sperm (Ebner *et al.*, 2011).

It has to be clarified that in cases of primary cilia dyskinesis, such as Kartagener syndrome and related structural problems, any treatment with PDE inhibitors will be ineffective (Yildirim *et al.*, 2009; Ebner *et al.*, 2015a). At the same concentration, PTX and theophylline have comparable activity, however, the half-life of theophylline is 10-fold higher.

PTX/theophylline are usually used pre-ICSI when testicular or frozen sperm, or sperm from retrograde ejaculation, is to be used which often shows poor motility, if any at all. Any improvement in outcome cannot be attributed to the PDE inhibitor itself but to the improved sperm selection process and time-saving for this process since sperm reacting to these PDE inhibitors immediately become motile.

Safety

Carryover of PTX and theophylline to oocytes during ICSI and contact with embryos should be kept to a minimum. Incubation of embryos in PDE inhibitors over several days was associated with developmental retardation or embryo arrest in a mouse model (Fisher and Gunaga, 1975). Parthenogenetic activation of mouse eggs has also been reported (Scott and Smith, 1995). Of note, exposure times and concentrations of sperm-activating agents used in IVF labs are significantly lower than those applied in the animal studies mentioned above.

In humans, no malformations have been observed in babies born from embryos fertilized with sperm treated with theophylline (Ebner *et al.*, 2011; Sandi-Monroy *et al.*, 2019). In the case of PTX, the malformation rate per live birth (one study, n = 122 newborns) was 3.3% (4/122; 95% CI 0.9% to 8.2%) (Navas *et al.*, 2017), which was considered a non-increased risk as compared to historical IVF data.

Recommendation

There are no studies evaluating artificial sperm activation treatment in a general male infertility population. Sperm activation with PDE inhibitors has been shown to be of benefit in cases of primary or secondary total asthenozoospermia which are not the result of axonemal structure defects (Ebner *et al.*, 2015a). It is crucial to conduct continuous monitoring and follow-up to assess the long-term effects and safety of children born through this approach.

Artificial sperm activation is currently not recommended for routine clinical use.

Artificial sperm activation is recommended for patients with primary or secondary total asthenozoospermia which are not the result of axonemal structure defects.

Advanced methods of sperm evaluation and selection

According to the World Health Organization (WHO) standards, analysis of the human semen sample is included in a male fertility evaluation. Traditionally, sperm count, sperm motility and morphology are analysed to assess male reproductive function and to evaluate fertility potential and choice of suitable treatment modalities for an infertile couple (WHO, 2021). While sperm analysis results can help select the MAR treatment (IUI, IVF, or ICSI) that is the most efficient method, at a minimum cost and with minimal intervention, the analysis has limited ability to effectively predict the fertilizing capability of the individual sperm sample. This has led to the development of other sperm analysis tests such as *in vitro* sperm functional assays, sperm nuclear maturity, DNA and chromatin normality, and sperm membrane functionality tests.

Sperm preparation is a method to optimize the identification of the sperm with the best potential and eliminate factors that are detrimental to fertilization. Traditional sperm preparation methods include density gradient centrifugation (DGC) and swim-up. Additional more sophisticated methods have been developed, such as sperm hyaluronic acid binding assay (HBA), MACS, microfluidics and electrophoretic sperm isolation and intracytoplasmic morphologic sperm injection (IMSI), aiming at achieving more accurate selection of functional spermatozoa. These advanced sperm selection approaches are based on sperm membrane characteristics, sperm size and motility (Vaughan and Sakkas, 2019).

Sperm hyaluronic acid binding assay and physiological ICSI

Hyaluronan or hyaluronic acid (HA) constitutes a major component of cumulus cells, and has been shown to selectively bind mature sperm with an intact acrosome and better morphology (Huszar *et al.*, 2003). The HA assay is based on the mature and intact sperm surface containing a receptor for HA or hyaluronidase, which binds to HA coated on the surface. The hyaluronan/HBA score has been suggested as an *in vitro* test to predict sperm fertilizing potential. The score is expressed as the value of the number of bound motile sperm versus the number of unbound motile sperm. Huszar *et al.* (2003) showed that the HBA score correlated with sperm motility and strict normal sperm morphology, suggesting that HBA binding reflects the semen quality indicated by routine semen analysis.

The sperm HA binding assay has also been used for sperm selection before ICSI, the so-called physiological ICSI (PICSI). The principle of the PICSI method is that binding to HA mimics the

natural mechanism of sperm selection, assuming that sperm expressing the HA receptor would be of high quality.

Efficacy

Several studies found a correlation between the HBA score and overall seminal quality (Ye *et al.*, 2006; Nijs *et al.*, 2010), while others investigated fertilization rates in IVF/ICSI in relation to the HBA score (Ye *et al.*, 2006; Kovacs *et al.*, 2011; Boynukalin *et al.*, 2012; Esterhuizen *et al.*, 2015). None of the studies found any predictive value of the HBA for fertilization or pregnancy, nor did the test aid in selecting an ART method (IVF or ICSI). One study, using washed semen rather than unprocessed ejaculate, reported an association between significantly lower hyaluronan-binding ability in samples resulting in lower IVF fertilization rates (<50% of oocytes fertilized) and SDF, indicating some relevance for the test (Pregl Breznik *et al.*, 2013). Also, the study by West *et al.* (2022) reported that lower HBA scores and sperm DNA quality were associated with poorer sperm quality that compromised treatment outcomes.

Evidence from the Cochrane systematic review and meta-analysis suggests that PICSI or sperm selection using HBA may have little or no effect on LBR (RR 1.09; 95% CI 0.97 to 1.23; 2 RCTs; n = 2903; $I^2 = 0\%$; low-quality evidence) but may reduce miscarriage (RR 0.62; 95% CI 0.46 to 0.82; 3 RCTs; n = 1065; $I^2 = 0\%$; low-quality evidence) (Lepine *et al.*, 2019). There have been studies reporting some benefits of HA-based selection to mitigate deleterious effects of damaged sperm DNA on treatment outcomes, particularly among older women (West *et al.*, 2022) or in patients with abnormal SDF (Hozyen *et al.*, 2022).

Safety

No safety issues have been shown. However, the manufacturer's recommendation that the optimal temperature for sperm HBA binding is 30°C should be taken into consideration when performing ICSI using the PICSI dish. There are a variety of available commercial products which select sperm based on HA receptor expression.

Recommendation

The sperm hyaluronic binding assay has limited clinical value with regard to the prediction of fertilization or pregnancy, or guiding of treatment selection, which is further hampered by limitations in the standardization of the test. The method may offer an advantage in some categories of patients. Similarly, PICSI, as a sperm selection method, may have little or no effect on live birth or CPR.

Sperm hyaluronic binding assay is currently not recommended for routine clinical use.

Physiological ICSI is currently not recommended for routine clinical use.

Magnetic-activated cell sorting

MACS uses colloidal magnetic microbeads conjugated with annexin V. The semen sample is passed through a column containing annexin V microbeads and apoptotic sperm expressing externalized phosphatidylserine are retained within the column and are thus deselected. The remaining selected sperm were shown to have better nuclear DNA integrity (Berteli *et al.*, 2017).

Efficacy

It has been suggested that the use of MACS on unprocessed semen or combined with DGC leads to the retrieval of spermatozoa with higher motility, normal morphology, and a lower SDF compared to DGC alone (Degheidy *et al.*, 2015; Berteli *et al.*, 2017; Anbari *et al.*, 2021). However, the effect of MACS on pregnancy and LBR is unclear. Based on currently available studies, a recent Cochrane systematic review and meta-analysis reported insufficient evidence of an effect of MACS sperm selection on LBR (RR 1.95; 95% CI 0.89 to 4.29; 1 RCT; n = 62; very low-quality evidence), CPR (RR 1.05; 95% CI 0.84 to 1.31; 3 RCTs; n = 413; $I^2 = 81\%$; very low-quality evidence), or miscarriage (RR 0.95; 95% CI 0.16 to 5.63; 2 RCTs; n = 150; $I^2 = 0\%$; very low-quality evidence) (Lepine *et al.*, 2019). An absence of a beneficial effect of MACS on pregnancy was confirmed by subsequent studies (Gil Juliá *et al.*, 2022; Norozi-Hafshejani *et al.*, 2022).

Safety

There are no data available regarding the safety of using MACS.

Recommendation

There is insufficient evidence of an impact of MACS on pregnancy and LBRs compared to traditional sperm preparation methods.

Magnetic-activated cell sorting is currently not recommended for routine clinical use.

Microfluidics

Microfluidics involves the study and control of small fluid volumes, ranging from picolitres to microliters, inside micrometre-sized channels (Sackmann *et al.*, 2014). Microfluidics-based technologies have been adapted for sperm selection and preparation, without the need for centrifugation, aiming to mimic the geometry of micro-confined regions within the female reproductive tract (Vaughan and Sakkas, 2019).

Efficacy

The use of microfluidic chambers may improve total motile sperm count, morphology and DNA integrity, and reduce ORP compared to conventional DGC (Quinn *et al.*, 2018; Gode *et al.*, 2019; Gode *et al.*, 2020). A study showed that the microfluidics technique significantly reduced the double strand SDF as compared to raw samples and swim-up (Pujol *et al.*, 2022). In a more recent RCT in 128 patients undergoing ICSI for male factor infertility, similar fertilization rates and number of good quality embryos were shown, but with a significant benefit in LBR of 59.4% compared to 35.9% in the control group (swim-up) ($P = 0.006$) (Aydın *et al.*, 2022). However, in an observational study of donor egg recipients (331 women), no benefit of microfluidics selection was found (CPR 55.6% compared to 58.9% in the DGC control group) (Srinivas *et al.*, 2022).

Safety

There are no data available regarding the safety of using microfluidics.

Other aspects

It has been hypothesized that relying solely on motility and size for sperm sorting by microfluidics will likely be replaced by further innovations, such as the addition of chemo-attractants, the integration of optics for dynamic high-speed imaging, or the use of electrical analysis to study the sperm flagellar beat frequency (Vaughan and Sakkas, 2019).

Recommendation

While a single small RCT has demonstrated a small increase in LBR, an observational study showed no benefit of using microfluidics for sperm selection. Further research is required to validate these findings and provide a more robust evidence base before making widespread recommendations.

Microfluidics can be considered.

Intracytoplasmic morphologic sperm injection

Intracytoplasmic morphologically selected sperm injection (IMSI) exploits a sperm selection method termed 'motile sperm organelle morphology examination' (MSOME). The method involves the observation and selection of sperm based on the absence of vacuoles in the sperm head at high magnification (>6000×) (Bartoov *et al.*, 2001).

Efficacy

A Cochrane systematic review and meta-analysis showed that IMSI results in similar LBR (RR 1.11; 95% CI 0.89 to 1.39; 5 RCTs; n = 929; I^2 = 1%; very low-quality evidence), CPR (RR 1.23; 95% CI 1.11 to 1.37; 13 RCTs; n = 2775; I^2 = 47%; very low-quality evidence), miscarriage rates per couple (RR 1.07; 95% CI 0.78 to 1.48; 10 RCTs; 2297; I^2 = 0%; very low-quality evidence) and miscarriage rate per pregnancy (RR 0.90; 95% CI 0.68 to 1.20; 10 RCTs; n = 783; I^2 = 0%, very low-quality evidence) compared to conventional ICSI (Teixeira *et al.*, 2020). Similar evidence was shown by other systematic reviews and meta-analyses (McDowell *et al.*, 2014; Duran-Retamal *et al.*, 2020).

The method of IMSI can be time-consuming and impacts workflow, especially in laboratories that do not use the method routinely.

Safety

There are no data available regarding the safety of using IMSI.

Recommendation

Based on the current available data, there is uncertainty regarding the clinical benefit of IMSI compared to conventional ICSI. Further research in this field is necessary to gain a better understanding of the potential benefits of IMSI as well as its implications.

Intracytoplasmic morphologic sperm injection is currently not recommended for routine clinical use.

Growth factor-supplemented embryo culture medium

Preimplantation human embryo development is regulated by growth factors of embryonic and maternal origin. These growth factors, such as EGF, TGF-α, IGF-I, IGF-II, PDGF-B, LIF, VEGF, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and their receptors are expressed in embryos and the female reproductive tract. Studies in animal models suggest that supplementation of embryo culture media with exogenous growth factors promotes embryo development and implantation (Hardy and Spanos, 2002). More limited data exist in the context of clinical IVF treatment.

Efficacy

A recent Cochrane systematic review and meta-analysis showed that the addition of GM-CSF in the embryo culture medium did not increase LBR (OR 1.19; 95% CI 0.93 to 1.52; 2 RCTs; n = 1432; I^2 = 69%; low-quality evidence) and did not reduce miscarriage rate (OR 0.75; 95% CI 0.41 to 1.36; 2 RCTs; n = 1432; I^2 = 0%; low-quality evidence) compared to culture in conventional media without GM-CSF (Armstrong *et al.*, 2020).

Safety

As growth factors act in both positive and negative synergy to produce an effect, the addition of a single growth factor to embryo culture media is questionable and will not necessarily elicit a beneficial effect. It is suggested that, if not well regulated, exogenous growth factors could have adverse effects on embryo development (Sunde *et al.*, 2016). In a recent study, it was shown that the addition of GM-CSF to embryo culture media resulted in a change in cell number and cell lineages, as well as an ectopic expression of NANOG transcription factor among trophectoderm cells in pre-implantation mouse embryos (Pock *et al.*, 2022).

The Cochrane review analysed the data on multiple gestations (OR 1.24; 95% CI 0.73 to 2.10; 2 RCTs; n = 1432; I^2 = 35%; very low-quality evidence), preterm birth (OR 1.20; 95% CI 0.70 to 2.04; 2 RCTs; n = 1432; I^2 = 76%; very low-quality evidence), birth defects (OR 1.33; 95% CI 0.59 to 3.01; I^2 = 0%; 2 RCTs; n = 1432; low-quality evidence), and aneuploidy (OR 0.34; 95% CI 0.03 to 3.26; I^2 = 0%; 2 RCTs; n = 1432; low-quality evidence) and reported no increased incidence of any adverse events but with a large degree of uncertainty (Armstrong *et al.*, 2020).

Recommendation

There is insufficient evidence for both the efficacy and safety of using culture media supplemented with GM-CSF. Further research is needed to better understand the potential benefits and risks associated with culture media supplements.

Growth factor-supplemented embryo culture medium is not recommended.

Assisted hatching

Failure of the embryo to hatch leads to entrapment within the zona pellucida (ZP) and implantation failure. Assisted hatching (AH) involves artificial disruption of the ZP to facilitate the escape of the blastocyst from the ZP after transfer. AH has been proposed as a method for increasing implantation and PRs in clinical IVF treatment (Cohen *et al.*, 1988; Hammadeh *et al.*, 2011).

Assisted hatching is performed either mechanically, chemically or using a laser. The type of ZP disruption can involve thinning, creating a small hole, a large hole, or complete removal of the ZP.

Efficacy

The most recent Cochrane review showed no significant effect of AH with regards to LBR compared to no AH (OR 1.09; 95% CI 0.92 to 1.29; 14 RCTs; n = 2849; I^2 = 20%; low-quality evidence), with slightly improved CPR (OR 1.20; 95% CI 1.09 to 1.33; 39 RCTs; n = 7249; I^2 = 55%; low-quality evidence) (Lacey *et al.*, 2021). From a subgroup analysis, it was suggested that in women with a poor prognosis AH may slightly improve the CPR, but not LBR, when compared with no AH (OR 1.68; 95% CI 1.38 to 2.04; 14 RCTs; n = 2108; I^2 = 25%) (Lacey *et al.*, 2021).

There is uncertainty about a difference in miscarriage rate among women who underwent AH compared with those who did not (OR 1.13; 95% CI 0.82 to 1.56; 17 RCTs; n = 2810; $I^2 = 0\%$; very low-quality of evidence).

Safety

There is concern for an increase in monozygotic twinning after AH, but the number of cases is too small to reach solid conclusions (Hviid *et al.*, 2018; Lacey *et al.*, 2021). The association of AH with ectopic pregnancy, congenital and chromosomal abnormalities and embryo damage could not be evaluated owing to lack of available data (Lacey *et al.*, 2021).

Recommendation

Assisted hatching has no significant impact on LBR. In addition, there may be risks to AH such as higher rates of multiple pregnancies and monozygotic twinning.

Assisted hatching is not recommended.

Genetic testing/treatments
Pre-implantation genetic testing for aneuploidy

Human preimplantation embryos carry a high number of chromosomal abnormalities of either meiotic or mitotic origin. While the rates found in the literature at the cleavage stage can go as high as 80%, it is mainly influenced by maternal age and at the blastocyst stage these rates are lower (Fragouli *et al.*, 2019). This has led to the valid assumption that de-selecting embryos carrying such chromosomal abnormalities would have a beneficial effect on the outcome of ART cycles. PGT-A has endured several re-iterations both at the level of the technology used and the preferred embryo stage for biopsy (Sermon *et al.*, 2016). Initially, FISH was applied for a selected number of chromosomes, usually on a single blastomere biopsied at the 8-cell stage (Geraedts and Sermon, 2016). This evolved to the use of comprehensive chromosome screening (CCS), first using array-comparative genomic hybridization (array-CGH) and later shallow whole genome sequencing (Fiorentino *et al.*, 2014), mostly on blastocyst biopsies (Coonen *et al.*, 2020). PGT-A was initially proposed for patients of advanced maternal age, since they are at the highest risk of producing embryos with meiotic abnormalities, but several other patient categories such as RIF, male infertility, and RPL are now also targeted (van Montfoort *et al.*, 2021).

Efficacy

The results of RCTs comparing PGT-A with conventional IVF treatment are summarized in Table 3, including whether outcomes were reported per ET or patient (per started cycle) and excluding older studies using FISH. The earliest RCTs using CCS showed some beneficial effects, such as sustained implantation rate (Dahdouh *et al.*, 2015) but were heavily criticized for either being in small groups, using the wrong outcome, or having serious methodological flaws (Yang *et al.*, 2012b; Forman *et al.*, 2013; Scott *et al.*, 2013; Mastenbroek and Repping, 2014). The later systematic review and meta-analysis by Cornelisse *et al.* included more robust RCTs, of which, however, only two used CCS, and concluded that there was no increased LBR after the first ET per woman randomized after PGT-A (Cornelisse *et al.*, 2020). The RCT by Rubio *et al.* (2017), which was excluded from the meta-analysis because the embryos were obtained after more than one OPU, also failed to show a higher LBR. Most recently, a large Chinese RCT in younger patients (20–37 years old) also failed to show improvement in LBRs per cycle (Yan *et al.*, 2021). However, a consensus exists that LBR is not an appropriate outcome measure for PGT-A, as it cannot improve a cohort of embryos, only select those that are euploid. Therefore, miscarriage rate and time-to-pregnancy have been proposed as alternative outcome measures, although they are not always included in the currently available RCTs or show contradicting results (Verpoest *et al.*, 2018; Munné *et al.*, 2019; Cornelisse *et al.*, 2020). The largest RCTs also received criticism: the ESTEEM study (Verpoest *et al.*, 2018) was criticized because polar body biopsy was chosen, the STAR study (Munné *et al.*, 2019) was criticized because patients were only randomized if they produced two blastocysts and the outcome was live birth per transfer (Wang *et al.*, 2020), and the Yan *et al.* because mosaic embryos were not transferred (Mastenbroek *et al.*, 2021).

PGT-A is hypothesized to shorten the time to pregnancy. This outcome has, so far, only been reported in the RCTs by Verpoest *et al.* (2018) and Rubio *et al.* (2017), who found no significant difference in time to pregnancy between the PGT-A and control group.

PGT-A is a costly procedure, demanding skilled personnel for the biopsy and genetic analysis, as well as an important investment in genetic analysis, which is often passed on to the patient (van de Wiel *et al.*, 2020). Cost-effectiveness analyses (models) suggest that PGT-A may be lowering costs in some specific patient categories, such as patients of advanced maternal age with a high number of blastocysts, by preventing futile ETs (Neal *et al.*, 2018; Somigliana *et al.*, 2019).

Safety

Several reports have flagged the differences in the diagnostic outcome of blastocyst biopsies between laboratories, especially pertaining to the diagnosis of mitotic mosaicism (Munné *et al.*, 2017), demonstrating, on one hand, the lack of standardization in both biopsy and analysis method, and on the other hand that viable embryos may have been discarded because of analytic errors (Mastenbroek *et al.*, 2021). Follow-up studies of pregnancies after PGT-A have not revealed adverse obstetric outcomes of the blastocyst biopsy (Natsuaki and Dimler, 2018), although there may be a small increase in the risk of intrauterine growth restriction that warrants investigation in larger patient groups (Hou *et al.*, 2021a).

Another meta-analysis, including 15 studies involving 3682 new-borns from PGT pregnancies, 127 719 new-borns from IVF/ICSI pregnancies and 915 222 spontaneously conceived new-borns, focussed on the safety of cleavage and blastocyst stage biopsy and PGT. An increased risk of certain adverse obstetric and neonatal outcomes was reported, namely low birthweight, preterm delivery, hypertensive disorders of pregnancy, and lower gestational age and birthweight in PGT pregnancies relative to spontaneously conceived pregnancies. In the comparison of PGT pregnancies to IVF/ICSI pregnancies, the reviewers reported a decreased risk of very preterm delivery and very low birthweight in PGT pregnancies, and an increased risk of hypertensive disorders of pregnancy (Zheng *et al.*, 2021).

Because of the introduction of blastocyst biopsy in conjunction with shallow sequencing, freeze-all of biopsied embryos is mostly applied in these cycles. This brings its own risks, as discussed in the paragraph on the freeze-all strategy.

Recommendation

The current available data for PGT-A using current methodology for genetic analysis indicate limited improvement in LBR. The supposition that PGT-A reduces miscarriages or time-to-pregnancy in

Table 3. Overview of the studies published to date that compare PGT-A with conventional IVF treatment.

RCT	Patients	Controls	Embryo biopsy	Genetic platform	LBR (unless otherwise indicated)	Miscarriage rate
Yang *et al.* (2012b)	55 good-prognosis patients, 1st IVF cycle Age: 31.2 ± 2.5	48 controls Age: 31.5 ± 2.7	Blastocyst	aCGH	Higher[1] 38/55 (69.1%) vs 20/48 (41.7%) ($P = 0.009$) (per ET)	No difference 1/55 (2.6%) vs 2/48 (9.1%) ($P = 0.597$)
Forman *et al.* (2013)	89 single euploid blastocyst transfer, normal ovarian reserve, ≤1 previous IVF failure Age: 35.1 ± 3.9	86 double blastocyst transfer Age: 34.5 ± 4.7	Blastocyst	qPCR	No difference[2] 60.7% vs 65.1% (RR 0.9; 95% CI 0.7 to 1.2) (per ET)	Not reported
Scott *et al.* (2013)	134 blastocysts/ 72 patients with normal ovarian reserve, ≤1 previous IVF failure Age: 32.2 ± 0.5	163 blastocysts/ 83 patients Age: 32.4 ± 0.5	Blastocyst	qPCR	Higher 61/72 (84.7%) vs 56/83 (67.5%) (RR 1.26; 95% CI 1.06 to 1.53; $P = 0.01$) (per ET)	No difference 7/61 (11.5%) vs 14/70 (20.0%); $P = 0.2$)
Rubio *et al.* (2017)	538 Day 3 embryos from 138 patients Age: 38–41	581 Day 3 embryos/ 140 patients Age: 38–41	Day 3	aCGH	No difference 44/138 (31.9%) vs 26/140 (18.6%) (OR 2.381, 95% CI 1.343 to 4.223)	Lower 1/37 (2.7%) vs 16/41 (39.0%) (OR 0.06, 95% CI 0.008 to 0.48)
Verpoest *et al.* (2018)	205 patients (177 transfers) Age: 38.6 ± 1.4	191 patients (249 transfers) Age: 38.6 ± 1.4	Polar body	aCGH	No difference 50/205 (24%) vs 45/ 191(24%) (RR 1.06; 95% CI 0.75 to 1.50; $P = 0.75$) (per patient)	Lower 14/205 (7%) vs 27/ 191 (14%) (RR 0.48; 95% CI 0.26 to 0.90; $P = 0.02$)
Munné *et al.* (2019)	330 patients undergoing IVF with at least two blastocysts that could be biopsied Age: 33.7 ± 3.59	331 patients undergoing IVF with at least two blastocysts that could be biopsied Age: 33.8 ± 3.58	Blastocyst	NGS	No difference[3] 137/274 (50%) vs 143/313 (46%) (per ET) per ITT (per patient): 138/ 330 (41.8%) vs 144/ 331 (43.5%)	No difference 27/274 (9.9%) vs 30/ 313 (9.6%)
Yan *et al.* (2021)	606 women with three or more good-quality blastocysts Age: 29.1 ± 3.6	606 women with three or more good-quality blastocysts Age: 29.2 ± 3.5	Blastocyst	NGS	Lower (per patient) 458/606 (77.2%) vs 496/606 (81.8%) (absolute difference, −4.6 percentage points; 95% CI −9.2 to −0.0; $P < 0.001$)	Lower 8.7% and 12.6%, (RR 0.69; 95% CI 0.49 to 0.98)

[1] Ongoing pregnancy (≥20 weeks gestational age).
[2] Ongoing pregnancy rate per randomized patient after the first ET.
[3] Ongoing pregnancy rate (OPR) at 20 weeks' gestation per ET.
aCGH: array comparative genomic hybridization; ET: embryo transfer; LBR: live birth rate; NGS: next-generation sequencing; OR: odds ratio; RR: relative risk; RCT: randomized controlled trial; PGT-A: preimplantation genetic testing for aneuploidy; Age = years.

specific patient groups, such as those with advanced maternal age, is based on *post hoc* analyses (Munné *et al.*, 2019) and requires further investigation to establish its validity.

Pre-implantation genetic testing for aneuploidy is currently not recommended for routine clinical use.

Non-invasive pre-implantation genetic testing

As an alternative to blastocyst biopsy, less invasive or non-invasive methods were proposed for performing genetic analysis on either blastocoel fluid (Gianaroli *et al.*, 2014) or spent culture media (Shamonki *et al.*, 2016), dubbed non-invasive PGT (niPGT).

Efficacy

As of now, niPGT is still considered to be in development and not suitable for clinical application (Leaver and Wells, 2020) although some recent reports claim better accuracy and even better concurrence between spent culture media and the inner cell mass (Huang *et al.*, 2019; Rubio *et al.*, 2019; Chen *et al.*, 2021). One clinical trial is ongoing (NCT03520933; Rubio *et al.*, 2019).

Safety

The diagnostic accuracy of niPGT has not reached a sufficient level to be considered for selection of embryos. When considering only the biopsy procedure itself, it can be assumed that niPGT-A, where no embryonic cells are removed, represents a lower risk for the ensuing pregnancy and baby.

Recommendation

At present, niPGT is to be considered in the research phase. Further studies and validation are needed before considering its widespread use in clinical practice.

Non-invasive PGT is currently not recommended for routine clinical use.

Mitochondrial DNA load measurement

Fragouli *et al.* reported that euploid blastocysts that failed to implant carried a higher mean load of mtDNA molecules, nevertheless with high overlap between groups (Fragouli *et al.*, 2015). This observation would fit with the 'quiet embryo' hypothesis that states that normally developing embryos have a lower metabolism (Leese *et al.*, 2022).

Efficacy

A correlation between mtDNA load and BMI, maternal age, aneuploidy of the embryo and embryo quality has been demonstrated (de Los Santos *et al.*, 2018; Lee *et al.*, 2019), even if other studies failed to find a correlation between mtDNA load in euploid embryos and implantation rate (Victor *et al.*, 2017; Klimczak *et al.*, 2018). While mtDNA loads may physiologically vary in relation to the viability of embryos, which represents an interesting field of research, they are not a reliable clinical marker to predict pregnancy (Treff *et al.*, 2017; De Munck *et al.*, 2019; Lee *et al.*, 2019; Zhou *et al.*, 2021; Ritu *et al.*, 2022).

mtDNA load measurements should not be confused with PGT for monogenic disorders (PGT-M) for mtDNA diseases (Treff *et al.*, 2012; Sallevelt *et al.*, 2017; Spath *et al.*, 2021).

Recommendation

At present, mitochondrial DNA load measurement is to be considered in the research phase. Further studies and validation are needed before considering its widespread use in clinical practice.

Mitochondrial DNA load measurement is currently not recommended for routine clinical use.

Time-lapse imaging with or without embryo selection software

Time-lapse imaging (TLI) involves a specialized incubation system that takes frequent digital images of the embryos in culture. A time-lapse video can be created from the images, which removes the need to take the embryos out of the incubator to analyse embryonic development. It has been proposed that TLI has two advantages, both of which may potentially improve LBR: TLI gives the embryo a more stable environment as it limits exposure to changes in temperature, pH, and osmolarity, and using various morphokinetic parameters, such as the timing of cell divisions and intervals between cell cycles, may improve embryo selection presumed to improve LBR and time-to-PR by selecting the embryos with the highest implantation potential first. A wide range of algorithms have been designed for embryo selection, but they appear to be laboratory dependent, probably owing to differences in culture conditions such as culture media and environment (Lundin and Park, 2020). More information on the use of algorithms for embryo selection with TLI can be found in the ESHRE recommendations paper for the use of time-lapse technology (ESHRE Working group on Time-lapse technology *et al.*, 2020).

Although TLI has not been shown to improve LBRs, it provides a tool for research, teaching, standardizing assessment, facilitating laboratory workflows and quality control (ESHRE Working group on Time-lapse technology *et al.*, 2020). These functions are not considered an add-on, at least if there is no additional cost for the patients based on the laboratory using TLI. Many clinics advertise TLI on their websites as a method that will improve embryo selection and can lead to improved outcomes (van de Wiel *et al.*, 2020). In some clinics, patients are charged an additional cost when opting in for TLI.

Efficacy

The most recent Cochrane systematic review and meta-analysis on TLI concluded there is insufficient good-quality evidence of differences in LBR/ongoing PR (OPR) (OR 0.91; 95% CI 0.67 to 1.23; 3 RCTs; n = 826; $I^2 = 33\%$; low-quality evidence), miscarriage (OR 1.90; 95% CI 0.99 to 3.61; 3 RCTs; n = 826; $I^2 = 0\%$; low-quality evidence) and stillbirth (OR 1.00; 95% CI 0.13 to 7.49; 1 RCT; n = 76; low-quality evidence) to choose between TLI, with or without embryo selection software, and conventional incubation (Armstrong *et al.*, 2019). Overall, the evidence is considered low to very low quality, and primary outcomes were often not LBR, cumulative LBR or OPR. From available data, no significant difference was observed when comparing TLI with morphological assessment of still TLI images versus conventional incubation and assessment with regards to LBR/OPR (OR 0.91; 95% CI 0.67 to 1.23; 3 RCTs; n = 826; $I^2 = 33\%$; low-quality evidence) or miscarriage rate (OR 1.90; 95% CI 0.99 to 3.61; 3 RCTs; n = 826; $I^2 = 0\%$; low-quality evidence). Using TLI with embryo selection software was not superior to TLI with morphological assessment of still TLI images or conventional incubation and assessment with regards to LBR. Based on the quality of evidence of the included studies, these findings should be interpreted with caution.

Safety

Kirkegaard *et al.* (2012) reported no difference in safety between TLI and embryo culture in conventional benchtop incubators.

Recommendation

Incubators that utilize TLI have been demonstrated to be a convenient and effective tool for observing the continuous development of embryos. However, the use of TLI, with or without embryo selection software, has not shown conclusive evidence of improving the LBR or the time-to-pregnancy.

Time-lapse imaging is not recommended as a tool to improve live birth rates.

An overview of all recommendations on laboratory tests and interventions with their level of evidence, benefit versus harm and other considerations that contributed to their formulation is available in Table 4.

Clinical management

Platelet-rich plasma

Platelet-rich plasma (PRP) is a technique based on the isolation and concentration of autologous platelets, obtained after centrifuging a sample of peripheral blood. The centrifugation process is suggested to initiate the platelet degranulation process, which releases growth factors that in turn can increase cell mitosis, angiogenesis, chondrogenesis, and chemotaxis or stimulate proliferation and growth. In the context of infertility, it has been hypothesized that PRP may improve folliculogenesis and/or endometrial development.

PRP is administered as an intrauterine infusion (see also uterus flushing) for women with thin/refractory endometrium or RIF and as an intraovarian injection in women with poor ovarian response or POI.

Table 4. Overview of all recommendations on laboratory tests and interventions with their level of evidence, benefit versus harm and other considerations that contributed to their formulation.

Intervention		Benefits versus harms (efficacy versus safety)	Level of evidence for efficacy (LBR/CPR)[1]	Level of evidence for safety[1]	Considerations	Recommendation
Artificial oocyte activation		Beneficial in patients with previous fertilization failure or low fertilization rate and embryo developmental problems Safety to be considered (mechanism unclear), but not reported	⊕⊕◯◯	⊕⊕◯◯	Current studies show variation in ionophore stimulus with respect to concentration, exposure time, and number of exposures.	Artificial oocyte activation is **currently not recommended for routine clinical use**. Artificial oocyte activation is **recommended** for complete activation failure (0% 2PN), very low fertilization (<30% fertilization), or globozoospermia.
Mitochondrial replacement therapy		Few data, no benefit on LBR No data on safety	⊕◯◯◯	No data	In some cases, the acceptors' mtDNA haplotype takes over the donors' mtDNA	Mitochondrial replacement therapy to affect oocyte quality is **not recommended**.
***In vitro* activation of dormant follicles**		No comparative studies Safety, adverse effects, and long-term effects: no data	⊕◯◯◯	No data	For POI patients, options are limited.	*In vitro* activation of dormant follicles is **not recommended**.
IVM	Clinical IVM	No comparative studies Abnormal fertilization and development arrest reported	⊕◯◯◯	No data	It has been suggested that IVM encompasses a lower financial and emotional burden as compared to standard IVF/ICSI.	Clinical IVM and rescue-IVM or natural cycle IVF/M are **currently not recommended for routine clinical use**.
	Rescue IVM	LBR is lower, effect on miscarriage rate is unclear, most studies report increased miscarriages/decreased implantation. Safety of rescue IVM is questionable, since these oocytes commonly have meiotic defects and are of poor quality.	⊕◯◯◯	⊕◯◯◯	/	
Sperm DNA testing and treatment		Diagnostic potential inconclusive No adverse events expected	⊕⊕◯◯	No data	Different assays have different discriminatory capacity, different lab conditions and sperm source can influence the outcome of the test	Sperm DNA damage testing is **currently not recommended for routine clinical use**.
Artificial sperm activation		Higher LBR/CPR No data on safety Malformations reported in animal studies	⊕◯◯◯	⊕◯◯◯	/	Artificial sperm activation is **currently not recommended for routine clinical use**. Artificial sperm activation is **recommended** for patients with primary or secondary total asthenozoospermia which are not the result of axonemal structure defects.
Sperm evaluation and selection	Hyaluronic acid binding assay and physiological ICSI	No evidence benefit on LBR, reduced miscarriage rate No harms reported	⊕⊕◯◯	No data	Hyaluronic acid binding assay has limited standardization.	Sperm hyaluronic binding assay is **currently not recommended for routine clinical use**. Physiological ICSI is **currently not recommended for routine clinical use.**

Continued

Table 4. Continued

Intervention		Benefits versus harms (efficacy versus safety)	Level of evidence for efficacy (LBR/CPR)[1]	Level of evidence for safety[1]	Considerations	Recommendation
	MACS	No evidence of benefit on LBR or miscarriage rate No harms reported	⊕◯◯◯	⊕◯◯◯	/	Magnetic-activated cell sorting is **currently not recommended for routine clinical use**.
	Microfluidics	Only one RCT reporting benefit on LBR No harms reported	⊕⊕◯◯	No data	/	Microfluidics for sperm selection and preparation **can be considered**.
	IMSI	No evidence of benefit on LBR or miscarriage rate No complications reported	⊕◯◯◯	⊕◯◯◯	The method of IMSI can be time-consuming and impacts laboratory workflow.	Intracytoplasmic morphologic sperm injection is **currently not recommended for routine clinical use**.
Growth factor supplemented embryo culture medium		No evidence of benefit on LBR or miscarriage rate Theoretical harms, but not reported	⊕⊕◯◯	⊕⊕◯◯	As growth factors act in both positive and negative synergy to produce an effect, addition of a single growth factor to embryo culture media is questionable and will not necessarily elicit a beneficial effect.	Growth factor-supplemented embryo culture medium is **not recommended**.
Assisted hatching		No evidence of benefit on LBR or miscarriage rate Complications: increased multiple pregnancy rate	⊕⊕◯◯	⊕◯◯◯	/	Assisted hatching is **not recommended**.
Genetic testing and treatments	PGT-A	Most RCTs did not report benefit on LBR, but some suggest reduced miscarriage rate Harms include disposal of viable embryos and IUGR	⊕⊕◯◯	⊕◯◯◯	Lack of standardization in biopsy and analysis method	Pre-implantation genetic testing for aneuploidy is **currently not recommended for routine clinical use**.
	niPGT-A	No data regarding effect on LBR or miscarriage rate Considered to be safer than PGT-A	No data	No data	/	Non-invasive PGT is **currently not recommended for routine clinical use**.
	Mitochondrial DNA load measurement	No data regarding effect on LBR or miscarriage rate Expected complications are similar to PGT-A	No data	No data	/	Mitochondrial DNA load measurement is **currently not recommended for routine clinical use**.
Time-lapse imaging		No evidence of benefit on LBR or miscarriage rate No evidence or rationale for harm	⊕⊕◯◯	No data	/	Time-lapse imaging is **not recommended** as a tool to improve live birth rates.

[1] Quality of Evidence Grades: ⊕⊕⊕⊕, body of evidence is of high quality (at least evidence from RCTs); ⊕⊕⊕◯, body of evidence is of moderate quality (evidence from RCTs or a number of observational studies showing a similar large effect); ⊕⊕◯◯, body of evidence is of low quality (mainly observational data); ⊕◯◯◯, body of evidence is of very low quality (few observational data).
CPR: clinical pregnancy rate; LBR: live birth rate; IMSI: intracytoplasmic morphologically selected sperm injection; IUGR: intra-uterine growth restriction; MACS: magnetic-activated cell sorting; mtDNA: mitochondrial DNA; niPGT-A: non-invasive PGT-A; PGT-A: pre-implantation genetic testing for aneuploidy; RCT: randomized controlled trial.

Intrauterine administration of PRP for thin/refractory endometrium or RIF

Most of the studies published regarding the role of PRP in women undergoing ART have focused on the intrauterine administration of PRP in women either with RIF or with thin/refractory endometrium. Recently, the intervention has also been applied to women with RPL (Nazari *et al.*, 2022a).

Efficacy

In a systematic review, including three RCTs and four cohort studies involving women undergoing IVF/ICSI, a significantly higher probability of CPR was reported with PRP as compared to controls receiving no, or another, active intervention (RR 1.79; 95% CI 1.37 to 2.32; 7 studies; n = 625; $I^2 = 16\%$; $P < 0.001$) (Maleki-Hajiagha *et al.*, 2020). There was no difference between women who received PRP and women without intervention regarding miscarriage (RR 0.72; 95% CI 0.27 to 1.93; 3 studies; n = 217; $I^2 = 0\%$; $P = 0.51$). More recently published RCTs reported either no difference between groups (Dieamant *et al.*, 2019; Javaheri *et al.*, 2020) or beneficial results on CPR (Nazari *et al.*, 2020; Bakhsh *et al.*, 2022; Nazari *et al.*, 2022a), OPR (Zamaniyan *et al.*, 2021), or LBR (Nazari *et al.*, 2022b) in favour of PRP. While, overall, published data support the use of PRP as an alternative treatment strategy for women with thin endometrium and RIF, it should be acknowledged that studies involved small sample sizes, heterogeneous patient populations and there is a possible overrepresentation of one research group in the data (Nazari *et al.*, 2019, 2020, 2022b). Also, the largest RCT including 438 patients has been registered as aiming to include 30 patients per arm and eventually published with a more than 10 times higher sample size (Nazari *et al.*, 2022b). Owing to the low-quality evidence and the lack of a proper multicentre RCT, it is unclear whether intrauterine PRP has a role in refractory or thin endometrium, or in cases of RIF.

Safety

The use of PRP in other fields of medicine has not been associated with any safety issues or risks. However, no safety evidence exists regarding the exposure of embryos in an endometrial cavity following PRP injection (and the related growth factors). In addition, no safety evidence exists regarding the potential short- or long-term effects of injection of PRP in the uterus.

Recommendation

While the available data regarding intrauterine PRP in the context of ART show promise, it is important to acknowledge the significant issues related to their quality and the overall lack of safety data. Further investigation and well-designed studies are necessary to assess the efficacy and ensure the safety of this procedure before considering its use in routine clinical practice.

Intrauterine administration of platelet-rich plasma is not recommended.

Intraovarian PRP injection for poor ovarian response or premature ovarian insufficiency

Intraovarian injection of PRP has been suggested as a method of ovarian rejuvenation for poor ovarian responders or women with POI given the fact that upon the activation of platelets, the alpha granules release several biologically active factors that play crucial roles in modulating folliculogenesis.

Efficacy

To date, no RCTs have been published regarding the potential role of intraovarian PRP injection in women with POI or poor ovarian response. A systematic review of four studies (one case-control and three uncontrolled studies involving 696 women) concluded that intraovarian PRP infusion increases the mature oocyte yield, fertilization rates, and good-quality embryo formation rate (Panda *et al.*, 2020). An additional uncontrolled study showed comparable results (Navali *et al.*, 2022). The lack of evidence from RCTs regarding the efficacy of intraovarian PRP injection, as well as the predominance of uncontrolled (quasi-experimental uncontrolled) studies does not allow firm conclusions regarding its potential efficacy.

Safety

The use of PRP in other fields of medicine has not been associated with any safety issues or risks. However, no safety evidence exists regarding the exposure of embryos in an endometrial cavity following PRP injection (and the related growth factors). In addition, no safety evidence exists regarding the potential short- or long-term effects of injection of PRP in the ovarian stroma.

Recommendation

Currently, there is a lack of RCTs or controlled studies that demonstrate the efficacy of intraovarian PRP. Furthermore, the available data regarding the safety of intraovarian PRP in the context of ART are limited. Further investigation and well-designed studies are necessary to assess its efficacy and ensure its safety before considering its use in routine clinical practice.

Intraovarian administration of platelet-rich plasma is not recommended.

Duostim

Duostim and its efficacy have been previously described in the ESHRE Guideline on Ovarian Stimulation (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020). Duostim, also termed double stimulation or 'Shanghai protocol', is the sequencing of two stimulation protocols within the same menstrual cycle: first in the follicular phase then, second, after the OPU in the luteal phase of the same cycle. The protocol theoretically allows the retrieval of more oocytes in a shorter time and has been used mainly for poor responders and (urgent) fertility preservation patients.

Recommendation

In terms of oocyte quality, there is some reassuring evidence for Duostim, but overall, there is a lack of efficacy and safety data. Therefore, while Duostim may be considered for urgent fertility preservation, further research is needed, particularly in the context of poor responders (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020). The findings from ongoing research will contribute to a better understanding of its efficacy and safety.

Duostim is currently not recommended for routine clinical use.

Adjuncts during ovarian stimulation

Whether the addition of adjuncts in ovarian stimulation is meaningful in terms of efficacy and safety has been previously

investigated, with a full description of published data (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020).

The authors did not find any relevance for the addition of the following compounds before and/or during ovarian stimulation: metformin, growth hormone, testosterone, dehydroepiandrosterone (DHEA), aspirin, indomethacin, and sildenafil. For some compounds, available data showed no benefit, while for others (indomethacin, and sildenafil) no studies have been performed. Safety data are lacking for most of these compounds.

Recommendation

The current evidence does not support the routine use of adjuncts such as metformin, growth hormone, testosterone, DHEA, aspirin, indomethacin, and sildenafil before or during ovarian stimulation. Furthermore, there are serious safety concerns with the use of some of these adjuncts, such as sildenafil. However, the use of these adjuncts based on individual patient characteristics or in specific clinical circumstances may warrant further investigation. Further research is needed to better understand the efficacy and safety of these adjuncts in the context of ovarian stimulation.

Adjuncts (metformin, growth hormone, testosterone, DHEA, aspirin, indomethacin, and sildenafil) before or during ovarian stimulation are not recommended.

Intravaginal and intrauterine culture device

There are two devices for *in vivo* culture of gametes and embryos, namely intravaginal and intrauterine culture, which replace part or all of the culture system that would normally take place in the incubator.

Intravaginal culture device

Intravaginal culture uses a 3 × 4 cm, gas-permeable, air-free plastic chamber. The oocytes and sperm, or ICSI-inseminated oocytes, are placed in the device, which is inserted into the vagina where it is held in place by a cup, similar to a diaphragm. The chamber allows CO_2 and O_2 to enter and regulates pH. The device is removed after 3–5 days at which point the embryos are evaluated and transferred or stored accordingly.

The device was originally designed to simplify IVF treatment. It has been suggested to give psychological benefits to the woman as she feels more involved in the early development of her embryos (Lucena *et al.*, 2012; Vieira and Colucci, 2013). The device is also suggested for same-sex female couples, where the woman who will not be the gestational mother carries the device to be more involved in gestation, so-called 'shared motherhood' (Babcock Gilbert and Polotsky, 2019; Jellerette-Nolan *et al.*, 2021).

Efficacy

Lucena *et al.*, published the first preliminary results using mild stimulation and showed that various IVF parameters, such as pregnancy, live birth and single LBRs, were similar to the US average (Lucena *et al.*, 2012). García-Ferreyra *et al.* (2015) used ICSI embryos and found comparable Day 3 development and PRs. A pilot RCT of 10 patients showed that fertilization and PRs were higher with conventional IVF treatment as compared to fertilization in the device (CPR: 43% (per patient ET procedure) versus 30% (per cycle)) (Mitri *et al.*, 2015). The authors also used questionnaires to document the woman's experience and reported that the women felt fertilization was more natural as a result of feeling closer to their embryos while carrying the device. The developers of the device performed an RCT on 40 women who underwent mild stimulation, with blastocyst quality as the primary outcome (Doody *et al.*, 2016). They found the control group embryos were of a higher grade but that LBRs were similar.

A large descriptive study examined 463 patients who underwent 526 cycles, and reported comparable results to *in vitro* culture, even if there was no control group. Some of the clinics in this study used ICSI and there was a trend to use milder stimulation (Jellerette-Nolan *et al.*, 2021). The study indicated that the intravaginal culture device is being utilized in 65 centres across the USA. However, it emphasized the necessity for a comprehensive cost-effectiveness analysis. It is important to note that implementing such technology in settings with limited financial resources, where low-cost IVF is prioritized, could still present challenges due to the requirement of an embryologist and an IVF laboratory.

Intravaginal culture devices are promoted as a more natural and cost-effective approach to ART. Formal cost-efficacy evaluations have not been performed to date. Also, intravaginal culture devices do not eliminate the need for an IVF laboratory or skilled embryologist, nor do they reduce exposure to synthetic culture media, all of which are still needed to load the gametes into the device (Lucena *et al.*, 2012).

Safety

An initial description of perinatal outcomes of 50 singleton and 16 twin gestations reported no concerning trends in adverse birth outcomes for the singletons, while for the twins a high rate of low birthweight and preterm delivery was reported (Kaye *et al.*, 2022).

Intrauterine culture device

Efficacy

The use of a similar device for intrauterine culture was reported by Blockeel *et al.*, who performed a small study involving intrauterine culture on 13 patients and found results similar to the *in vitro* group (Blockeel *et al.*, 2009). There are no further published studies using this device.

Safety

The device is approved for clinical use in the UK (HFEA), Spain (AEMPS, Consejerias de Sanidad), Denmark (Sundhedsstyrelsen), Czech Republic (MZCR), and Poland (URPL).

Recommendation

Given the limited quality of the available information, there is insufficient evidence to support the use of intravaginal or intrauterine culture devices as a substitute for standard IVF treatment in terms of clinical outcomes and efficacy. Further investigation and well-designed studies are necessary to assess the efficacy of these devices.

Intravaginal or intrauterine culture devices are currently not recommended for routine clinical use.

Additions to transfer media (hyaluronic acid)

Despite what on many occasions may seem to be optimal conditions at ET, i.e. the replacing of a high-quality embryo onto a 'good-looking' endometrium with correct thickness, implantation

often fails. The implantation process is constituted by apposition, adhesion, and invasion, involving many factors and signalling substances and it is difficult to know what fails in a particular patient/cycle. It has been speculated that additions of possible adherence ('sticky') compounds to the transfer media could help to promote and support the implantation process. Potential compounds have mostly been naturally occurring substances such as albumin, fibrin, collagen, and hyaluronan. However, studies investigating a correlation between the secretion of these substances in patients and implantation failure are lacking. Furthermore, it can be questioned whether externally added substances have the same effect as those secreted *in vivo*.

HA is one of the major macromolecules present in the female reproductive tract, and has been shown to increase in the uterus at the time of implantation in humans (Salamonsen *et al.*, 2001). In addition to being a promotor of cell-to-cell adhesion, HA produces a viscous solution that has been proposed to inhibit the expulsion of the embryo (Stojkovic *et al.*, 2002). It can be present in culture media in lower concentrations but also used at higher concentrations at ET. The embryo is preincubated in the HA-enriched transfer medium for 10 min or for up to 4 h before ET.

Efficacy

Studies of the adherence compounds albumin, fibrin sealant, and collagen are scarce, and none have found evidence for increased implantation or LBRs (Menezo *et al.*, 1989; Abou-Setta *et al.*, 2014; Huang *et al.*, 2016).

A recent Cochrane review (Heymann *et al.*, 2020) including 26 RCTs and 6704 women undergoing assisted reproduction compared ET media with no addition of HA to either a low (0.125 mg/ml) or high ('functional' = 0.5 mg/ml) concentration. The overall quality of evidence of the studies included was low to moderate, mainly owing to imprecision and/or heterogeneity. In studies with live birth as the endpoint, an increased LBR was found when using transfer media with a high concentration of HA, compared to low concentration or no addition (RR 1.21; 95% CI 1.1 to 1.70; 10 RCTs; n = 4066; $I^2 = 33\%$; moderate-quality evidence; number needed to treat 14. The increase was seen both for early cleavage stage ETs and for blastocyst transfers, as well as for good and poor prognosis patients. Regarding the time of exposure, three out of eight studies where less than 10 minutes of exposure was used found no significant effect of the addition of high levels of HA.

A slightly reduced risk of miscarriage was found (RR 0.82; 95% CI 0.67 to 1.00; 7 RCTs; n = 3091; $I^2 = 66\%$; low-quality evidence), but this result should be interpreted with caution as it is dominated by the outlier results of a single study (Heymann *et al.*, 2020).

Some of the studies were mixed fresh and frozen-thawed transfers, however three studies were performed only on frozen ET cycles (n = 713), and these studies showed no evidence of a beneficial effect. This was supported by a recent RCT including 550 frozen ET cycles, where Yung *et al.* (2021) found no improvement in LBR with 0.5 mg/ml HA compared to standard transfer medium.

Heymann *et al.* (2022) later summarized the data separately for donor oocyte cycles and autologous oocyte cycles and concluded that, in donor oocyte cycles, HA addition showed little effect on LBR (RR 1.12; 95% CI 0.86 to 1.44; 2 RCTs; n = 317; $I^2 = 50\%$; low-quality evidence) and CPR (RR 1.06; 95% CI 0.97 to 1.28; 3 RCTs; n = 351; $I^2 = 23\%$; low-quality evidence).

Safety

It has been speculated that the use of an adherence compound could allow implantation of lower-quality embryos, and thereby cause an increased rate of miscarriages. However, the present results do not support this.

Multiple PRs were found to be increased (RR 1.45; 95% CI 1.24 to 1.70; 7 RCTs; n = 3337; $I^2 = 36\%$; moderate-quality evidence), which was attributed to the combination of transfer of more than one embryo and the presence of high concentrations of HA in the transfer medium.

Apart from miscarriages, in the Cochrane analysis (Heymann *et al.*, 2020) two RCTs reported on ectopic pregnancies, and one on foetal malformations. The pooled results showed no evidence for an increase in these adverse events when using HA-enriched transfer media (RR 0.86; 95% CI 0.40 to 1.84; 3 RCTs; n = 1487; $I^2 = 0\%$; low-quality evidence).

Recommendation

Current data indicate that addition of HA as an adherence compound in ET media in IVF treatment increases LBR following fresh transfers, without a significant effect on adverse outcomes. No effect was seen following frozen ETs. The higher multiple PRs after the use of HA-supplemented transfer medium should be further investigated.

Hyaluronic acid addition to transfer media is recommended. Monitoring of the multiple pregnancy rate is still advisable.

Endometrial scratching

Endometrial scratching, also termed endometrial injury, has been proposed to improve the chance of implantation of the embryo in patients undergoing IVF treatment. Although unsupported by evidence and debated, endometrial scratching is thought to initiate changes likely to improve implantation. This hypothesis is based on the potential of induction of endometrial decidualization, the triggering of a wound-healing response, associated with a beneficial 'inflammatory response' in the endometrium, the modulation of gene expression involved in the preparation of the endometrium for embryo implantation, and the improvement of synchronicity between the endometrium and the transferred embryo by retardation of endometrial maturation (after being advanced by ovarian stimulation, causing asynchrony) (Lensen *et al.*, 2021c).

Numerous RCTs and systematic reviews have been published on endometrial scratching. These studies have explored various aspects such as the comparison of timing and the number of procedures made, the technique used for the endometrial injury, and identification of populations that may benefit from it.

Efficacy

The most recent Cochrane systematic review and meta-analysis included a total of 37 RCTs (8786 women). In most of the studies, endometrial scratching was performed by pipelle biopsy in the luteal phase of the cycle before an IVF cycle. The primary analysis was restricted to studies with low risk of bias (Lensen *et al.*, 2021c). The effect of endometrial scratching on LBR was unclear as the result was consistent with no effect, a small reduction, or an improvement (OR 1.12; 95% CI 0.98 to 1.28; 8 RCTs; n = 4402; $I^2 = 15\%$; moderate-quality evidence). Similarly, the effect of endometrial scratching on CPR was unclear (OR 1.08; 95% CI 0.95 to

1.23; 8 RCTs; n = 4402; I^2 = 0%; moderate-quality evidence). It was concluded that endometrial scratching probably results in little to no benefit in risk of miscarriage (OR 0.88; 95% CI 0.68 to 1.13; 8 studies; n = 4402; I^2 = 0%; moderate-quality evidence) (Lensen *et al.*, 2021c).

Numerous systematic reviews have addressed if endometrial scratching is beneficial for all patients or only for specific subgroups.

In one recent systematic review, the authors addressed whether a likely effect of endometrial scratching was influenced by the procedure being performed more than once (Nahshon *et al.*, 2020). The review included 17 RCTs comprising 3016 patients and was limited to RCTs examining the effect of endometrial scratching in women with at least one previous failed IVF attempt. Endometrial scratching, once or twice, was mostly performed in the luteal phase but not exclusively, and in four studies hysteroscopy was performed in both groups. When comparing the effect of endometrial scratching with controls, LBR was significantly improved after endometrial injury (RR 1.18; 95% CI 1.04 to 1.34; 14 RCTs; n = 2769; I^2 = 43%; P = 0.009). However, when considering only studies that included patients with at least two previous failed IVF cycles, no statistical difference in LBR was found between groups (RR 1.30; 95% CI 0.87 to 1.94; 7 RCTs; n = 1235; I^2 = 61%; P = 0.20). Subgroup analysis by the number of times endometrial scratching was performed showed no difference in LBR (RR 1.13; 95% CI 0.96 to 1.32; 9 RCTs; n = 2035; I^2 = 49%; P = 0.15) between the endometrial scratching and control groups when endometrial scratching was performed once. However, when endometrial scratching was performed twice, a significantly higher LBR (RR 1.30; 95% CI 1.06 to 1.59; 5 RCTs; n = 734; I^2 = 30%; P = 0.01) was found in the endometrial injury group. The miscarriage rate did not differ between the endometrial scratching and control groups in any of the analyses.

In another recent review, the evidence regarding endometrial scratching in women undergoing their first IVF cycle was summarized (Pluddemann and Onakpoya, 2020). This was done by combining data from a large multicentre RCT (Lensen *et al.*, 2019) with data from an earlier systematic review (Vitagliano *et al.*, 2019). The combined data showed that endometrial scratching had no statistically significant positive effect on LBRs (risk difference (RD) 0.05; 95% CI −0.02 to 0.13; P = 0.17) (Pluddemann and Onakpoya, 2020). Further data for women undergoing a first cycle confirmed no significant effect on LBR in the trial (unadjusted RR 1.04; 95% CI 0.89 to 1.21; n = 1048), and when combining the trial with published data (OR 1.03; 95% CI 0.87 to 1.22; 9 RCTs; n = 2473; I^2 = 0%) (Metwally *et al.*, 2022).

A beneficial effect of endometrial scratching on LBRs in women with more than two previous failed ETs was shown in another earlier review (Vitagliano *et al.*, 2018). Yet, the combined data from the RCT by Lensen *et al.* with those of the review by Vitagliano *et al.* did not support endometrial scratching as an intervention for improving LBRs in women with more than two implantation failures (Pluddemann and Onakpoya, 2020). These findings corroborate those of a recent review by van Hoogenhuijze *et al.* where the effect of endometrial scratching was assessed for three different patient groups: no prior IVF treatment, one failed full IVF/ICSI cycle or two or more failed full IVF/ICSI cycles. Fourteen RCTs involving 2537 participants were included but no difference between endometrial scratching and control was found for LBR, CPR or miscarriage between any of the groups (van Hoogenhuijze *et al.*, 2019).

Endometrial scratching (by pipelle) is a relatively easy procedure to perform. While the procedure itself is considered cheap, the cost-effectiveness is difficult to assess owing to the uncertainty regarding the clinical effectiveness. One study showed the incremental cost-effectiveness ratio for an endometrial scratch was 6524€per additional live birth (van Hoogenhuijze *et al.*, 2022).

Safety

Minimal to moderate bleeding and pain may occur in relation to endometrial scratching. When the procedure is performed by hysteroscopy, there is a small risk of infection.

Recommendation

Even though the most recent Cochrane meta-analysis included 37 RCTs, there is still uncertainty regarding the effect of endometrial scratching on LBR owing to large heterogeneity among studies in methodology and timing of the intervention. Subgroup analyses also failed to identify patient groups that would benefit from endometrial scratching.

Endometrial scratching is currently not recommended for routine clinical use.

Flushing of the uterus

Flushing of the uterus has been performed with hCG, G-CSF, embryo culture supernatant and seminal plasma. Other agents have been used, but with too little data to report and these are not included here.

Intrauterine administration of hCG

hCG is considered the most important regulating factor of embryo-endometrium communication (Hou *et al.*, 2018) and is already secreted by the embryo before implantation. hCG is later synthesized by the syncytiotrophoblast and regulates implantation by facilitating trophoblast invasion, supporting trophoblast apposition and adhesion, and regulating proteins involved in implantation, thereby playing a fundamental role in embryo implantation and early pregnancy. Intrauterine (intracavity) administration of hCG via the ET catheter around the time of transfer has been suggested to improve success rates in IVF treatment.

Efficacy

A Cochrane systematic review and meta-analysis summarized the studies evaluating intrauterine administration of hCG and its effect on reproductive outcomes in women undergoing IVF treatment. To overcome the heterogeneity of the data, results were reported by day of transfer and hCG dosage (Craciunas *et al.*, 2018). LBRs in women having Day 3 ET with intrauterine hCG at a dose <500 IU were similar to controls without hCG administration (RR 0.76; 95% CI 0.58 to 1.01; 1 RCT; n = 280; I^2 = 0%; very low-quality) (Craciunas *et al.*, 2018), but LBR was higher with a higher dosage of hCG (≥500 IU) compared to controls (RR 1.57; 95% CI 1.32 to 1.87; 3 RCTs; n = 914; I^2 = 0%; moderate-quality evidence).

For blastocyst transfer with intrauterine hCG (≥500 IU) compared to controls having blastocyst transfer without hCG, no significant difference in LBR was observed (RR 0.92; 95% CI 0.80 to 1.04; 2 RCTs; n = 1666; I^2 = 0%; moderate-quality evidence) (Craciunas *et al.*, 2018). No RCTs investigated blastocyst transfer with the lower hCG dosage (<500 IU) (Craciunas *et al.*, 2018).

The Cochrane review concluded that there is moderate-quality evidence that in women undergoing cleavage-stage ET, intrauterine administration of hCG (dosage ≥500 IU) may improve the LBR and that there is insufficient evidence for a benefit

of hCG administration with blastocyst transfer. The meta-analysis reported several issues with the studies, such as unclear reporting of study methods and lack of blinding (Craciunas *et al.*, 2018).

Since the publication of the Cochrane review in 2018, four more meta-analyses have been published (Hou *et al.*, 2018; Gao *et al.*, 2019; Xie *et al.*, 2019; Tan *et al.*, 2019a). One of them, including only fresh cycles, showed no benefit in clinical pregnancy and LBRs with intrauterine hCG compared to conventional IVF treatment (Hou *et al.*, 2018). The meta-analysis from Xie *et al.* (2019) was restricted to patients that experienced two or more implantation failures and showed they may benefit from the intrauterine administration of hCG before ET (LBR: RR 1.52; 95%CI 1.18 to 1.96; 1 RCT and 2 cohort studies; n = 870; $P = 0.001$). Gao *et al.* (2019) reported, based on 15 RCTs with a total of 2763 participants, that intrauterine hCG before ET resulted in significantly higher LBR (44.89% vs. 29.76%), OPR (48.09% vs. 33.42%), and implantation rate (31.64% vs. 22.52%) compared to no intervention. Most studies in the previously mentioned meta-analyses investigated the effect of hCG ≥500 IU and the results of the pooled analysis were not stratified by cleavage stage or blastocyst stage transfer (Hou *et al.*, 2018; Gao *et al.*, 2019; Xie *et al.*, 2019; Tan *et al.*, 2019a). Comparable results, based on similar included studies, were reported by Tan *et al.* (2019a). Additionally, no significant difference was found in LBR in subgroups receiving cleavage-stage or blastocyst-stage ET with or without intrauterine hCG before ET (RR 0.99; 95% CI 0.34 to 2.86; 2 RCTs; n = 603 and RR 0.93; 95% CI 0.80 to 1.07; 1 RCT; n = 1186, respectively).

Overall, the findings from multiple clinical trials on the efficacy of intrauterine hCG administration at the time of ET to improve embryo implantation remain controversial.

Two recent RCTs evaluated intrauterine administration of hCG (dosage 1000 IU and 500 IU, respectively) immediately after OPU, rather than at ET as in the other studies. The study using the higher dosage reported no benefit with regards to LBR or any other outcome, while the trial using the lower dosage reported an increased CPR (49%) compared to saline intrauterine infusion (22.9%) (Hosseinisadat *et al.*, 2021; Torky *et al.*, 2022).

In Gao *et al.*, the miscarriage rate was significantly lower (OR 0.57; 95% CI 0.33–0.99) with intrauterine hCG administration as compared to controls (Gao *et al.*, 2019), but this was not reported in other reviews (Craciunas *et al.*, 2018; Hou *et al.*, 2018).

Safety

Ectopic PRs do not seem to be influenced by intrauterine hCG administration but the evidence is of very low-quality and events are too few to reach firm conclusions (Craciunas *et al.*, 2018; Hou *et al.*, 2018).

Recommendation

Current evidence for the efficacy of intrauterine administration of hCG is conflicting. The evidence for its benefits in specific patient subgroups is also inconclusive. Considering the safety concerns, further studies are necessary.

Intrauterine administration of hCG is not recommended.

Intrauterine administration of G-CSF

G-CSF is a cytokine that interacts with a specific cell-surface receptor. The rationale for using G-CSF is that it is hypothesized to induce trophoblast proliferation, invasion, and maintenance during pregnancy. Additionally, it is thought to improve endometrial receptivity for patients with RIF by promoting endometrial vascular remodelling, embryo adhesion and invasion, and regulating endometrial immunity, and it can also maintain endometrial growth by inhibiting apoptosis. G-CSF is involved in regulating the expression of genes associated with embryo adhesion, cell migration, tissue remodelling and angiogenesis, essential for implantation.

Efficacy

Four systematic reviews and meta-analyses have recently been published on the subject (Jiang *et al.*, 2020; Rocha *et al.*, 2020; Hou *et al.*, 2021b; Melo *et al.*, 2022). The most recent review by Melo *et al.* (2022), including two RCTs with good prognosis patients, two RCTs with at least one implantation failure, and one RCT with thin endometrium patients, reported that intrauterine G-CSF may result in a higher LBR/OPR than placebo or no intervention (RR 1.52; 95% CI 1.11 to 2.10; 5 RCT; $I^2 = 12\%$), although the certainly of the evidence was found to be low. The review by Hou *et al.* (2021b) included nine RCTs with 976 patients with RIF. There were no significant differences in the LBR (RR 1.43; 95% CI 0.86 to 2.36; 3 RCT; n = 372) and the miscarriage rate (RR 1.13; 95% CI 0.25 to 5.21; 4 RCT; n = 472) in their pooled analyses (Hou *et al.*, 2021b). The systematic review by Jiang *et al.* found positive results for G-CSF administration on CPR in patients with RIF (Jiang *et al.*, 2020).

Rocha *et al.* focussed on patients with a thin endometrium. They did not perform a meta-analysis, included also non-RCTs and reported an overall positive effect of G-CSF (Rocha *et al.*, 2020). A subgroup analysis of the systematic review by Melo *et al.* (2022) on women with a thin endometrium treated with intrauterine G-CSF suggested that this is the group in whom the increase in the LBR is most substantial (RR 2.57; 95% CI 1.24 to 5.29; 1 RCT; n = 304), although the evidence was judged to be of low certainty owing to the serious risk of bias and the low number of events. Overall, conclusions are limited as the studies' sample sizes are small and are a mix of cleavage and blastocyst transfer in both fresh and frozen cycles.

Further trials, published after the meta-analyses, have reported a benefit of intrauterine administration of G-CSF on the day of OPU in patients with RIF (Torky *et al.*, 2022), while another trial reported no improvement in the clinical outcomes of frozen ET in patients with a thin endometrium (Zhu *et al.*, 2021).

Safety

No firm conclusions can be drawn on safety aspects.

Although fatigue and bone and muscle pain are common side effects of G-CSF treatment in general, very few adverse events were reported in the included studies investigating the use of intrauterine G-CSF, presumably because the systemic level of G-CSF is very low after intrauterine administration (Melo *et al.*, 2022).

Recommendation

Current evidence concerning the intrauterine administration of G-CSF is inconclusive because of the studies' small sample sizes and mixed cleavage and blastocyst transfer in both fresh and frozen cycles. Further research is needed to better understand its potential efficacy and safety.

Intrauterine administration of granulocyte colony-stimulating factor is not recommended.

Endometrial administration of embryo culture supernatant

Embryo culture supernatant (i.e. spent embryo culture media) is another option evaluated for uterus flushing. During the procedure, performed at various times before ET, ~20 µl of the embryo culture supernatant is injected into the uterus. The intrauterine administration of embryo culture supernatant is hypothesized to facilitate implantation through embryonic factors secreted into the culture medium.

Efficacy

The literature on endometrial injection of embryo culture supernatant is limited and what we know is condensed in a recent Cochrane systematic review and meta-analysis including 5 RCTs involving 526 women (Siristatidis *et al.*, 2020). No RCTs on embryo culture supernatant have been published since the Cochrane review.

There was no evidence of an effect on LBR/OPR with the endometrial application of embryo culture supernatant before ET versus standard care or no intervention (OR 1.11; 95% CI 0.73 to 1.70; 3 RCTs; n = 340; I^2 = 84%; very low-quality evidence). Results suggest that if the LBR/OPR following placebo or no treatment is assumed to be 42%, the chance following the endometrial injection of embryo culture supernatant before ET would vary between 22% and 81% (Siristatidis *et al.*, 2020).

There was no evidence of an increased risk of miscarriage (OR 0.89; 95% CI 0.44 to 1.78; 4 RCTs; n = 430; I^2 = 58%; very low-quality evidence) with endometrial administration of embryo culture supernatant compared to no intervention (Siristatidis *et al.*, 2020). Results suggest that if the chance of miscarriage following placebo or no treatment is assumed to be 9%, the chance following injection of embryo culture supernatant would vary between 3% and 30%.

In several studies, it was unclear if the culture media had been administered by injection or as a uterine infusion.

Safety

There was no evidence of an increased risk of ectopic pregnancy (OR 0.32; 95% CI 0.01 to 8.24; n = 250; 2 RCTs; I^2 = 41%; very low-quality evidence) with endometrial administration of embryo culture supernatant compared to no intervention (Siristatidis *et al.*, 2020).

Recommendation

There are insufficient data to support the use of embryo culture supernatant for intrauterine application to enhance success rates in IVF treatment. Further research is needed to establish its efficacy and safety.

Endometrial administration of embryo culture supernatant is not recommended.

Endometrial exposure to seminal plasma

Seminal plasma is known to contain factors (cytokines, chemokines, prostaglandins, growth factors), considered important for regulating endometrial receptivity (e.g. Nederlof *et al.*, 2017; Szczykutowicz *et al.*, 2019). The hypothesis is therefore that exposure to seminal plasma could potentially 'prime' the endometrium, facilitating implantation and live birth.

Efficacy

The most recent Cochrane systematic review and meta-analysis included 11 RCTs with a total of 3215 women exposed to seminal plasma at the time of ET (Ata *et al.*, 2018). The Cochrane review reported no or little difference with regards to LBR (RR 1.10; 95% CI 0.86 to 1.43; 3 RCTs; n = 948; I^2 = 0%; low-quality evidence) and miscarriage rates (RR 1.01; 95% CI 0.57 to 1.79; 4 RCTs; n = 1209; I^2 = 0%; low-quality evidence). The studies were very heterogeneous with regards to the inclusion/exclusion criteria of patients and the interventions. The interventions included unprotected vaginal intercourse around the time of ET, untreated ejaculate applied vaginally on the day of oocyte collection, and seminal plasma applied to the uterus or the cervix and vagina.

Safety

From the Cochrane meta-analysis, there was insufficient evidence to determine if the application of seminal plasma influenced the risk for ectopic pregnancy (RR 1.59; 95% CI 0.20 to 12.78; 5 RCTs; n = 1521; I^2 = 0%; very low-quality evidence) (Ata *et al.*, 2018). There was no or little difference in multiple PRs (RR 1.11; 95% CI 0.76 to 1.64; 5 RCTs; n = 1642; I^2 = 9%; low-quality evidence). While the reviewers found no data on infection or other adverse events following seminal plasma application at ET, seminal plasma hypersensitivity may be triggered by contact with seminal fluid. Seminal plasma hypersensitivity presents with localized vaginal and/or systemic allergic symptoms on exposure to protein components of seminal plasma and has been reported following exposure to seminal fluid during unprotected sexual intercourse (Lavery *et al.*, 2020).

Recommendation

There is insufficient evidence regarding both the efficacy and safety of administering seminal plasma into the vagina. Further research is needed to better understand the potential efficacy and safety of endometrial exposure to seminal plasma.

Endometrial exposure to seminal plasma is not recommended.

Stem cell mobilization

Stem cell therapy for premature ovarian insufficiency or diminished/poor ovarian reserve

In mouse models of POI, bone marrow transplantation facilitated follicle development and rescued long-term fertility (Xia *et al.*, 2015). In humans, there are also several cases reported of patients with POI caused by chemotherapy/radiotherapy who conceived spontaneously following autologous stem cell transplantation (Hershlag and Schuster, 2002; Veitia *et al.*, 2007).

Because mesenchymal stem cells are a major subgroup of stem cells present in bone marrow, they were hypothesized to be contributing to this 'ovarian rejuvenation'. Therefore, it was hypothesized that infusion of bone marrow-derived stem cells (BMDSCs), both mesenchymal stem cells (MSC) and haematopoietic stem cells, into the ovary could help maintain or promote follicular rescue in patients with impaired or aged ovarian reserves (Fàbregues *et al.*, 2020). Administration of the stem cells to the ovary can be achieved through transvaginal ultrasound-guided injection, ovarian injection via laparoscopy, intra-arterial

catheterization of the ovarian artery, or a combination of these techniques (Fàbregues et al., 2020).

Efficacy

No RCTs or comparative studies are available.

In an experimental study, antral follicles were cultured together with different concentrations of bone marrow-derived MSCs. The presence of the MSCs in *in vitro* culture significantly promoted the survival rates, increased the growth velocity, and improved the viability of preantral follicles (Xia et al., 2015).

A case report by Gupta *et al.* describes a live birth after the injection of bone marrow-derived MSCs into the ovary of a postmenopausal woman by laparoscopy (Gupta et al., 2018). Edessy *et al.* showed that after laparoscopic injection of bone marrow-derived MSCs into the ovary of 10 patients with POI, two women resumed menstruation, and one of these achieved a live birth (Edessy et al., 2016). In a comparative clinical study, including 31 poor ovarian responders, menstrual blood-derived MSCs were injected into the ovary in the study group and compared to normal ICSI treatment in the control group. Seven out of 15 women achieved a live birth in the study group, compared to 2 out of 16 women (either spontaneously or via IVF treatment) in the control group (Zafardoust et al., 2020).

Herraiz *et al.* (2019) injected bone marrow-derived MSCs into the ovarian artery of patients with poor ovarian reserve. Ovarian activity improved in 81.3% of women, resulting in three spontaneously conceived pregnancies and two after ET.

Stem cell therapy for thin endometrium

In women of reproductive age, the endometrium undergoes stripping during every menstrual cycle and can be rebuilt without scarring in subsequent cycles. It is hypothesized that endometrial stem cells have a crucial role in this uterine homeostasis and regeneration, and that thin endometrium is the consequence of the loss of endometrial stem cells (Zhang et al., 2021).

Efficacy

No RCTs or comparative studies are available.

In a rat model of endometrial injury, stem cell-loaded grafts with umbilical cord-derived MSCs were transplanted to the damaged endometrium. Sixty days after the transplant, the endometrium appeared normal in the transplant group, while the controls showed severe intrauterine adhesions (Xin et al., 2019). Similarly, in a prospective before and after self-controlled study in humans, umbilical cord-derived MSCs were seeded onto a collagen scaffold and transplanted on Days 7–12 of menstruation into the uterine cavity of 17 patients with refractory adhesions. This procedure was repeated in the next menstrual cycle. One month later, a hysteroscopy with an endometrial biopsy was performed and patients were allowed to proceed with frozen ET. The endometrial thickness was significantly increased with MSC treatment from 4.08 ± 0.26 to 5.87 ± 0.77 mm. Four patients achieved pregnancy, one spontaneous and three after frozen ET, resulting in three live births and one spontaneous second-trimester abortion (Zhang et al., 2021).

A larger case series included 29 women with previously failed IVF cycles and refractory thin endometrium, in whom 'subendometrial inoculation' of autologous endometrial-derived MSCs was performed. The MSCs were suspended in 1 ml autologous PRP and transferred via transmyometrial catheter into the uterine cavity. Treatment with MSCs produced a significant increase in endometrial thickness, from 5.25 ± 1.24 to 9.93 ± 0.77 mm), and a total of 10 live births and 7 ongoing pregnancies (Tersoglio et al., 2020).

In a prospective non-comparative study in 11 women with refractory adhesions and five women with endometrial atrophy, autologous bone marrow-derived MSCs were injected into the spiral arterioles by catheterization. During follow-up, three women conceived spontaneously, resulting in one live birth, one ongoing pregnancy and one second-trimester miscarriage. Seven pregnancies were obtained after 14 ETs, resulting in one live birth, one ongoing pregnancy, three biochemical pregnancies, one ectopic pregnancy and one miscarriage (Santamaria et al., 2016).

Safety

There were no acute symptoms after intraovarian injection, such as pain, nausea, infection, bleeding, or fever, according to a single study (Zafardoust et al., 2020). Different procedures for administering stem cells have been described, which are all invasive with serious risks of complications.

Furthermore, there are serious concerns regarding the long-term effect of injections of stem cells and the risk of tumorigenesis.

A detailed description of the health of infants born after such treatment modalities is not available.

Recommendation

The biological rationale for stem cell therapy in women with POI, thin endometrium or diminished/poor ovarian reserve is unclear. Furthermore, the available data on efficacy are limited and primarily derived from observational studies with small sample sizes. More importantly, there are serious safety concerns with this technique. Further preclinical studies are necessary to assess the relevance and potential efficacy of this technique.

Stem cell therapy for premature ovarian insufficiency, diminished/poor ovarian reserve or thin endometrium is not recommended.

Steroids

Steroids are used in women with autoimmune diseases, even before or during treatment, but this is not considered an add-on treatment.

Glucocorticoids are a class of steroid hormones that have been used with the aim of improving folliculogenesis and PRs in women undergoing IVF/ICSI treatment. However, there are inconsistent data on whether the administration of glucocorticoid during ovarian stimulation yields any superiority for LBRs when compared with standard treatment cycles. Glucocorticoids have also been examined in patients considered to have an immunological factor prohibiting pregnancy or live birth.

Efficacy

A Cochrane systematic review and meta-analysis reported that the LBR was comparable across groups assigned to glucocorticoids supplementation (different dosages) or placebo (OR 1.08; 95% CI 0.45 to 2.58; 2 RCTs; n = 310; low-quality evidence) (Kalampokas et al., 2017). Another meta-analysis of women undergoing IVF treatment reported that CPR was not different in women using glucocorticoids and those that did not (OR 1.12; 95% CI 0.75 to 1.67; 2 RCTs; n = 202) (Achilli et al., 2018).

Safety

With regards to the safety of glucocorticoid administration, animal studies have reported foetal growth retardation,

cardiovascular, metabolic, neuroendocrine disorders, and teratogenic effects. In humans, increased risk of miscarriage, preterm births, gestational hypertension, and diabetes have been reported, even if the data are limited (Kim, 2021).

Recommendation

While there is some indication of potential benefits in patients with autoimmune disease, it is important to note that the existing data on the use of glucocorticoids in ART is limited and based on small, non-controlled studies with inconsistent criteria.

Glucocorticoids are not recommended in ART.

Elective freeze-all

Freeze-all is a strategy where all embryos obtained in a cycle are frozen, avoiding a fresh ET. This procedure was initially used to prevent OHSS and was not considered an add-on treatment. It is still considered a valid preventative strategy for this indication but has in addition evolved to 'Freeze-all for all' or 'elective freeze-all', applying the procedure irrespective of any OHSS risk. The rationale is that the endometrium and embryo are asynchronous in the gonadotrophin-stimulated cycle prior to oocyte collection because of the high levels of sex steroid hormones (Devroey *et al.*, 2011). Thus, segmentation of the cycle and postponement of ET is hypothesized to give higher success rates for IVF treatment. To address the efficacy and cost-benefit of the freeze-all strategy used during IVF treatment, systematic reviews, meta-analyses and RCTs comparing reproductive outcomes in freeze-all with fresh ET were considered for inclusion. For the aim of this article, freeze-all in the context of women with PCOS or preventing OHSS was not evaluated as this is not considered an add-on.

Efficacy

Four large cohort studies based on the SART, HFEA and Victoria (Australia) data have shown the same tendency that the freeze-all strategy seems to be beneficial in high responders but not in intermediate or low responders (Acharya *et al.*, 2018; Smith *et al.*, 2019; Li *et al.*, 2019b; Le *et al.*, 2022). This was confirmed in a meta-analysis from 2019 where the authors found that a significantly higher probability of live birth was present in high, but not normal responders, after the first frozen ET in a freeze-only cycle strategy as compared to a fresh ET (RR 1.13; 95% CI 0.90 to 1.41; 3 RCT; n = 3118) (Bosdou *et al.*, 2019). The meta-analysis included 3398 women, in which the RCT from China on women with PCOS accounted for more than 3000 patients (Chen *et al.*, 2016).

A meta-analysis from 2018 based on seven studies comparing women who underwent freeze-all and those who had fresh-ET found that the LBR was significantly higher in the freeze-all group (RR 1.18; 95% CI 1.08 to 1.30; 6 RCTs; n = 2194; $I^2 = 40\%$; $P = 0.0003$) (Zhang *et al.*, 2018).

However, the most recent Cochrane systematic review and meta-analysis found little or no difference in cumulative LBR between the 'freeze-all' strategy and the conventional fresh ET (OR 1.08; 95% CI 0.95 to 1.22; 8 RCTs; n = 4712; $I^2 = 0\%$; moderate-quality evidence) (Zaat *et al.*, 2021). Their summary finding was that the cumulative LBR following the 'freeze all' strategy would be 57–63% versus 58% following the conventional strategy. The non-superiority of the freeze-all strategy was also confirmed in the two most recent RCTs performed in the UK and Denmark with 619 and 460 patients, respectively (Stormlund *et al.*, 2020; Maheshwari *et al.*, 2022). Both studies reported LBRs after the first ET after OPU but no cumulative LBRs, and the Danish study was included in the meta-analyses by Zaat *et al.* (2021) but not in the analyses on cumulative LBRs.

The reason for the differences between the two meta-analyses is most likely that the one by Zaat *et al.* used cumulative LBR as the primary outcome and included also the most recent RCTs with women with a regular menstrual cycle and normo-ovarian response and similar LBR and OPR in the freeze-all and fresh-ET group. In contrast, the review by Zhang *et al.*, did not include cumulative live birth as an outcome and the majority of the included RCTs focussed on women with PCOS or younger patients with a high ovarian reserve (Zhang *et al.*, 2018; Zaat *et al.*, 2021).

The Cochrane review concludes that, by design, time-to-pregnancy is shorter in the conventional strategy compared to the 'freeze-all' strategy when the cumulative LBR is comparable. This corresponds well with a recent RCT including 460 women with a regular menstrual cycle and a mean age of 32 years where the median time-to-pregnancy was significantly longer in the freeze-all strategy group (86 days; IQR 77–107) compared with the fresh transfer strategy group (28 days; IQR 27–30; $P < 0.001$) (Stormlund *et al.*, 2020). It is fair to conclude that with similar LBR and OPR and longer time-to-pregnancy and the added freezing/thawing procedures, the cost with a 'freeze-all' for all strategy will exceed the costs of conventional fresh ET. This was confirmed in the most recent RCT on the topic where the elective freeze-all approach was more costly and was unlikely to be cost-effective (Maheshwari *et al.*, 2022).

Safety

Regarding safety, the Cochrane review showed that the risks of hypertensive disorder in pregnancy (HDP) (OR 2.15; 95% CI 1.42 to 3.25; 3 RCTs, n = 3940; $I^2 = 29\%$; low-quality evidence) and large-for-gestational age (OR 1.96; 95% CI 1.51 to 2.55; 3 RCTs; n = 3940; $I^2 = 0\%$; low-quality evidence) were higher after the freeze-all strategy than after fresh ET and also a higher mean birthweight was observed after freeze-all (MD 127 g; 95% CI 77.1 to 177.8; 5 RCTs; 1607 singletons; $I^2 = 0\%$; moderate-quality evidence) (Zaat *et al.*, 2021). The increased risk of HDP and high birthweight is higher with hormone replacement therapy (HRT)-frozen ET, which was shown in a recent systematic review (Busnelli *et al.*, 2022). This may to a certain extent be related to the lack of a corpus luteum in HRT-frozen ET (von Versen-Höynck *et al.*, 2019). A systematic review on perinatal outcomes specifically also reported an association of frozen ET with large-for-gestational-age babies, but also caesarean section and pre-eclampsia, while the incidence of preterm birth and small-for-gestational-age babies was lower (Li *et al.*, 2021a).

The risk of OHSS is lower with the 'freeze-all' strategy compared to the conventional IVF/ICSI strategy (OR 0.26; 95% CI 0.17 to 0.39; 6 RCTs; n = 4478; $I^2 = 0\%$; low-quality evidence) (Zaat *et al.*, 2021).

Recommendation

The available evidence shows that the cumulative LBR and LBR with the freeze-all strategy are not superior to fresh ET, while the time to achieve pregnancy is likely to be longer. Moreover, elective freeze-all carries obstetric and perinatal risks such as hypertensive disorders in pregnancy, large for gestational age, and macrosomia. The freeze-all strategy should only be considered when there is a clear clinical indication, such as a higher risk of OHSS or endometrial pathology, and in cases involving PGT. Adopting the freeze-all strategy should be done judiciously, considering individual patient factors and the potential risks

involved. For the aim of this article, freeze-all in the context of women with PCOS or preventing OHSS was not evaluated as this is not considered an add-on.

Elective freeze-all is currently not recommended for routine clinical use.

ICSI for non-male factor infertility

ICSI is an ART technique that has created a breakthrough in the field as it improved fertilization rates and PRs in couples with severe male factor infertility (Palermo *et al.*, 1992). However, despite the stable incidence of male factor infertility over the last decades, the use of ICSI increased from 35% of all ART cycles in 1997 to >70% in 2018 (The European IVF-Monitoring Consortium for the European Society of Human Reproduction and Embryology *et al.*, 2022), considered to result from its increased use among patients with non-male infertility (Boulet *et al.*, 2015).

Efficacy

Even if most evidence that has been published regarding the efficacy of ICSI focuses on couples with male factor infertility, its role in the case of a normal sperm analysis remains questionable. The first large multicentre RCT failed to find any differences in implantation and CPRs in women scheduled for IVF treatment for non-male factor infertility (Bhattacharya *et al.*, 2001). Following this report, several studies have been published that evaluate the role of ICSI in certain patient categories such as poor ovarian responders, advanced maternal age, or couples with unexplained infertility (Franasiak *et al.*, 2022). However, no clear benefit has been demonstrated in favour of ICSI as compared to IVF treatment in these studies.

Published evidence from large retrospective cohort studies failed to reveal any benefit in pregnancy, live birth or cumulative LBR following the use of ICSI in poor responders (Luna *et al.*, 2011; Sfontouris *et al.*, 2015; Drakopoulos *et al.*, 2019), while others even suggested higher PRs or LBRs after conventional IVF treatment in this population (Artini *et al.*, 2013; Butts *et al.*, 2014).

Similarly, in patients with advanced maternal age, ICSI did not improve fertilization rates and clinical outcomes as compared to IVF treatment (Tannus *et al.*, 2017; Gennarelli *et al.*, 2019), with some studies even reporting lower LBRs following the use of ICSI (Supramaniam *et al.*, 2020). The most recent RCT comparing IVF and ICSI treatment in advanced-age women (>39 years old) showed that both fertilization techniques result in comparable fertilization rates and numbers of top-quality embryos (Haas *et al.*, 2021).

In women with unexplained infertility, although an early systematic review supported that ICSI was superior to IVF treatment in terms of fertilization rates and fertilization failure (Johnson *et al.*, 2013), results should be interpreted with caution owing to the high heterogeneity among included studies, and the lack of cumulative data regarding pregnancy outcomes (Franasiak *et al.*, 2022).

Finally, a large RCT that randomly assigned 1064 couples with non-male factor infertility to ICSI and conventional IVF treatment was published in 2021 (Dang *et al.*, 2021). According to this RCT, ICSI resulted in comparable LBRs (RR 1.11; 95% CI 0.93 to 1.32; $P = 0.27$) and comparable fertilization failure (RR.0.85; 95% CI 0.53 to 1.38; $P = 0.60$) as compared to IVF treatment (Dang *et al.*, 2021).

Although the use of ICSI is widespread today, the mean laboratory time is significantly longer for ICSI compared to conventional IVF treatment (Bhattacharya *et al.*, 2001). From a detailed treatment cost analysis of conventional IVF and ICSI treatment, it was calculated that the cost of ICSI was 8.3% higher than IVF treatment (Bouwmans *et al.*, 2008).

Safety

Concerns have been raised regarding the safety of ICSI over IVF treatment, with several reports suggesting that perinatal or neonatal outcomes may be associated with the paternal characteristics linked to male factor infertility (Rumbold *et al.*, 2019). Perinatal outcomes appear to be comparable between IVF and ICSI treatment as reported in a large retrospective study published in 2020 (Liu *et al.*, 2020a). Similarly, a meta-analysis including 46 studies (Wen *et al.*, 2012) and the most recent RCT including >1000 patients (Dang *et al.*, 2021) failed to find any difference between the two techniques regarding perinatal outcomes.

In terms of long-term child development, although an early study supported a potentially delayed development of children born after ICSI as compared with natural conception (Bowen *et al.*, 1998), this was not confirmed by later reports (Bosch *et al.*, 2020; Leunens *et al.*, 2006). Furthermore, a systematic review has shown that neurodevelopment, growth, vision, and hearing appear similar between ICSI and spontaneously conceived children. Concerning general physical health and metabolic and reproductive endpoints, the clinical significance is unclear and remains to be determined (Catford *et al.*, 2018).

In terms of imprinting disorders and DNA methylation, although a study supported that children born from ICSI demonstrated higher DNA methylation in an imprinted gene (Whitelaw *et al.*, 2014), a meta-analysis published in 2014 showed that although there was an increase in imprinting disorders in children conceived through IVF and ICSI treatment, there was insufficient evidence for an association between ART and methylation in other imprinted genes (Lazaraviciute *et al.*, 2014). Most recent evidence suggests that ART (including ICSI) is associated with limited epigenetic variation at birth and these largely resolve by adulthood (Novakovic *et al.*, 2019).

Finally, given that the majority of this data comes from studies including patients with male factor infertility, we cannot determine whether the recorded defects are related to ICSI or to the infertile condition itself.

Recommendation

There is no evidence regarding the advantages of ICSI for non-male factor infertility in terms of pregnancy outcomes, LBRs, and cumulative LBRs. In addition, ICSI is associated with higher costs compared to conventional IVF treatment. There may be specific treatments where ICSI is indicated, such as for PGT cycles.

ICSI is not recommended for non-male factor infertility.

Antioxidant therapy

OS has been implicated in the deterioration of sperm count, motility, morphology, fertilization, and embryo development and suggested to be associated with the risk of infertility, miscarriage, and RIF (Wang *et al.*, 2019; Scaruffi *et al.*, 2021). Lifestyle factors, pollution, stress, allergies, and clinical varicocele are considered to increase OS (Agarwal *et al.*, 2012).

Antioxidants are a group of organic nutrients that include vitamins, minerals and polyunsaturated fatty acids, which are suggested to reduce oxidative damage and balance the negative outcomes related to OS (Showell *et al.*, 2020). However, the methodology used in the measurement of OS, particularly in sperm samples, the ideal combination of antioxidant therapy and their efficacy is controversial.

Efficacy

For female subfertility, a Cochrane systematic review was uncertain whether oral antioxidants (1–3 cycles) improve LBR compared with placebo or no treatment/standard treatment (OR 1.81; 95% CI 1.36 to 2.43; 13 RCTs; n = 1227; $I^2 = 29\%$; $P < 0.001$; very low-quality evidence) (Showell *et al.*, 2020). Pooling all studies (n = 35) for CPR showed that in the studies reporting LBR (n = 13), there was a small overestimation of the effect of antioxidants, which might be the same for LBR. There was no difference between the groups in terms of miscarriage (OR 1.13; 95% CI 0.82 to 1.55; 24 RCTs; n = 3229; $I^2 = 0\%$; $P = 0.46$; very low-quality evidence), and no particular type of antioxidant was superior to the others (Showell *et al.*, 2020).

For male subfertility, a Cochrane review reported that oral antioxidants (3-12 months) may lead to increased LBRs compared to placebo or no treatment (OR 1.43; 95% CI 1.07 to 1.91; 12 RCTs; n = 1283; $I^2 = 49\%$; very low-quality evidence) (de Ligny *et al.*, 2022). When studies at high risk of bias were removed from the analysis, there was no evidence of increased live birth (Peto OR 1.22; 95% CI 0.85 to 1.75; 8 RCTs; n = 827; $I^2 = 32\%$; $P = 0.27$). There was no evidence of an increased risk of miscarriage (OR 1.46; 95% CI 0.75 to 2.83; 6 RCTs; n = 664; $I^2 = 3\%$; very low-quality evidence). There was also no evidence that different antioxidants had differing effects (de Ligny *et al.*, 2022).

Several studies aimed to identify a particular group of patients which may potentially benefit from antioxidant therapy by stratification according to BMI, smoking, lifestyle factors, basal DFI, presence of varicocele etc. However, most of the studies showed a small sample size and retrospective design, used various combinations of antioxidants, and semen parameters or DFIs were used as surrogate success parameters rather than the PR itself (Majzoub and Agarwal, 2018).

Safety

The Cochrane review revealed that antioxidants may lead to an increase in gastrointestinal discomfort when compared to placebo or no treatment (OR 2.70; 95% CI 1.46 to 4.99; 16 RCTs; n = 1355; $I^2 = 40\%$; low-quality evidence) (de Ligny *et al.*, 2022).

Recommendation

Antioxidant therapy lacks substantial and reliable evidence demonstrating a significant enhancement in LBRs.

Antioxidant therapy is not recommended in ART.

Complementary and alternative medicine

The terms complementary and alternative therapies are sometimes used interchangeably and together (complementary and alternative medicine (CAM)). They both offer an approach different to conventional medicine; an alternative therapy is a procedure that is used instead of conventional treatment and a complementary therapy is a treatment that can be used alongside conventional treatment. They include a range of procedures such as acupuncture, reflexology, nutritionist services, Chinese herbal medicine (CHM), mindfulness, hypnotherapy, massage, yoga, reiki healing, meditation, neuro-linguistic programming therapy, kinesiology, and detoxing.

In ART, complementary therapies are often advertised by fertility clinics with suggestions that they can relax the patient and improve their well-being but also claims that they may improve IVF outcomes (Stein and Harper, 2021). The UK patient survey by the HFEA has shown that acupuncture was the second most common IVF add-on undertaken (HFEA, 2018) and an Australian study showed that acupuncture and CHM were in the top three used ART add-ons (Lensen *et al.*, 2021a). In the UK, practitioners offering complementary therapies are often external to the IVF unit, so clinics do not usually have control over the information they give to patients (Stein and Harper, 2021).

Various explanations have been put forward as to how complementary therapies could increase ART success. Some claim that acupuncture may increase blood flow to the uterus and ovaries (Stener-Victorin *et al.*, 2006), regulate fertility hormones (Stener-Victorin and Wu, 2010) and may help patients with PCOS owing to its effects on beta-endorphin production, which may affect GnRH secretion (Lim *et al.*, 2019).

Efficacy

Assessing complementary therapies through RCTs is challenging, especially with respect to a suitable control group and consistent methodology. For example, there have been at least 34 RCTs and about 25 systematic reviews to determine whether acupuncture can improve IVF PRs but the methods reported have been very heterogeneous: using a sham or placebo control (using acupuncture points that are not relevant or using a placebo acupuncture device); using manual or electrical stimulation; treatment being undertaken in cycles before the oocyte collection cycle, during ovarian stimulation, or around the time of the ET; and variations in the number of needle insertions.

The four meta-analyses from the last 2 years on acupuncture have either shown no effect or improved CPR but with low-quality evidence and method heterogeneity (Jang *et al.*, 2020; Coyle *et al.*, 2021; Li *et al.*, 2021c; Wang *et al.*, 2021b). For example, Coyle *et al.* (2021) reported that acupuncture around the time of ET was not significantly different to placebo acupuncture in terms of LBR (RR 0.87; 95% CI 0.75 to 1.01; 4 RCTs; n = 1835; $I^2 = 0\%$; high-quality evidence), CPR (RR 0.99; 95% CI 0.88 to 1.11; 6 RCTs; n = 2473; $I^2 = 51\%$; moderate-quality evidence), or miscarriage rate (RR 1.23; 95% CI 0.89 to 1.71; 4 RCTs; n = 502; $I^2 = 30\%$; high-quality evidence).

With regards to herbal medicine, a systematic review reported (overall) there may be a benefit of the intervention compared to no treatment/placebo for LBR (RR 1.34; 95% CI 1.05 to 1.72; 5 studies; n = 837; $I^2 = 35\%$; low-quality evidence) and CPR (RR 1.38; 95% CI 1.29 to 1.49; 35 studies; n = 3596; $I^2 = 0\%$; low-quality evidence) but commented that additional RCTs with robust methodology and long-term follow up are still required (Kwon *et al.*, 2020). Specifically for CHM, a review reported increased CPRs with the treatment (OR 2.04; 95% CI 1.67 to 2.49; 20 RCTs; n = 1721; $I^2 = 0\%$; low-quality evidence) (Cao *et al.*, 2013).

There have been several retrospective cohort studies on other complementary therapies but very few RCTs.

Treatment costs were found to range from <£50 (58€) for individual appointments to hundreds of pounds for treatment packages (Stein and Harper, 2021).

Safety

Adverse events reported after acupuncture include dizziness, nausea, and subcutaneous haematoma (Lim *et al.*, 2019). For herbal medicines, Kwon *et al.* (2020) reported only eight out of the 43 included studies reported adverse events, mostly gastrointestinal complaints, with low prevalence. Cao *et al.* (2013) stated that no conclusion could be drawn with respect to the reproductive toxicity of CHM.

Recommendation

For acupuncture, the existing evidence is contradictory regarding its potential to enhance the LBR. As for Chinese and herbal medicine, it is essential to conduct RCTs with rigorous methodologies and long-term follow-up to ascertain the treatments' efficacy and safety. For the other complementary therapies and alternative medicine included, there are insufficient clinical studies on their efficacy and safety to draw any conclusions.

Acupuncture, Chinese and herbal medicine and other complementary therapies are not recommended.

An overview of all recommendations on clinical management with their level of evidence, benefit versus harm and other considerations that contributed to their formulation is available in Table 5.

Discussion

Since the first IVF baby was born in 1978, the field of ART has undergone significant advancements, leading to improved safety and effectiveness of treatments for a greater variety of patients. These developments have greatly benefitted individuals struggling with infertility.

Innovation plays a crucial role in the field of ART and will continue to do so. The purpose of this article is not to discourage ongoing or future research. On the contrary, for add-ons that have a clear rationale, further studies should be encouraged.

However, premature implementation of innovations can lead to the widespread use of interventions that have not been proven safe, effective, or relevant for ART. In addition, when considering the introduction of new methods, it is also important to assess the level of risk and invasiveness, both in terms of treatment outcomes and patient well-being. This article identifies and examines 27 such tests and interventions and provides 42 recommendations. These tests and interventions, referred to as add-ons, are currently considered optional for an ART cycle. They often lack evidence on their efficacy and safety and are typically accompanied by additional costs for patients.

Through a careful investigation and summary of the rationale, efficacy, and safety data of the listed interventions, it is apparent that most of them cannot be recommended for routine clinical practice, meaning they should not be offered to the majority of patients (Supplementary Data File S2). Some interventions have raised (serious) safety concerns or failed to demonstrate any beneficial effects. Others lack sufficient data to support their integration into clinical practice and require further exploration through pre-clinical or clinical research, which involves ethical approval, a clearly defined protocol, and long-term follow-up. Until these studies establish clear clinical relevance and the potential for successful pregnancies, such interventions should not routinely be offered to patients. In arguing for further research, it is recognized that conducting strict research is not always feasible owing to difficulties with low numbers of patients or rare conditions, or in obtaining research funding. It is the responsibility of clinics to always monitor and follow up on non-established interventions and share the results with other clinics to gather sufficient data for meaningful conclusions.

In addition to the absence of efficacy and safety data, this article reveals that several of the included tests and interventions lack a scientific rationale or possess questionable or incorrect theoretical foundations. Although significant advancements in ART have arisen serendipitously, research and innovation should ideally be driven by sound scientific rationale and/or a valid theoretical basis.

To summarize, this article emphasizes the limitations of a set of tests and interventions currently available and provided to patients in the context of ART. ESHRE urges that all tests and interventions provided in clinical practice undergo thorough evaluation for efficacy, safety, relevance, and cost-effectiveness, and that sharing this evaluation becomes a standard part of patient counselling. A clear distinction should be made between tests and interventions that have demonstrated benefits for patients and those that have not, and the latter should only be provided within a research context. Only evidence-based add-ons should be provided to patients in clinical practice.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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Authors' roles

K.L. and A.P. chaired the ESHRE Add-ons Working Group. N.V. and N.L.C. provided methodological support. All other authors contributed equally to writing the paper, and all approved the final version for publication.

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Conflict of interest

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Table 5. Overview of all recommendations on clinical management with their level of evidence, benefit versus harm and other considerations that contributed to their formulation.

Intervention		Benefits versus harms (efficacy versus safety)	Level of evidence for efficacy (LBR/CPR)[1]	Level of evidence for safety[1]	Considerations	Recommendation
Platelet-rich plasma	Intrauterine PRP administration	Evidence of benefit on CPR, no evidence of an effect on miscarriage rate No evidence of harm	⊕◯◯◯	⊕◯◯◯	Current studies include a small sample size and heterogenous study population in addition to different dosages of PRP	Intrauterine administration of platelet-rich plasma is **not recommended**.
	Intraovarian PRP administration	Mostly uncontrolled studies, no data on effect on LBR or miscarriage rate No evidence of harm	No data	No data		Intraovarian administration of platelet-rich plasma is **not recommended**.
Duostim		No data of benefit on LBR or miscarriage rate Harms are expected to be similar to standard OS	No data	No data	An RCT comparing duostim with two conventional stimulations has not been performed to date	Duostim is **currently not recommended for routine clinical use**.
Adjuncts during ovarian stimulation		Conflicting evidence on LBR Safety concerns	⊕⊕◯◯	⊕⊕◯◯	/	Adjuncts (metformin, growth hormone, testosterone, DHEA, aspirin, indomethacin, and sildenafil) before or during ovarian stimulation are **not recommended**.
Intravaginal and intrauterine culture device	Intravaginal culture device	No evidence of a benefit on LBR No data on harms	⊕◯◯◯	No data	An embryologist and an IVF lab are still required	Intravaginal or intrauterine culture devices are **currently not recommended for routine clinical use**.
	Intrauterine culture device	One small study showing no benefit on LBR No data on harms	⊕◯◯◯	No data		
Additions to transfer media (HA)		With a high dose of HA, a benefit on LBR and reduced risk of miscarriage was found Complications: increased multiple pregnancy rate	⊕⊕⊕◯	⊕⊕⊕◯	The use of HA should be combined with a single embryo transfer policy	Hyaluronic acid addition to transfer media is **recommended**. Monitoring of the multiple pregnancy rate is still advisable.
Endometrial scratching		Inconclusive data of benefit on LBR, with no effect on miscarriage rate Complications: moderate pain, bleeding, risk of infection	⊕⊕⊕◯	⊕⊕◯◯	Timing of scratch and methodology of the procedure differed between studies.	Endometrial scratching is **currently not recommended for routine clinical use**.
Flushing of the uterus	Intrauterine administration of hCG	Some benefit for cleavage stage (not blastocyst) transfer at >500 IU No evidence of harm	⊕⊕⊕◯	⊕◯◯◯	Timing of administration, dosage of hCG and timing of embryo transfer differed between studies.	Intrauterine administration of hCG is **not recommended**.
	Intrauterine administration of G-CSF	RIF: No evidence of benefit on LBR Thin endometrium: may improve LBR (1 RCT) Very few side effects reported	⊕⊕◯◯	⊕◯◯◯	/	Intrauterine administration of granulocyte colony-stimulating factor is **not recommended**.
	Endometrial administration of embryo culture supernatant	No evidence of benefit on LBR or miscarriage rate No evidence of harm	⊕◯◯◯	⊕◯◯◯	In several studies, it was unclear how the culture media were administered, by injection or as a uterine infusion.	Endometrial administration of embryo culture supernatant is **not recommended**.

Continued

Table 5. Continued

Intervention		Benefits versus harms (efficacy versus safety)	Level of evidence for efficacy (LBR/CPR)[1]	Level of evidence for safety[1]	Considerations	Recommendation
	Endometrial exposure to seminal plasma	No evidence of a benefit on LBR or miscarriage rate Complications: no evidence of an effect on multiple pregnancy rate, potential risk of allergic reaction	⊕⊕◯◯	⊕⊕◯◯	Available evidence is very heterogenous with regards to the inclusion/exclusion criteria of patients, and the interventions	Endometrial exposure to seminal plasma is **not recommended**.
Stem cell mobilization	Stem cell therapy for POI or DOR	Available evidence comes from case reports and uncontrolled studies with very little information on the actual procedures and long-term follow-up is lacking	No data	No data	/	Stem cell therapy for premature ovarian insufficiency, diminished/poor ovarian reserve or thin endometrium is **not recommended**.
	Stem cell therapy for thin endometrium		No data	No data		
Steroids		No benefit on LBR/CPR Safety concerns: increased risk of miscarriage, preterm births, gestational hypertension, …	⊕⊕◯◯	⊕◯◯◯	/	Glucocorticoids are **not recommended** in ART treatment.
Elective freeze-all		No benefit on LBR of freeze-all over fresh transfer Complications: higher risk of hypertensive disorder in pregnancy, large-for-gestational age, and higher mean birth weight	⊕⊕⊕◯	⊕⊕◯◯	With similar LBR and OPR and longer time to pregnancy and the added freezing/thawing procedures the cost with a 'freeze-all' for all strategy will exceed the costs in conventional fresh embryo transfer.	Elective freeze-all is **currently not recommended for routine clinical use**.
ICSI for non-male factor infertility		Conflicting evidence of effect on LBR, even if most studies report no significantly higher LBR with ICSI Safety is similar to IVF	⊕⊕◯◯	⊕⊕◯◯	Mean laboratory time is significantly longer for ICSI compared to conventional IVF, in addition to an increase in cost.	ICSI is **not recommended** for non-male factor infertility.
Antioxidant therapy		Effect on LBR in females is uncertain, no evidence of effect on LBR in males; no evidence of effect on miscarriage rate. Complications: gastrointestinal discomfort has been reported	⊕◯◯◯	⊕⊕◯◯	Most of the studies showed a small sample size, retrospective design, used various combinations of antioxidants and semen parameters or DFI were used as surrogate success parameters rather than pregnancy rate	Antioxidant therapy is **not recommended** in ART treatment.
Complementary and alternative medicine	Acupuncture	Conflicting evidence of effect on LBR, no evidence of effect on miscarriage rate Minor adverse effects have been reported	⊕⊕◯◯	⊕◯◯◯	Assessing complementary therapies through RCTs is challenging, especially with respect to a suitable control group and consistent methodology.	Acupuncture, Chinese and herbal medicine and other complementary therapies are **not recommended**.
	Other complementary therapy	No data	No data	No data		
	Alternative medicine	May improve LBR/CPR No data regarding safety	⊕⊕◯◯	No data		

[1] Quality of Evidence Grades: ⊕⊕⊕⊕, body of evidence is of high quality (at least evidence from RCTs); ⊕⊕⊕◯, body of evidence is of moderate quality (evidence from RCTs or a number of observational studies showing a similar large effect); ⊕⊕◯◯, body of evidence is of low quality (mainly observational data); ⊕◯◯◯, body of evidence is of very low quality (few observational data).
CPR: clinical pregnancy rate; LBR: live birth rate; DFI: DNA fragmentation index; DHEA: dehydroepiandrosterone; DOR: diminished ovarian reserve; G-CSF: granulocyte-colony stimulating factor; HA: hyaluronic acid; IMSI: intracytoplasmic morphologically selected sperm injection; OPR: ongoing pregnancy rate; PRP: platelet-rich plasma; RIF: repeated implantation failure; RCT: randomized controlled trial; POI: premature ovarian insufficiency.

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