Good practice recommendations for add-ons in reproductive medicine

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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 application of *in vitro* fertilisation (IVF) in 1978 (Steptoe and Edwards, 1978).

Despite this, no underlying cause of infertility is identified for many couples who can therefore not be helped with appropriate treatments and even in patients with clear indications, the success of IVF is limited. The latest data from the European IVF monitoring (EIM) consortium reported that pregnancy rates (PR) per aspiration ranged from 7.8% to 47.2% and delivery rates from 6.3% to 31.3% in fresh cycles after IVF or ICSI (The European IVF-Monitoring Consortium for the European Society of Human Reproduction and Embryology et al., 2022, The European IVF-Monitoring Consortium for the European Society of Human Reproduction and Embryology et al., 2021). Pregnancy and delivery rates per thawing for frozen embryo transfer varied between 24.4% and 49.5% and between 17.8% and 40.6%, 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 a cumulative delivery rate of 32.3%, calculated over all cycles, calculated as the ratio between the total number of deliveries from fresh and frozen embryo transfers (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 assisted reproduction technology (ART) or spontaneous conception (Ferreira et al., 2016). In a study based on the Swedish IVF registry, it was shown that the cumulative live birth rate (LBR) per 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, including mainly single embryo transfer (SET) cycles (Saket et al., 2021). Belgian registry data similarly showed a LBR of 33.2% per started cycle (De Neubourg et al., 2021). A multicentre study reported a cumulative LBR of 43.9% after a single OPU and including all fresh and frozen (day 3 or day 5/6) ETs performed within a two year period after OPU (Polyzos et al., 2018).

The cumulative rate per a complete treatment was analysed in 2002 by Olivius *et al.*, showing that 63% of the couples were estimated to achieve childbirth after three available conventional IVF or ICSI cycles, including all (day 2) transfers (Olivius et al., 2002). De Neubourg *et al.*, also estimated the cumulative LBR for the total of six reimbursed OPU and ET cycles. The cumulative LBR for six cycles was estimated to be 55.4 or 76.8% (depending on the assumptions made for incomplete date) (De Neubourg, et al., 2021) (Malchau et al., 2017).

However, due to the still substantial risk of failure of any ART cycle, treatment remains a distressing event both for patients and their treating clinicians. For some patients, this risk of failure combined with the financial aspects of ART may drive them towards dropping out of treatment, while for others this fuels their desire for other presumed better treatment options. Clinicians may be driven - sometimes also by commercial motives - to go beyond usual treatments (Iacoponi et al., 2022).

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. Treatment add-ons are defined here as being not clinically relevant for an IVF/ICSI cycle but as optional additional procedures that are sometimes offered on top of standard fertility procedures, most often at an additional cost for the patient. A wide range of add-ons are on offer including tests, drugs,

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equipment, complementary or alternative therapies, laboratory, and surgical interventions, but having in common to claim to improve pregnancy or live birth rate, reduce the risk of miscarriage or shorten the time to pregnancy. Evidence on whether add-ons are safe or effective is often anecdotal or absent (Harper et al., 2017, Harper et al., 2012).

While not unique to private or commercial ART settings, the uptake of add-ons is estimated to be lower in public ART centres where tests and treatments are more often performed as stipulated in reimbursement schemes. The context is an additional factor in the use of add-on tests and treatments. For example, in some countries ICSI is only performed when indicated, i.e., in couples with diagnosed male factor infertility or fertilisation 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. As such, ICSI is not an add-on in the first setting, but should be considered so in the latter, in particular if extra costs are charged to the patients.

This paper outlines a set of add-on tests and treatments, describes the rationale for their implementation and the evidence of their efficacy and safety. The paper further makes recommendations for clinical practice including under which conditions and precautions they could be applied in clinical practice, or whether they should be further investigated in a research context. Add-ons tests and treatments are described in three subgroups: diagnostic tests, laboratory tests and interventions, and add-ons in clinical management.

Methodology

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. 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. During a second in-person meeting the feedback from the stakeholder review was discussed and the paper was finalised for publication.

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. In summarizing data for a specific add-on, priority was given to systematic reviews and 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 used. For efficacy, (cumulative) LBR was considered the primary outcome, and only when not reported in the studies, pregnancy rates were reported. Abbreviations used throughout this paper are listed in Supplementary data 1.

The final draft was published on the ESHRE website between 1 November and 1 December 2022 for stakeholder review. [TO BE ADDED IN THE FINAL VERSION] comments 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 2 [TO BE ADDED IN THE FINAL VERSION].

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Results

Diagnosis and diagnostic tests

(1) Screening hysteroscopy

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

Efficacy

According to a Cochrane review, hysteroscopy before IVF may increase LBR (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 where a mixture of unselected patients, first IVF cycle and recurrent implantation failure (RIF) patients, 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 done 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 a borderline significant benefit of hysteroscopy with respect to miscarriage rate (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 trials included in the Cochrane review (El-Toukhy and El Tokhy, 2016, Smit et al., 2016), a recent RCT confirmed similar LBR when hysteroscopy was performed before IVF or not (23.9 vs. 19.3%, respectively; n=171; p=0.607) (Ben Abid et al., 2021).

A meta-analysis focusing on patients with RIF, reported a significantly higher LBR after hysteroscopy compared to RIF patients that did not have hysteroscopy (RR 1.29; 95% CI 1.03 to 1.62; 4 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 prior to a first IVF treatment (Smit, et al., 2016).

Safety

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

Other aspects

In a recent study including 5151 women attending for outpatient hysteroscopy, although pain was reported by most women (4490; 87 %), 41% of these women rated 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 was reported despite all women receiving paracetamol/codeine prior to the procedure (Ben Abid, et al., 2021).

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There are no data on the cost-effectiveness of screening hysteroscopy. In the study by Smit *et al.*, cost effectiveness analysis had been planned but was not performed or reported because of the absence of effect of hysteroscopy (Smit, et al., 2016).

Recommendation

Based on the two recent multicentre RCTs of high quality showing no benefit with regards to live birth rate, screening hysteroscopy prior to IVF treatment is not recommended. In patients experiencing recurrent implantation failure, hysteroscopy may be beneficial as shown in the meta-analysis by Cao *et al. (Cao, et al., 2018)*.

(2) Endometrial receptivity tests

The principal mechanisms underlying human endometrium receptivity are complex and not well understood. Still, tests have emerged that investigate endometrial receptivity. Such tests report whether the endometrium is pre-receptive, receptive, or proliferative, and guides personalized ET (pET), i.e. timing of the ET according to the receptiveness (Craciunas et al., 2019). These tests have been mainly applied to patients presenting with RIF (Cohen et al., 2020, Cozzolino et al., 2020, Eisman et al., 2021, Hashimoto et al., 2017) but also to recipients of donated oocytes (Neves et al., 2019) and good prognosis patients (Bassil et al., 2018).

Efficacy

Several smaller retrospective studies on small numbers of patients failed to demonstrate a positive effect of endometrial receptivity tests (Bassil, et al., 2018, Bergin et al., 2021, Cohen, et al., 2020, Cozzolino, et al., 2020, Eisman, et al., 2021, Neves, et al., 2019), with a few studies showing some benefit of endometrial receptivity tests and pET with regards to pregnancy rates (Barrenetxea et al., 2021, Hashimoto, et al., 2017). Craciunas *et al.* summarised 5 studies published up to 2019, but the authors were unable to perform a meta-analysis due to clinical and methodological heterogeneity in patient populations (number of previously failed cycles), reported comparisons and unit of analysis (per couple or per cycle) (Craciunas, et al., 2019). 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 standard ET.

Most recently an 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). The test and pET seemed however to increase the cumulative LBR that considered both the first ET and cumulative rates after 1-year follow-up. This paper received significant criticism both on the design of the RCT (Lensen et al., 2021b) and on the fundamental utility of the endometrial receptivity test (Ben Rafael, 2021).

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 PGT-A (Neves, et al., 2019, Tan et al., 2018), which proved to have a larger effect on implantation rate than the endometrial receptivity test alone.

Safety

The endometrial biopsy procedure is considered safe and serious complications are rare (Williams and Gaddey, 2020). Since following an endometrial receptivity test, ET is performed in a subsequent cycle, the impact of the procedure on a subsequent pregnancy is considered minimal.

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Recommendation

Due to the lack of clear benefit, endometrial receptivity tests are not recommended. Furthermore, the currently available tests do not consider the full complexity of the process involving crosstalk between the endometrium and the embryo, as well as the timing, place, and depth of the biopsy.

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

Immunological test

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 like 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 view emerged that the 'foetus is rejected' unless there is modification of the maternal immune response. More recently, a claim has been made that the dominant leukocytes in the endometrium, uterine Natural Killer cells (uNK), can kill the foetus. 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, 2022).

Immunological tests applied in reproductive medicine include NK-cell levels and function in blood, typing for Killer-cell immunoglobulin-like receptor (KIR) and human leukocyte antigen (HLA) genotypes, regulatory T cells (Tregs), Th1/Th2 ratios, and cytokines. There is no clear rationale for performing any of these tests (Moffett and Shreeve, 2015). However, the local uterine immune populations are quite different than that in blood. NK -cells are measured as either numbers, percentages, ratios or with functional assays. The proportion of blood mononuclear leukocytes 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 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 the 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 they do in normal or abnormal pregnancies. Indeed, NK -functions depend in large part on inherited highly variable NK-receptors (KIR) that will differ between individuals.

Efficacy

A recent meta-analysis summarised 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 uterine NK-cells is 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, timing of biopsy and the definition of RIF varied, and BMI was not considered.

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Safety

Most of these parameters are evaluated through a blood test, apart from uterine NK -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, Killer immunoglobulin-like receptors (KIRs), was introduced by some clinics is that they are highly polymorphic meaning that women have their own repertoire of KIR genes. Some members of this family bind to HLA-C ligands expressed by the invading placental trophoblast cells (Moffett and Colucci, 2015). Several studies of pregnancy disorders like 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 compromise is still unknown. All the evidence so far points to the increased number of uNK-cells in early pregnancy acting in a physiologic 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 donors where the risk of pre-eclampsia is high (~25%) (Alecsandru and García-Velasco, 2017). These genetic tests cannot be recommended until more studies are performed in large clinically well-characterised cohorts of similar ethnic groups with appropriate controls. Detailed reasons for why these tests should not be introduced at present are outlined (Moffett, et al., 2016).

Recommendation

Peripheral blood tests for immune parameters, uNK-cell testing and KIR and HLA typing are not recommended in the context of fertility or RIF. For uNK tests, no reliable normal reference ranges have been agreed on and any changes could be an effect rather than causative and merely reflect altered global differentiation of the secretory endometrium after ovulation.

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 lipid emulsion (Intralipid) infusion, Intravenous Immunoglobulin (IVIG), leukocyte immunisation therapy (LIT), tacrolimus, anti-tumour necrosis factor (anti-TNF) agents, Granulocyte colony stimulating factor (G-CSF), and hydroxychloroquine. More recently, some of these treatments, (e.g., LIT, G-CSF) have been infused into the uterus.

Efficacy

The recommendation not to use any of these immune treatments is also the conclusion of a recent systematic review and meta-analysis of interventional studies that were considered of very low to low quality (Melo et al., 2022). The use of intralipids was evaluated in 2 RCTs including 244 patients in which the pooled effect of intralipids on the LBR was uncertain (RR, 1.78; 95% CI, 0.95–3.34; I2=26%). The use of IVIG has mostly been investigated in cohort studies, pointing towards a higher LBR. However, only 1 RCT was identified, including 51 patients, and demonstrated no clear effect of IVIG on the LBR (RR, 1.28; 95% CI, 0.32–5.16; low-certainty evidence). Recombinant human LIF was administered in 1 RCT, that showed a possible lower LBR (RR, 0.47; 95% CI, 0.24–0.91; n=150; low-certainty evidence). Two RCTs,

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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, 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 assisted reproductive technology has many known side-effects, some of which are serious (Moffett and Shreeve, 2015). Side-effects for Intralipid therapy include hepatomegaly, jaundice, cholestasis, splenomegaly, thrombocytopenia, leukopenia 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 four out of 100 pregnancies in mothers using the agent after organ transplantation (Ali et al., 2018).

Recommendation

Based on the absence of a rationale or clinical relevance for blood tests for a range on immune parameters, the uncertainty over which tests to use or how to interpret them, and the general uncertainty regarding the role of uterine NK-cells in endometrial function and implantation, no immune treatments can be recommended.

Thus, immunomodulating treatments (e.g., Intralipid, IVIG, rh-LIF, PBMCs, anti-TNF) are not recommended based on the absence of any rationale, documented side-effects, and no clinical benefit.

Laboratory tests and interventions

(4) Artificial oocyte activation (AOA)

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

Oocyte activation occurs physiologically as a synergy between the sperm and oocyte, and 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 fertilisation. Nevertheless, human oocytes are tolerant to perturbations in Ca^{2+}-balance as long as it is guaranteed that the total amount of Ca^{2+} availability is uncompromised and passes a critical threshold. Consequently, Ca^{2+} 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 storages and/or external culture medium.

There are several ways to perform AOA, none of which will result in physiological Ca^{2+}-oscillations. On the other hand, mechanical, electrical, or chemical stimuli will generate a single Ca^{2+}-peak (Kashir et al., 2022).

The least invasive but also least effective method to initiate AOA would be to modify the ICSI technique itself by making the injection process more invasive which should cause depletion of Ca^{2+} from internal

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storages due to the additional mechanical manipulations with the injection pipette (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 the above-mentioned AOA-methods are associated with a high degeneration rate or require special equipment the most common approach is the one using chemical compounds for AOA, the most common ones being Ca^{2+}-ionophores such as calcimycin or ionomycin.

In this context it has been reported that the most commonly used chemical agent for AOA in the clinic is calcimycin (also known as A23187), which 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). A ready-to-use solution of calcimycin has also been used clinically with good success rates (Ebner et al., 2012). Alternatively, ionomycin became more widely used in ART and due to its higher specificity for Ca^{2+}-ions (as compared to calcimycin) it was found to be more potent (Nikiforaki et al., 2016).

According to the literature, application of chemical AOA can be considered in cases of complete fertilisation failure, poor fertilisation outcome (<30%), and cases of severe male factor infertility. Independent of the kind of ionophore used for AOA, its clinical application is characterised by the ease of the procedure. In principle, immediately after ICSI (0-60 min) injected oocytes are transferred to a pre-equilibrated ionophore solution for a 10-30 min culture after which a series of washing steps is done.

Efficacy

A meta-analysis pooling results of 14 studies showed that AOA with any kind of calcium ionophore increased LBR (OR 2.65; 95% CI 1.53 to 4.60; 14 studies; n=3621; I^2=80%; p=0.0005, publication bias detected) and pregnancy rate (OR 2.14; 95% CI 1.38 to 3.31; 17 studies; n=4233; I^2=80%; p=0.0006) (Shan et al., 2021). In subgroup analysis, AOA with calcium ionophore significantly increased the birth rate in patients with previous fertilisation failure or low fertilisation 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 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 fertilisation failure, globozoospermia is the only indication that requires ionophore-based AOA to achieve fertilisation. With sperm from a globozoospermic patient, AOA with ionomycin resulted in a higher amplitude of the intracellular Ca^{2+}-rise during ICSI and therefore could be the first-line option, even if the fertilisation rate was not significantly different from AOA with calcimycin (30% vs. 11.8%, respectively) (Nikiforaki, et al., 2016).

Safety

Ca^{2+}-ionophores can bind Ca^{2+}-cations and due to their hydrophobic properties, they form a complex at the lipid bilayer of the membrane. Due to a conformation of their tertiary structure, ionophores then transport Ca^{2+}-molecules across the membrane and release it into the cytosol (Brasseur et al., 1983). Thus, ionophores themselves do not 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) or morphokinetics (Shebl et al., 2021) in literature. Furthermore, no increase in birth defects has been reported (Deemeh et al., 2015, Li et al., 2019a, Long et al., 2020, Mateizel et al., 2018, Miller et al., 2016) and cognition as well as language and motor skills were normal in children aged 3-

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10 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, due to the nature of the Ca^{2+}-signal, ionophores should only be used with proper indication.

However, 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 methylation level of imprinted gene H19 in cleavage-stage embryos but not in blastocysts in a small-scale human study (Liang et al., 2022).

Other aspects

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

Ionophores are also used to increase 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 makes sense since 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.

Recommendation

Artificial oocyte activation using Ca^{2+}-ionophores is not recommended for most ART-patients. There are data showing that artificial oocyte activation can be effective for cases of complete activation failure (0% 2PN), very low fertilisation (<30% fertilisation), or globozoospermia.

(5) 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 fertilisation 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.* (Craven et al., 2017) and Siristatidis *et al.* (Siristatidis et al., 2021).

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 commercially available (Woods and Tilly, 2015). A RCT comparing autologous mitochondria transfer with regular ICSI was discontinued prematurely due to negative results (Labarta et al., 2019). This technique is now suspended and not discussed further here.

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Efficacy

Only few papers have been published so far. One birth after spindle transfer was reported in a couple where the woman was carrying a mtDNA mutation (Zhang et al., 2017). Other reports of healthy births after mitochondrial replacement therapy are only available from newspapers and websites. A report on spindle transfer applied the technique in one patient carrying a mtDNA mutation and two patients with fertilisation failure. Although the authors demonstrated full replacement of the mitochondria in all cases, the study was pre-clinical and all embryos obtained were used for further investigations (Tang et al., 2022).

Safety

In view of 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 nor guaranteed (Siristatidis, et al., 2021). This is added to the significant concern regarding ethical questions (Adashi and Cohen, 2018, Craven, et al., 2017).

Other aspects

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

Recommendation

Mitochondrial replacement therapy for oocyte quality "boosting" is not recommended (and in many instances not allowed) outside strict research protocols ensuring the safety of the patients and donors involved, as well as guaranteeing long term follow-up of their offspring.

(6) In vitro activation of dormant follicles (IVA)

In patients with premature ovarian insufficiency (POI), ovarian stimulation and IVF/ICSI have limited efficacy which is attributed to inactive or dormant follicles that cannot be stimulated to produce mature oocytes. Growing evidence supports the 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 which 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; this is termed drug-free IVA. Recently, the technique was also applied to patients with poor ovarian response.

Efficacy

Due to the low chances of spontaneous pregnancy 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. The total 'classical' series of IVA consists of 51 women to whom a total of 3 babies were born, whereas drug-free IVA has been evaluated in five studies in which 15 babies were born to 126 women with POI (Wang, et al., 2021a). Those figures represent a pregnancy rate of 10.2%.

A recent RCT in 34 women with poor ovarian response showed an increase in antral follicle count (AFC) in the intervention ovary 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-Mullerian hormone (AMH) and follicle-stimulating hormone (FSH) levels or reproductive outcomes (LBR 6.7% vs. 18.7% in the IVA and control groups, respectively) (Díaz-García et al., 2022).

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Safety

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

Other aspects

There is 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.

Recommendation

In vitro activation of primordial follicles is not recommended to be routinely applied in patients with premature ovarian insufficiency or poor ovarian reserve outside strict research protocols based on limited efficacy, potentially high cost and safety concerns.

(7) In vitro maturation (IVM)

In vitro maturation (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 context of fertility preservation as an alternative 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). For the indication of PCOS/high responders and 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, and safer treatment. IVM can be considered an add-on in these situations.

Clinical IVM

Efficacy

In a study, 536 women in their first IVM cycles (942 cycles) were included (Wiser et al., 2011). The clinical pregnancy rates in women aged 20–25 years were 42.1%, 26–35 years were 33.8% and in those 36–39 years were 35.1%. The ongoing pregnancy rates in women aged 20–25 years were 36.8%, 26–35 years were 30.0% and in those 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%/oocyte retrieval or 16.6%/ET) involving 30 gestational sacs with 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 arrested in stage MI, in the second woman, one oocyte reached maturity, unfortunately the ICSI procedure resulted in abnormal fertilization.

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Hourvitz *et al.* described a case series of IVM in seven women with abnormal follicular development (Hourvitz et al., 2010). Three women had an embryo transfer of three to four embryos. Two women had a live birth.

IVM has also been successfully applied to a woman with unresponsive antral follicles to endogenous and exogenous FSH (Grynberg et al., 2013).

Safety

Currently available data do not report an increase in imprinting errors after IVM, or difference in the neonatal health and developmental outcome of children conceived with the technique as compared to those conceived through IVF/ICSI (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.

Other aspects

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 (ASRM, 2021). A recent cost-effectiveness study showed IVM is less expensive than IVF in women with a high AFC (Braam et al., 2021). IVM requires specific expertise.

Rescue-IVM or natural cycle IVF/M

Rescue-IVM has been used in poor responders or poor prognosis patients to increase the number of embryos available for transfer (Braga et al., 2010).

Efficacy

In a case series, 13 normo-ovulatory women with inadequate follicular growth or follicular arrest underwent rescue-IVM. Pregnancy was achieved in six patients. Two of these were biochemical pregnancies, while the remaining four pregnancies resulted in birth of five healthy babies (Hatırnaz et al., 2018).

Several studies reported on the combination of IVF and IVM in regularly ovulating women (Álvarez et al., 2013), (Shin et al., 2013), (Yang et al., 2012a), (Reichman et al., 2010), (Xu et al., 2010). Alvarez *et al.* compared reproductive data between cycles with embryos derived from exclusively mature oocytes or immature oocytes at retrieval. Clinical pregnancy rate was 33.1% in group 1 and 12.4% in group 2. Abortion rate was 22% in group 1 and 66% in group 2 (Álvarez, et al., 2013). Shin *et al.* retrospectively analysed 463 cycles where at least one immature oocyte was retrieved and 24 ICSI cycles with only immature oocytes at retrieval. Out of the 24 cycles with only immature oocytes retrieved, one ended in a chemical pregnancy (Shin, et al., 2013). Yang *et al.* compared reproductive outcomes between patients with mature and without mature oocytes at retrieval. clinical pregnancy rates between the groups were not different (40.1% (126/314) versus 34.5% (19/55). However, LBR per embryo transfer (29.6% = 93/314 vs. 16.4% = 9/55) and miscarriage per clinical pregnancy (26.2% = 33/126 vs. 52.6% =10/19) were significantly lower in the group without mature oocytes at retrieval (Yang, et al., 2012a). Reichman *et al.* observed that in cycles with a larger proportion of IVM embryos transferred, implantation rates and ongoing pregnancy rates were lower, and in cycles with complete IVM transfers, implantation and ongoing pregnancy rates were zero (Reichman, et al., 2010). Xu *et al.* included 323 women (364 cycles) with regular menstrual cycles for natural-cycle IVF/M. Clinical pregnancy rate was 35.9% (Xu, et al., 2010).

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In a prospective cohort study, 77 regular cycling women underwent a combination of IVF and IVM (Tang-Pedersen et al., 2012). When cycles with immature versus mature oocytes at retrieval were compared, clinical pregnancy rates per ET were 6.7% and 10.7%, respectively and the LBR were also 6.7% and 10.7%, respectively.

In a retrospective cohort study, including 63 women with oocyte maturation arrest in previous cycles, rescue-IVM was performed, and results were stratified by type of oocyte maturation arrest. Mature oocytes were only obtained in women with type 5 oocyte maturation arrest. Six women underwent embryo transfer, however, no clinical pregnancies occurred (Hatirnaz et al., 2021).

In a small study, 25 consecutive infertile women with a low functional ovarian reserve undergoing rescue IVM were included (Lee et al., 2016). In 10/25 cycles, only immature oocytes were retrieved. Following ICSI, 20 embryos were available for transfer. Only one clinical pregnancy was achieved, resulting in a live birth.

In a prospective cohort study, 146 poor prognosis patients received rescue IVM (n=50) or double ovarian stimulation (n=96) (Liu et al., 2020b). Comparing the IVM part in group 1 with the luteal phase stimulation part in group 2, there was no significant difference seen in live birth (10% vs. 16.9%) or clinical pregnancy rate (10% vs. 21.5%).

Liu *et al*. reported a case series of eight poor responders, of which three achieved a pregnancy after retrieval of immature oocytes and rescue-IVM (Liu et al., 2003).

Li *et al.* reported three cases of rescue IVM in poor responders (Li et al., 2011). The first woman had three mature oocytes (1 mature and 2 after IVM) that could be fertilised by ICSI, the second woman had two mature oocytes retrieved and the third women had three mature oocytes after IVM that could be fertilised by ICSI. All available embryos were transferred at the same time and resulted in two live births and an ongoing pregnancy.

In a large cohort study, 440 poor-responder patients with <5 mature and at least 1 immature oocyte undergoing ICSI were divided in 2 groups (Braga, et al., 2010). In group 1, only mature oocytes were injected, and in group 2 cycles were included where at least one immature remained in culture for spontaneous maturation and injected for ICSI. No significant differences were found between mature and rescue-IVM groups for clinical pregnancy rate (16.7% vs. 16.5%) or miscarriage rate (25.5% vs. 29.4%). However, the number of transferred embryos was higher in the rescue IVM group (1.87±1.24 vs. 2.35±1.22). In 17 cycles, only embryos derived from RSM oocytes were available for transfer and two pregnancies were achieved (Braga, et al., 2010).

Rescue IVM has been used to overcome empty follicle syndrome (Al-Hussaini et al., 2019), in a patient with auto-immune POI (Chansel-Debordeaux et al., 2021)

Safety

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

Recommendation

In vitro maturation is not recommended for infertile patients without specific indications (PCOS/high responders or fertility preservation) in absence of long-term safety data, procedural reliability, and effectiveness.

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(8) 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 due 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 (SSB) and double-strand breaks (DSB). 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 (Agarwal, et al., 2020, Casanovas et al., 2019).

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, Homa et al., 2019, Mahfouz et al., 2010). It was reported that oxidation reduction potential (ORP) cut-off value of 1.36 mV/106 sperm/mL could predict fertilisation (Morris et al., 2019). However, other studies reported little (Arafa et al., 2019, Majzoub et al., 2018) or no correlation between ORP and SDF (Homa, et al., 2019).

Efficacy

A systematic review and meta-analysis showed that infertile men had higher SDF compared to fertile counterparts (mean difference (MD) -1.67; 95% CI -2.12 to -1.21; 28 studies; n=4177; I^2=97%), and the SDF threshold level to discriminate infertile from fertile men was set to 20% (area under the curve (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 ART outcomes. However, the predictive value of SDF on pregnancy, live birth or miscarriage, is as yet inconclusive as the quality of evidence is low and there is significant heterogeneity between different studies included in systematic reviews and meta-analyses (Osman et al., 2015, Ribas-Maynou et al., 2021, Simon et al., 2017, Zhao et al., 2014, Zini, 2011).

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

In patients with abnormal SDF, an RCT showed that applying advanced sperm selection techniques (physiological ICSI (PICSI) and magnetic-activated cell sorting (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). A similar trial reported no difference in CPR with the use of MACS (Mei et al., 2022).

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Safety

No safety issues have been reported.

Other aspects

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

Recommendation

As there is insufficient evidence for the relevance of sperm DNA fragmentation tests to predict pregnancy or guide treatment decisions, the routine use of these tests is not recommended outside strict research protocols.

(9) Artificial sperm activation

Immotile sperm are one of the key problems in severe male factor infertility because embryologists face the problem to distinguish between immotile but viable sperm and non-viable sperm. Typically, aids such as ICSI needles, hypoosmotic solutions or laser pulses are used to identify viable spermatozoa with functional membranes, however, only pharmacological activation using chemical compounds would allow to restore 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 immotility.

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 minutes and lasts for 1-2 hours. In clinical use, a small volume of the PDE inhibitors is added to the sperm sample or the suspension containing testicular tissue. Usually, incubation with PDE inhibitors is done in an ICSI dish to facilitate identification and catching of the sperm considered for the ICSI procedure. Before injection spermatozoa are washed in culture medium and/or polyvinylpyrrolidone (PVP) to avoid carry over PTX or theophylline to the oocyte.

Efficacy

A randomized controlled prospective study on patients with mild to moderate asthenozoospermia revealed that usage of spermatozoa artificially stimulated with PTX resulted in a significantly higher rate of clinical pregnancy (73.3% vs. 60% respectively, p=0.04) (Amer et al., 2013).

In a sibling oocyte approach, ICSI with frozen-thawed sperm that were activated with a ready-to-use theophylline resulted in significantly higher rates of fertilisation (79.9% vs. 63.3%), blastocyst formation (63.9% vs. 46.8%), clinical pregnancy (53.9% vs. 23.8%), and LBR (53.9% vs. 19.1%) as compared to ICSI with 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 (Ebner et al., 2015a, Yildirim

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et al., 2009). At the same concentration both compounds have comparable activity, the half-life of theophylline, however, is 10-fold higher.

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 applied in the above-mentioned animal studies.

In human, no malformations have been observed in babies born from embryos fertilised 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) was 3.3% (4/122; 95% CI 0.9–8.2%) (Navas et al., 2017) which was considered a non-increased risk as compared to historical IVF data.

Other aspects

PTX/theophylline are usually used pre-ICSI when testicular or frozen sperm or sperm from retrograde ejaculation are to be used which often show 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.

Recommendation

Sperm activation with phosphodiesterase inhibitors should not be used as a routine technique in IVF, however, it should be the first-line treatment in cases of primary or secondary total asthenozoospermia which are not the result of axonemal structure defects (Ebner, et al., 2015a).

(10) 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 in effectively predicting the fertilising capability of the 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 next step to optimise sperm quality and eliminate factors that are detrimental to fertilisation. 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), magnetic-activated cell sorting (MACS), microfluidics and electrophoretic sperm isolation and intracytoplasmic morphologic sperm injection (IMSI), aiming to help more accurate selection of functional spermatozoa. These advanced sperm selection approaches are based on the sperm membrane characteristics, sperm size and motility (Vaughan and Sakkas, 2019).

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Sperm hyaluronic acid binding assay (HBA) and physiological ICSI (PICSI)

Hyaluron or Hyaluronic acid (HA) constitutes a major component of cumulus cells, and has been shown to selectively bind mature sperm with intact acrosome and better morphology (Huszar et al., 2003). The assay is based on the mature and intact sperm surface containing a receptor of HA or hyaluronidase, which binds to HA coated on a surface. The hyaluronan/hyaluronic acid binding assay (HBA) score has been suggested as an *in vitro* test to predict sperm fertilising potential. The score is expressed as the value of the number of bound motile sperm versus number of unbound motile sperm. Huszar *et al.* 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 (Huszar, et al., 2003).

The sperm HA binding assay has also been used for sperm selection before ICSI, so-called physiological ICSI (PICSI). The principle of the 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 of the HBA score with seminal quality and investigated fertilisation rates in IVF/ICSI in relation to the HBA score (Boynukalin et al., 2012, Esterhuizen et al., 2015, Kovacs et al., 2011, Nijs et al., 2010, Ye et al., 2006). None of the studies found any predictive value of the HBA for fertilisation or pregnancy, nor did the test aid in selecting an ART treatment method (IVF or ICSI). One study, using washed semen rather than unprocessed ejaculate, reported significantly lower hyaluronan-binding ability in samples resulting in lower IVF fertilisation rates (less than 50% of oocytes fertilised) compared to higher fertilisation rates, indicating some relevance for the test (Pregl Breznik et al., 2013). Also, the study by West *et al*. reported that lower HBA scores and sperm DNA quality were associated with poorer sperm quality that compromised treatment outcomes (West et al., 2022).

The evidence from a Cochrane review suggests that PICSI or sperm selection using HBA may have little or no effect on live birth (RR 1.09; 95% CI 0.97 to 1.23; 2 RCTS; n=2903; I^2=0%, low quality evidence) or clinical pregnancy (RR 1.00; 95% CI 0.92 to 1.09; 4 RCTS; n=3492; 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). The absence of an improvement in LBR was confirmed in a large multi-centre study published the same year (HABSelect study; OR 1.12; 95% CI 0.95 to 1.34; n=2772; p=0.18) (Miller et al., 2019). There have been studies reporting some benefit 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 sperm DNA fragmentation (Hozyen, et al., 2022).

Safety

No safety issues have been shown. There are a variety of available commercial products which select sperm based on HA receptor expression.

Recommendation

Based on the limited standardisation and the limited clinical value with regards to the prediction of fertilisation or pregnancy, or selection of treatment method, using the sperm hyaluronic binding assay is not recommended.

PICSI is not recommended as a sperm selection method since it has been shown to have little or no effect on live birth or clinical pregnancy rates.

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Magnetic-activated cell sorting (MACS)

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 lower SDF compared to DGC alone (Anbari et al., 2021, Berteli, et al., 2017, Degheidy et al., 2015). However, the effect of MACS with regards to pregnancy and live birth rates is unclear. Based on currently published studies, a recent Cochrane review reported no significant effect of the 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

No safety issues have been shown.

Recommendation

The routine use of MACS for sperm selection is not recommended based on insufficient evidence of an effect on pregnancy and live birth compared to traditional preparation methods.

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 appears to improve total motile sperm count, morphology and DNA integrity, and reduce ORP compared to conventional DGC (Gode et al., 2019, Gode et al., 2020, Quinn et al., 2018). A study, without a control group, showed the microfluidics technique significantly reduced the dsSDF as compared to raw samples and swim up (Pujol et al., 2021). In a second step of this study, sperm selection based on microfluidics and ICSI in cohort of 163 patients diagnosed previously with ≥60% dsSDF resulted in a LBR of 42.0% and a miscarriage rate of 14.4% (Pujol, et al., 2021). In a more recent RCT in 128 patients undergoing ICSI for male factor infertility, similar fertilisation 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 a study of donor egg recipients, no benefit of microfluidics selection was found (CPR 55.6% compared to 58.9 % in the DGC control group) (Srinivas et al., 2022).

Safety

No safety issues have been reported.

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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

Although based on a single rather small RCT, sperm selection using microfluidics may increase the LBR without any adverse outcomes. However, more research is needed to confirm these findings.

Intracytoplasmic morphologic sperm injection (IMSI)

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 using high magnification (> 6000x) (Bartoov et al., 2001).

Efficacy

A Cochrane review showed that IMSI does not improve LBR (RR 1.11; 95% CI 0.89 to 1.39; 5 RCTs; n=929; I^2=1%;very low quality evidence) and clinical pregnancy (RR 1.23; 95% CI 1.11 to 1.37; 13 RCTs; n=2775; I^2=47%; very low quality evidence), nor does it reduce 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 (Duran-Retamal et al., 2020, McDowell et al., 2014).

Safety

No safety issues have been reported.

Other aspects

The method of IMSI can be time-consuming and impacts laboratory workflow.

Recommendation

As the available data have not shown a benefit for clinical outcomes, intracytoplasmic morphologic sperm injection (IMSI) is not recommended.

(11) 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, LIF 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.

Efficacy

Supplementation of embryo culture medium with GM-CSF was shown to increase ongoing pregnancy (23% vs. 18.7%) and LBR (28.9% vs. 22.4%), with a more pronounced effect in women with previous miscarriage (Ziebe et al., 2013). A recent Cochrane review confirmed that 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%;

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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). In addition, there is uncertainty whether GM-CSF-supplemented culture media make any difference to clinical pregnancy (Armstrong, et al., 2020). In a more recent retrospective study, patients who underwent frozen-thawed blastocyst culture and transfer in medium supplemented with GM-CSF had significantly higher ongoing pregnancy (OR 1.64; 95% CI 1.13 to 2.41), and LBR (OR 1.67; 95% CI 1.14 to 2.45) compared to the control group (Okabe-Kinoshita et al., 2022).

Safety

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. It is suggested that if not well regulated, exogenous growth factors could have adverse effects on embryo development (Sunde et al., 2016).

The Cochrane review analysed the data on multiple gestation, preterm birth, birth defects and aneuploidy, and reported no increased incidence of these adverse events but with a large degree of uncertainty (Armstrong, et al., 2020).

Recommendation

As there is insufficient evidence to support the efficacy or safety, supplementing culture media with GM-CSF is not recommended.

(12) Assisted hatching

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

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

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

AH may lead to a higher multiple pregnancy rate (OR 1.38; 95% CI 1.13 to 1.68; 18 RCTs; n=4308; I^2=48%; low quality evidence) compared to no AH (Lacey, et al., 2021). In addition, there is concern for an increase in monozygotic twinning after AH, but the number of cases is too small to reach solid

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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 due to lack of available data (Lacey, et al., 2021).

Recommendation

Based on the lack of increase in live birth rate and since it may increase multiple pregnancy rates and monozygotic twinning rate, assisted hatching is not recommended.

(13) Genetic testing/treatments

Pre-implantation genetic testing for aneuploidy (PGT-A)

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%, at the blastocyst stage these rates are lower and mainly influenced by maternal age (Fragouli et al., 2019). This has led to the not unreasonable assumption that de-selecting embryos carrying such chromosomal abnormalities would have beneficial effect on the outcome of ART cycles. PGT-A has known several re-iterations both at the level of the technology used and the preferred embryo stage for biopsy (Sermon et al., 2016). Initially fluorescent in-situ hybridization (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, first using array-comparative genomic hybridisation (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 are summarized in Table 1, including whether outcomes were reported per ET or per patient (per started cycle). The earliest RCTs showed some beneficial effect, such as sustained implantation rate (Dahdouh et al., 2015), but were heavily criticized for either being on small groups, using the wrong outcome, or serious methodological flaws (Forman et al., 2013, Mastenbroek and Repping, 2014, Scott et al., 2013, Yang et al., 2012b). The later review by Cornelisse *et al.* included more robust RCTs and concluded that there was no increased LBR after the first embryo transfer per woman randomised after PGT-A (Cornelisse et al., 2020). With regards to the miscarriage rate per woman randomised, included RCTs showed contradicting results (Cornelisse, et al., 2020, Munné et al., 2019, Verpoest et al., 2018). Most recently, a large Chinese RCT in younger patients (20 – 37-year-old) also failed to show improvement in live birth rates per cycle (Yan et al., 2021). The largest RCTs also received criticism: the ESTEEM study (Verpoest, et al., 2018) was criticised because polar body biopsy was chosen, the STAR study (Munné, et al., 2019) was criticised 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).

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Table 1: . CGH: comparative genomic hybridisation, LBR: live birth rate, NGS: next generation sequencing

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)
(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%) versus 30/313 (9.6%)
(Yan, et al., 2021)	660 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 (≥20wks GA)
[2] ongoing pregnancy rate per randomized patient after the first ET
[3]Ongoing pregnancy rate (OPR) at 20 weeks' gestation per embryo transfer

Safety

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

Another meta-analysis focussing on the safety of cleavage and blastocyst stage biopsy and PGT reported an increased risk of certain adverse obstetric and neonatal outcomes, namely low birth weight, preterm delivery, hypertensive disorders of pregnancy and lower gestational age and birth weight in PGT

pregnancies relative to pregnancies after spontaneous conception. In the comparison of PGT pregnancies to IVF/ICSI pregnancies, the reviewers reported a decreased risk of very preterm delivery and very low birth weight 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 often applied in these cycles. This brings its own risks, as discussed in the paragraph on freeze-all strategy.

Other aspects

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

PGT-A is a costly procedure, demanding skilled personnel for the biopsy and genetic analysis, as well as an important investment in genetic analysis instrumentation which is often passed on to the patient (van de Wiel et al., 2020).

Recommendation

Based on the current evidence showing lack of improvement of live birth rates, or a decrease in miscarriage, routine use of PGT-A is not recommended. However, PGT-A may decrease time to pregnancy in specific patient groups.

Non-invasive pre-implantation genetic testing (niPGT)

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

Efficacy

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

Safety

It can be assumed that niPGT-A represents even lower risk for the ensuing pregnancy and baby.

Mitochondria DNA load measurement

Fragouli *et al.* reported that euploid blastocysts that failed to implant carried a higher load of mtDNA molecules (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

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

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

Recommendation

Both niPGT and mtDNA load measurements are to be considered in research phase.

(14) Time-lapse imaging (TLI) with or without embryo selection software

Time-lapse imaging (TLI) involves a specialised incubation system that takes frequent digital images of the embryos in culture. Put together, these images make a time-lapse video 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 which may potentially improve LBR: it 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 increase LBR and time to pregnancy rate by selecting and freezing the embryos with the highest implantation potential. A wide range of algorithms have been designed for embryo selection, but they appear to be lab dependent, probably due to differences in culture conditions such as culture media and environment (Lundin and Park, 2020).

In addition to being marketed as an option to improve live birth rates, which is considered an add-on intervention, TLI provides a tool for research, teaching, standardising assessment and facilitating laboratory workflows (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.

Efficacy

The most recent Cochrane review on TLI concluded there is insufficient good-quality evidence of differences in live birth or ongoing pregnancy, miscarriage and stillbirth, or clinical pregnancy 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 ongoing PR. 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/ongoing pregnancy rate (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, these findings should be interpreted with caution.

Safety

Kirkegaard *et al.* concluded that TLI was as safe as embryo culture in conventional incubators (Kirkegaard et al., 2012).

Other aspects

In the UK, 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.

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Recommendation

Time-lapse imaging has been shown to be a convenient and effective incubator which allows a continuous view of embryo development. However, TLI with or without embryo selection software has not been shown to improve the LBR.

Clinical management

(15) Platelet rich plasma (PRP)

Platelet-rich plasma (PRP) is a technique – used in orthopaedics – based on the isolation of autologous platelets at high concentration, obtained after centrifugation of peripherally collected 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 either improve folliculogenesis 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.

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, 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). More recently published RCTs all reported positive results in favour of PRP (Bakhsh et al., 2021, Dieamant et al., 2019, Javaheri et al., 2020, Nazari et al., 2020, Nazari et al., 2021, Nazari et al., 2022b, Zamaniyan et al., 2021). 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, Nazari, et al., 2020, Nazari, et al., 2021, Nazari, et al., 2022b). Also, the largest RCT including 438 patients has been registered as aiming to include 30 patients per arm and eventually published with more than 10 times higher sample size (Nazari, et al., 2021). 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.

Intraovarian PRP injection for poor ovarian response or premature ovarian insufficiency

Intraovarian injection of PRP has been suggested as 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 the 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 4 studies (1 case control and 3 uncontrolled studies) concluded that intraovarian PRP infusion increases the mature oocyte yield, fertilisation rates and good quality embryo formation rate (Panda et al., 2020). An additional

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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 (such as orthopaedics) 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.

Other aspects

Either intrauterine or intraovarian PRP is an experimental procedure without compelling evidence in its favour; still in several settings the procedure is offered with extra costs which are not justified by the evidence.

Recommendation

Even if the available data are promising, there are significant issues with their quality and in general, there is a lack of data showing safety in the ART context. Therefore, intrauterine or intraovarian platelet rich plasma (PRP) is not recommended outside strict research protocols in infertile women.

(16) 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, immediately after the oocyte pick up, 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

Apart from reassuring evidence on the quality of the oocytes retrieved, the guideline did not find any data on the efficacy and safety of the procedure, and hence recommended it can be used for urgent fertility preservation but requires further research in poor responders (The ESHRE Guideline Group on Ovarian Stimulation, et al., 2020).

(17) Adjuncts during ovarian stimulation

Whether the addition of adjuvants 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.

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Recommendation

The use of adjuncts before and/or during ovarian stimulation is not recommended. Adjuncts include metformin, growth hormone, testosterone, DHEA, aspirin, indomethacin, and sildenafil.

(18) Intravaginal and intrauterine culture device

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

Intravaginal culture device

Intravaginal culture uses a 3x4cm, 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 to 5 days at which point the embryos are evaluated and transferred or stored accordingly.

The device was originally designed to simplify IVF. 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 were similar to the US average (Lucena, et al., 2012). García-Ferreyra *et al.*, used ICSI embryos and found comparable day 3 development and pregnancy rates (García-Ferreyra et al., 2015). A pilot RCT of 10 patients showed that fertilisation and pregnancy rates were higher with conventional IVF as compared to fertilisation in the device (Mitri et al., 2015). The authors also used questionnaires to document the woman's experience and reported that the women felt fertilisation was more natural. The founders of the device performed an RCT on 40 women who underwent mild stimulation, with blastocyst quality as primary outcome (Doody et al., 2016). They found the control group embryos were of a higher grade but that live birth rates were similar.

A large descriptive study examined 463 patients who underwent 526 cycles, and they claimed comparable results to *in vitro* culture but 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). They reported that intravaginal culture device is currently used in 65 centres in the US and that there is a need for a formal cost-efficacy evaluation, but this would exclude most of the patients that intravaginal culture device is currently used for, and an embryologist and IVF lab are still required.

Safety

The intravaginal culture device was FDA approved in 2016 and CE marked in 2019. 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 birth weight and preterm delivery were reported (Kaye et al., 2022).

Other aspects

Intravaginal culture devices are promoted as a more natural and cost-conscious approach to ART, but it does not eliminate the need for an IVF laboratory or skilled embryologist, nor does it reduce exposure

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to synthetic culture media, which are still need to load the gametes into the device (Lucena, et al., 2012).

Recommendation

There is currently no evidence that the intravaginal culture devices have an advantage over standard IVF with regards to clinical outcomes. It could be used for its expected psychological benefits.

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 thirteen 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 CE marked and is approved for clinical use in the UK (HFEA), Spain (AEMPS, Consejerias de sanidad), Denmark (Sundhedsstyrelsen), Czech Republic (MZCR) and Poland (URPL).

Recommendation

There is currently no evidence that the intrauterine culture devices have an advantage over standard IVF with regards to clinical outcomes. It could be used for its expected psychological benefits.

(19) Additions to transfer media (hyaluronic acid)

Despite what on many occasions may seem to be optimal conditions at embryo transfer, 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 intrinsically secreted.

Hyaluronic acid (HA) is one of the major macromolecules present in the female reproductive tract. In addition to being a promotor of cell-to-cell adhesion, HA produces a viscous solution that has been proposed to inhibit expulsion of the embryo (Stojkovic et al., 2002). HA can be present in culture media in lower concentrations, but also used at higher concentration at embryo transfer. The embryo is preincubated in the HA enriched transfer medium for up to 4 hours before ET.

Efficacy

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

A recent Cochrane review (Heymann et al., 2020), including 26 RCTs and 6704 women undergoing assisted reproduction compared embryo transfer media with no addition of HA to either 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 due to imprecision and/or heterogeneity. In studies with live

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birth as 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). The increase was seen both for early cleavage stage embryo transfers and for blastocyst transfers, as well as for good and poor prognosis patients. The time of exposure to HA was of importance; 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).

Multiple pregnancy rates were found to be increased (RR 1.45; 95%CI 1.24 to 1.00; 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.

Some of the studies were mixed fresh and frozen-thawed transfers, however, 3 studies were performed only on frozen embryo transfer (FET) cycles (n=713), and these studies showed no evidence of a beneficial effect. This was supported by a recent RCT including 550 FET cycles, where Yung *et al.* found no improvement in LBR when comparing to standard transfer medium (Yung et al., 2021).

Heymann *et al.* 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 studies; n=317; I^2=50%; low quality evidence) and CPR (RR 1.06; 95% CI 0.97 to 1.28; 3 studies; n=351; I^2=23%; low quality evidence) (Heymann et al., 2022).

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.

Apart from miscarriages, in the Cochrane analysis (Heymann, et al., 2020), two studies reported on ectopic pregnancies, and one on foetal malformations. The pooled results showed no evidence for an increase of 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

Addition of HA as an adherence compound in embryo transfer media in IVF seems to increase the live birth/clinical pregnancy rates without a significant effect on adverse outcomes.

The increased multiple pregnancy rate should be further investigated but HA addition to transfer media is recommended to be performed only within a single embryo transfer policy program.

(20) Endometrial scratching

Endometrial scratching, also termed endometrial injury, has been proposed to improve the chance of implantation of the embryo in patients undergoing IVF. Although unsupported by evidence and debated, endometrial scratching is thought to initiate changes likely to improve implantation because of (1) induction of endometrial decidualization, (2) a wound-healing response, associated with a beneficial inflammatory response in the endometrium, (3) modulation of gene expression of a variety

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of genes involved in preparation of the endometrium for embryo implantation and (4) an improved synchronicity between the endometrium and the transferred embryo (Lensen et al., 2021c).

Numerous RCTs and systematic reviews have been published on endometrial scratching, including comparison of timing and the number of procedures made, how the endometrial injury is performed, and which population may benefit from it.

Efficacy

The most recent Cochrane review included a total of 37 studies (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 live birth was unclear as the result was consistent with no effect, or a small reduction, or an improvement (OR 1.12; 95% CI 0.98 to 1.28; 8 studies; n=4402; I^2=15%; moderate quality evidence). Similarly, the effect of endometrial scratching on clinical pregnancy was unclear (OR 1.08, 95%CI 0.95 to 1.23; 8 studies; n=4402; I^2=0%; moderate quality evidence). 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 certain 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 studies 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 studies; 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 studies; 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; p=0.15) between the endometrial scratching and control groups when endometrial scratching was performed once. However, when endometrial scratching was performed twice, significantly higher LBR (RR 1.30; 95% CI 1.06 to 1.59; p=0.01) was found in the endometrial injury group. Miscarriage rate did not differ between the endometrial scratching and control groups in any of the analyses.

In another recent review the aim was to update the evidence regarding endometrial scratching women undergoing their first IVF cycle (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 result showed that that endometrial scratching had no statistically significant positive effect on LBRs in the first IVF cycle (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).

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A beneficial effect of endometrial scratching on LBRs in women with more than two previous failed embryo transfers was shown in another earlier review (Vitagliano et al., 2018). Yet, the combined result of 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. 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).

Safety

Minimal to moderate bleeding and pain has been reported in relation to endometrial scratching. When the procedure is performed by hysteroscopy, a small risk of infection exists.

Other aspects

Endometrial scratching (by pipelle) is a relatively easy procedure to perform. While the procedure it itself is considered cheap, the cost-effectiveness is difficult to assesses due 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).

Recommendation

Routine use of endometrial scratching for patients undergoing IVF/ICSI cannot be recommended. A benefit of endometrial scratching for specific patient subgroups has not been equivocally shown and further studies are needed if it is to be recommended.

(21) Flushing of the uterus

Flushing of the uterus has been performed with human chorionic gonadotropin (hCG), G-CSF, embryo culture supernatant and seminal plasma. Other agents have been used, but with too few 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 synthesised 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.

Efficacy

A Cochrane review summarised the studies evaluating intrauterine administration of hCG and its effect on reproductive outcomes, in women undergoing IVF. To overcome the heterogeneity of the data, results were reported per 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 increased with higher dosage of hCG (≥500 IU) (RR 1.57; 95% CI 1.32 to 1.87; 3 RCTs; n=914;

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I^2=0%; moderate quality evidence). With regards to clinical pregnancy rate, there was no benefit observed with the lower hCG dosage (<500 IU), but a benefit was reported for the higher dosage.

For blastocyst transfer with intrauterine hCG (≥500 IU) compared to controls having blastocyst transfer without hCG, no benefit on LBR, nor clinical pregnancy rate, 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 embryo transfer, 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 Cochrane review was published in 2018, 4 more meta-analyses have been published (Gao et al., 2019, Hou, et al., 2018, Tan et al., 2019a, Xie et al., 2019). One of them, including only fresh cycles, showed no benefit in clinical pregnancy and LBRs with intrauterine hCG compared to conventional IVF (Hou, et al., 2018). The meta-analysis from Xie *et al.* 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; 3 RCTs; n=870; p=0.001) (Xie, et al., 2019). Gao *et al.* 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%), CPR (47.80% vs. 32.78%), and implantation rate (31.64% vs. 22.52%) compared to no intervention (Gao, et al., 2019). Comparable results, based on similar included studies, were reported by Tan *et al.* (Tan, et al., 2019a).

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, resp.) immediately after oocyte retrieval, rather than at ET as 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 increased clinical pregnancy rates compared to saline intrauterine infusion (Hosseinisadat et al., 2021, Torky et al., 2022).

Safety

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

In Gao *et al.,* the miscarriage rate was significantly lower (12.4% vs. 18.6%) 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).

Other aspects

Costs have not been discussed in any of the systematic reviews, but these are likely restricted to the cost of an extra catheter and the procedure itself.

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Recommendation

Intrauterine administration of hCG is not recommended. Further trials are necessary, with live birth as the primary outcome, to identify the groups of women who could benefit from this intervention.

Intrauterine administration of G-CSF

G-CSF is a glycoprotein functioning as a growth factor and cytokine with functional sites in the reproductive system. The rationale for using G-CSF is that it is believed to induce trophoblast proliferation, invasion, and maintenance during pregnancy. Additionally, it may improve endometrial receptivity for patients with RIF by promoting endometrial vascular remodelling, embryo adhesion and invasion and regulating endometrial immunity and it can maintain endometrial growth by inhibiting apoptosis. G-CSF also regulates expression of genes associated with embryo adhesion, cell migration, tissue remodelling and angiogenesis essential for implantation.

Efficacy

Three reviews and meta-analyses have recently been published on the subject (Hou et al., 2021b, Jiang et al., 2020, Melo, et al., 2022, Rocha et al., 2020). The most recent review by Melo *et al.*, including 2 RCT with good prognosis patients, 2 RCT with at least 1 implantation failure and 1 RCT with thin endometrium patients, reported that intrauterine G-CSF may result in a higher OPR or LBR than placebo or no intervention (RR, 1.52; 95% CI, 1.11–2.10; 5 RCT; I^2=12%), although the certainly of the evidence was found to be low (Melo, et al., 2022). The review by Hou *et al.* included nine RCTs with 976 patients with RIF (Hou, et al., 2021b). There were no significant differences in the LBR (RR 1.43; 95% CI 0.86 to 2.36) and the miscarriage rate (RR 1.13; 95% CI 0.25 to 5.21) in their pooled analyses (Hou, et al., 2021b). Subgroup analysis indicated that G-CSF improved the CPR for both the fresh and frozen embryo transfer cycles (fresh: RR 1.74; 95% CI 1.27 to 2.37; and frozen: RR 1.44; 95% CI 1.14 to 1.81), but the biochemical pregnancy rate of the RIF group was also higher than that of the control group (RR 1.85; 95% CI 1.28 to 2.68) (Hou, et al., 2021b). Jiang *et al.* found similar positive results for G-SCF administration on CPR in RIF patients; however, the miscarriage rates seemed higher although not significant (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.* 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, 1.24–5.29; 1 RCT; n=304), although the evidence was judged to be of low certainty owing to the serious risk of bias and low number of events (Melo, et al., 2022). Overall, conclusions are limited as studies' sample sizes are small and are a mix of cleavage and blastocyst transfer in both fresh and frozen cycles.

Another review including several interventions to optimize embryo transfer included 4 RCTs on this topic and concluded there is a mixed benefit of using G-CSF with low evidence quality (Tyler et al., 2022).

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

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Safety

No firm conclusions can be drawn on miscarriage rates or other safety aspects.

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

Recommendation

Based on the current literature, intrauterine infusion of G-SCF is not recommended outside strict research protocols, including for patients experiencing recurrent implantation failure, as there is no robust evidence showing it improves live birth rates in fresh or frozen cycles.

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 embryo transfer, approximately 20µL 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 review including five RCTs involving 526 women (Siristatidis et al., 2020). No RCTs on embryo culture supernatant have been published since the Cochrane review.

There was no significant effect on LBR/ ongoing pregnancy rate with 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 and OPR following placebo or no treatment is assumed to be 42%, the chance following the endometrial injection of embryo culture supernatant before embryo transfer would vary between 22% and 81%. Similar results were reported for CPR (OR 1.13; 95% CI 0.80 to 1.61; 5 RCTs; n=526, I^2=0%; very low-quality evidence) (Siristatidis, et al., 2020).

Safety

There was no 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) or 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). 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%.

Other aspects

Costs for the embryo culture supernatant have not been discussed in any of the RCTs nor in the Cochrane review but are assumed to be limited to the ET catheter used to administer the supernatant.

In several studies, it was unclear how the culture media were administered, by injection or as a uterine infusion.

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Recommendation

The current evidence does not support the use of embryo culture supernatant for intrauterine application to increase success rates in IVF.

Endometrial exposure to seminal plasma

The 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 embryo transfer (Ata et al., 2018). The Cochrane review reported no or little difference with regards to live birth rates (RR 1.10; 95% CI 0.86 to 1.43; 3 RCTs; n=948; I^2=0%, low-quality evidence), miscarriage rates (RR 1.01; 95% CI 0.57 to 1.79; 4 RCTs; n=1209; I^2=0%; low-quality evidence) or multiple pregnancy rates (RR 1.11; 95% CI 0.76 to 1.64; 5 RCTs; n=1642; I^2=9%; low-quality evidence). The studies were very heterogenous with regards to the inclusion/exclusion criteria of patients, and the interventions. The latter 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 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). While the reviewers found no data on infection or other adverse events following seminal plasma application at embryo transfer, 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

Since there is insufficient evidence of a benefit for live birth rate and potential risks, seminal plasma administration into the vagina or uterus is not recommended.

(22) 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 due to chemotherapy/radiotherapy which conceived spontaneously following autologous stem cell transplantation (Hershlag and Schuster, 2002, Veitia et al., 2007).

Because MSCs 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 BMDSCs, both MSCs and HSCs, into the ovary could help maintain or promote follicular rescue in patients with impaired (such as POI) or aged ovarian reserves (Fàbregues et al., 2020). Administration of the stem cells to the ovary can be achieved through transvaginal ultrasound-guided injection, ovary injection via

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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 concentration of bone marrow derived MSCs. The presence of the MSCs significantly promoted the survival rates, increased the growth velocity, and improved the viability of preantral follicles in *in vitro* culture (Xia, et al., 2015).

A case report by Gupta *et al.* describe a live birth after injection of BMDSCs into the ovary of a post-menopausal woman by laparoscopy (Gupta et al., 2018). Similarly promising results were obtained by Edessy *et al.* after laparoscopic injection of MBDSCs into the ovary of 10 patients with POI (Edessy et al., 2016). In a comparative clinical study, including 31 poor ovarian responders, menstrual blood derived MSCs were injected in 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 two out of 16 women (either spontaneously or via IVF) in the control group (Zafardoust et al., 2020).

Herraiz *et al.* injected BMDSCs into the ovarian artery of patients with poor ovarian reserve. Ovarian activity improved in 81.3% of women, resulting in three spontaneous pregnancies and 2 after embryo transfer (Herraiz et al., 2019).

Stem cell therapy for thin endometrium

In women of reproductive age, the endometrium undergoes stripping every menstrual cycle, and can be rebuilt without scarring in subsequent cycles. It is hypothesised that endometrial stem cells have a crucial role in this uterine homeostasis and regeneration, and that thin endometrium is the consequence of 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 on the damaged endometrium. Sixty days after transplant, the endometrium was normal seeming in the transplant group, while the controls groups showed severe intrauterine adhesions (Xin et al., 2019). Similarly, in a prospective study in humans, umbilical cord derived MSCs were seeded onto a collagen scaffold and transplanted on day 7-12 of menstruation in the uterine cavity of 17 patients with refractory adhesions. This procedure was repeated in the next menstrual cycle. One month later, a hysteroscopy was performed with endometrial biopsy, and patients were allowed to proceed with FET. The endometrial thickness was significantly increased with MSC treatment (4.08±0.26 mm vs. 5.87±0.77 mm). Four patients achieved pregnancy, one spontaneous and three after FET, resulting in three live births and one spontaneous second trimester abortion (Zhang, et al., 2021).

Sapozhak *et al.* presented a case of a woman with PCOS and atrophic endometrium. She was treated with a submucosal injection of autologous endometrium derived MSCs. After 13 days, the endometrial thickness was increased from 2mm to 6.3mm, 2 embryos were transferred resulting in a dichorionic twin pregnancy (Sapozhak et al., 2020). Similarly, a large 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 1ml autologous PRP and via transmyometrial catheter transferred into the uterine cavity. Treatment with MSCs

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produced a significant increase in endometrial thickness (5.25±1.24 vs. 9.93±0.77), 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 5 women with endometrial atrophy, autologous BMDSCs 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 the stem cells have been described, which are all invasive with serious risk 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 on the health of infants born after such treatment modalities is not available.

Recommendation

Stem cell mobilization is not recommended as the technique has no rationale and the only available data is derived from case series or retrospective case control studies with small sample size. Further preclinical studies should evaluate the relevance of this technique.

(23) 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 to improve folliculogenesis and pregnancy rates in women undergoing IVF/ICSI. However, there is inconsistent data on whether administration of glucocorticoid during ovarian stimulation yields any superiority for live birth rates 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 review reported that the LBR and CPR were comparable across groups assigned to glucocorticoids supplementation (different dosages) or placebo (LBR: OR 1.08; 95% CI 0.45 to 2.58; 2 RCTs; n=310; low quality evidence; CPR: OR 1.69; 95% CI 0.98 to 2.90; 2 RCTs; n=310; low quality evidence) (Kalampokas et al., 2017). Another meta-analysis focussing on women with RPL reported that the ongoing pregnancy rate 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). In patients with anti-thyroid antibodies, a recent review evaluating two prospective and one retrospective studies using various thresholds for concentration and applying different treatment modalities, an improved live birth rate was noticed with glucocorticoid treatment (OR 3.19; 95% CI 1.13 to 9.04; n=237; p=0.03) (Zhou et al., 2021a).

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Safety

With regards to the safety of glucocorticoid administration, animal studies have reported foetal growth retardation, cardiovascular, metabolic, neuroendocrine disorders, and teratogenic effects. In human, increased risk of miscarriage, preterm births, gestational hypertension, and diabetes have been reported, even if the data are limited (Kim, 2021).

Recommendation

Although the use of glucocorticoids might have some benefits in patients with autoimmune disease, the available data were based on small, non-controlled designs with inconsistent criteria. Therefore, the available data does not support routine administration of glucocorticoids in patients undergoing IVF among unselected or any particular group of patients.

(24) 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 ovarian hyperstimulation syndrome (OHSS), and 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 due to 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. To address efficacy and cost-benefit of the freeze-all strategy used during IVF, systematic reviews, meta-analyses and RCTs comparing reproductive outcomes in freeze-all with fresh ET were considered for inclusion. For the aim of this paper, studies evaluating freeze-all in the context of OHSS prevention were not considered.

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, Le et al., 2022, Li et al., 2019b, Smith et al., 2019) .

A meta-analysis from 2018 based on seven studies comparing women who underwent freeze-all and those who had fresh-ET found that the live birth and clinical pregnancy rates were significantly higher in the freeze-all group (LBR: RR 1.18; 95% CI 1.08 to 1.30; 6 RCTs; n=2194; I^2=40%; p=0.0003; CPR: RR 1.10; 95% CI 1.02 to 1.19; 6 RCTs; n=2041; I^2=41%; p=0.02) (Zhang et al., 2018).

The most recent Cochrane meta-analysis found 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 between 57% and 63% versus 58% following the conventional strategy. Neither was there a difference in ongoing pregnancy rate (OR 0.95; 95% CI 0.75 to 1.19; 4 RCTs; n=1245; I^2=31% ; moderate quality evidence) or miscarriage rate (OR 1.06; 95% CI 0.72 to 1.55; 2 RCTs; n=986; I^2=55%; very low-quality evidence) (Zaat, et al., 2021). The non-superiority of the freeze-all strategy was also confirmed in the two most recent RCTs performed in Europe on 460 and 619 patients (Maheshwari et al., 2022, Stormlund et al., 2020).

The reason for the differences between the two meta-analyses is most likely that the one by Zaat *et al.* used cumulative live birth rate as the primary outcome and included also the most recent RCTs with

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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).

Safety

Regarding safety aspects the Cochrane review showed that the risks of hypertensive disorder in pregnancy (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 higher mean birth weight was observed after freeze-all (MD 127g; 95% CI 77.1 to 177.8; 5 RCTs; 1607 singletons; I^2=0%; moderate quality evidence) (Zaat, et al., 2021). A review on perinatal outcomes specifically also reported an association of frozen ET with large for gestational age babies, but also caesarean section and preeclampsia, 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 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).

Other aspects

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 live birth rate 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 in conventional fresh embryo transfer. 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).

Recommendation

As the freeze-all strategy is not superior to fresh embryo transfer in terms of cumulative live birth rate, live birth rate and ongoing pregnancy rate, while time-to pregnancy is likely to be longer, elective freeze-all is not recommended. Obstetric and perinatal risks including hypertensive disorders in pregnancy, large for gestational age and macrosomia are higher after freeze-all. This method should only be adopted if there is a definite clinical indication, such as an increased risk of OHSS or endometrial pathology and in case of PGT cycles.

(25) ICSI for non-male factor infertility

ICSI is an ART technique that has created a breakthrough in the field as it improved fertilisation rates and pregnancy rates 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 increase 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 due to its increased use among patients with non-male infertility (Boulet et al., 2015).

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Efficacy

Even if most evidence has been published regarding the efficacy of ICSI focussed on couples with non-male factor infertility, its role in case of a normal sperm analysis remains questionable. The first large multicentre RCT failed to find any differences in implantation and clinical pregnancy rates in women scheduled for IVF for non-male factor infertility (Bhattacharya et al., 2001). Following this report, several studies have been published in order to 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.

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

Similarly, in advanced maternal age patients, ICSI did not improve fertilisation rates and clinical outcomes as compared with IVF (Gennarelli et al., 2019, Tannus et al., 2017), with some studies even reporting lower LBRs following the use of ICSI (Supramaniam et al., 2020). The most recent RCT comparing IVF and ICSI in advanced age women (>39 years old) has shown that both fertilisation techniques result in comparable fertilisation rates and number 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 in terms of fertilisation rates and fertilisation 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 has been 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 fertilisation failure (RR.0·85; 95% CI 0.53 to 1.38; p=0·60) as compared to IVF (Dang, et al., 2021).

Safety

Concerns have been raised regarding the safety of ICSI over IVF, 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 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 more recent reports (Bosch et al., 2020, Leunens et al., 2006). However, 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).

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In terms of imprinting disorders and DNA-methylation, although an early study supported that children born from ICSI demonstrated higher DNA-methylation in the 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, 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) are associated with limited epigenetic variation at birth and these largely resolve by adulthood (Novakovic et al., 2019).

Other aspects

Although the use of ICSI is widespread today, the mean laboratory time is significantly longer for ICSI compared to conventional IVF (Bhattacharya, et al., 2001). From a detailed treatment cost analysis of conventional IVF and ICSI, it was calculated that the cost of ICSI was 8.3% higher than IVF (Bouwmans et al., 2008); some clinics may charge up to 30% more for an ICSI cycle as compared with conventional IVF.

Recommendation

Since there are no significant benefits in terms of pregnancy, live birth and cumulative live birth rates and there is an increased cost with ICSI as compared to conventional IVF, ICSI should not be recommended in case of non-male factor infertility.

(26) Antioxidant therapy

Oxidative stress has been implicated in the deterioration of sperm count, motility, morphology, fertilisation, and embryo development and suggested to be associated with the risk of infertility, miscarriage, and RIF (Scaruffi et al., 2021, Wang et al., 2019). Lifestyle factors, pollution, stress, allergies, and clinical varicocele are considered to increase oxidative stress (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 oxidative stress (Showell et al., 2020). However, the methodology in the measurement of oxidative stress, particularly in sperm samples, the ideal combination of antioxidant therapy and their efficacy is controversial.

Efficacy

For female subfertility, a Cochrane review revealed that 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; low quality evidence) (Showell, et al., 2020). 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). 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 of evidence). There was also no evidence that different antioxidants had differing effects (de Ligny, et al., 2022).

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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).

Other aspects

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 DNA fragmentation indexes, presence of varicocele etc. However, 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 the pregnancy rate itself (Majzoub and Agarwal, 2018).

Recommendation

As there is no sufficiently reliable and good quality evidence to support an improved live birth rate, antioxidant therapy in male or female patients is not recommended.

(27) 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 (NLP) 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 wellbeing, but also claims that they may improve IVF outcome (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 3 used ART add-ons (Lensen et al., 2021a). In the UK, practitioners offering complementary therapies are often external to the IVF unit, and 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 PCOS patients due to its effects on beta-endorphin production, which may affect gonadotropin-releasing hormone (GnRH) secretion (Lim et al., 2016, 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 pregnancy rates 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

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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 two years on acupuncture have either shown no effect, or improved CPR but with low quality evidence and method heterogeneity (Coyle et al., 2021, Jang et al., 2020, Li et al., 2021c, Wang et al., 2021b). For example, Coyle *et al.* 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) (Coyle, et al., 2021).

Systematic reviews have further summarized the studies for specific patient populations, such as PCOS patients. A Cochrane review found no benefit on LBR (RR 0.97; 95% CI 0.76 to 1.24; 1 RCT; n=926; low quality evidence), but also commented there were too few RCTs to determine if acupuncture helped (Lim, et al., 2019).

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 Chinese herbal medicine, 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.

Safety

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

Other aspects

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

Recommendation

For acupuncture, there is conflicting evidence of whether it will improve live birth rate, therefore it cannot be recommended. For all other complementary therapies and alternative medicine, since there are no clinical studies, they cannot be recommended for use.

Discussion

From the birth of the first IVF baby in 1978; the field of Medically Assisted Reproduction (MAR) has evolved tremendously thanks to innovation. Treatments have improved both in safety and efficacy, which has benefited many people affected by infertility.

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Innovation is and will remain essential for the field of MAR, and this paper does not intend to discourage any ongoing or future research. In fact, for those add-ons that have a clear rationale, ESHRE would encourage further studies, if they are performed within a research context.

However, precocious introduction or implementation of innovation can result in commercial distribution of interventions that have not been shown to be safe, effective, and/or relevant. The current paper outlines 27 tests and interventions that fall under these categories, and that we have defined as add-ons, i.e., they are currently considered not essential, nor relevant for an ART cycle, are often missing evidence on efficacy and safety, and are most often offered with an additional cost for the patient.

We have carefully investigated and summarized the proposed rationale of the listed tests and interventions and the most reliable data on their efficacy and safety. From this analysis, most of the tests and interventions are not recommended for routine clinical practice, meaning they should not be offered to patients. For some interventions, the available data have highlighted safety concerns or have shown they are ineffective. Other interventions are lacking sufficient data to support their uptake in clinical practice, and these should be further explored either in pre-clinical research, or in a clinical research context, which includes ethics board approval, a clearly defined protocol and (long-term) follow-up. Until these studies have shown clear clinical relevance for the patients and their chances of having a healthy baby, such interventions should not be offered and definitely not at an additional cost to the patients.

In addition to the absence of efficacy and safety data, the current paper has shown that for several of the included interventions, a scientific rationale and/or a valid theoretical basis was lacking, questionable or has meanwhile been found to be incorrect. Even if some great achievements have originated from serendipity, research and innovation should preferably be driven by a scientific rationale and/or have a valid theoretical basis. In general, there is a need for more basic research in the field of MAR, for example with regards to the immunological and inflammatory processes during implantation and pregnancy and the relevance of the genetic composition of the embryo.

In summary, this paper highlights the limitations of a set of interventions currently offered to patients in the context of MAR. ESHRE urges that all interventions offered in clinical practice are thoroughly evaluated for efficacy, safety, relevance, and cost-effectiveness and that this is standard part of patient counselling. A clear distinction should be made between those where evidence has found a benefit to patients versus those where it has not. The latter should only be offered in a research context. Only evidence-based add-ons should be offered to patients in clinical practice.

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2712 ## Supplementary data 1

Abbreviation	Explanation
AFC	Antral follicle count
AH	Assisted hatching
AOA	Artificial oocyte activation
ART	Assisted reproductive technology
BMDSC	Bone marrow-derived stem cells
Ca^{2+}	Calcium
CGH	Comparative genomic hybridisation
CI	Confidence interval
CAM	Complementary and alternative medicine
CPR	Clinical pregnancy rate
DFI	DNA fragmentation index
DGC	Density gradient centrifugation
DSB	Double strand breaks
ET	Embryo transfer
FET	Frozen embryo transfer
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte macrophage-colony stimulating factor
hCG	Human chorionic gonadotropin
HFEA	Human Fertilisation and Embryology Authority
HA	Hyaluronic acid
HBA	Hyaluronan/hyaluronic acid binding assay
HLA	Human leukocyte antigen
IMSI	Intracytoplasmic morphologically selected sperm injection
IQR	Interquartile range
IUI	Intra-uterine insemination
IVA	*In vitro* activation
IVIG	Intravenous immunoglobulin
IVF	*In vitro* fertilisation
IVM	*In vitro* maturation
KIR	Killer-cell immunoglobulin-like receptor
LBR	Live birth rate
LIT	Leukocyte immunisation therapy
MACS	Magnetic-activated cell sorting
MAR	Medically assisted reproduction
MD	Mean difference
MSC	Mensenchymal stem cells
mtDNA	Mitochondrial DNA
NGS	Next generation sequencing
niPGT	Non-invasive pre-implantation genetic testing
NK-cells	Natural killer cells
OHSS	Ovarian hyperstimulation syndrome
OPR	Ongoing pregnancy rate
OPU	Oocyte pick-up
OR	Odds ratio

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ORP	Oxidation reduction potential
OS	Oxidative stress
PBMC	Peripheral blood mononuclear cell
PCOS	Polycystic ovary syndrome
PDE	Phosphodiesterase
pET	Personalised embryo transfer
PGT-A	Preimplantation genetic testing for aneuploidy
PICSI	Physiological ICSI
POI	Premature ovarian insufficiency
PR	Pregnancy rate
PRP	Platelet-rich plasma
PTX	Pentoxifylline
PVP	Polyvinylpyrrolidone
RIF	Recurrent implantation failure
RPL	Recurrent pregnancy loss
RR	Relative risk/risk ratio
SART	Society for Assisted Reproductive Technology
SCD	Sperm chromatin dispersion test
SCSA	Sperm chromatin structure assay
SDF	Sperm DNA fragmentation
SET	Single embryo transfer
SSB	Single strand breaks
TLI	Time-lapse imaging
TNF	Tumour necrosis factor
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling
uNK-cells	Uterine natural killer cells
ZP	Zona pellucida

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