



International Journal of Reproductive Medicine & Gynecology

Review Article

Adjuvants in Assisted Reproductive Technology -

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Submitted: 09 August 2019; **Approved:** 30 August 2019; **Published:** 12 September 2019

Cite this article: Nagarajan G, Thanikachalam P, Kesavan VR, Vijayakumar R, Pandiyan R, et al. Adjuvants in Assisted Reproductive Technology. Int J Reprod Med Gynecol. 2019;5(2): 032-045.

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ABSTRACT

Introduction: Use of adjuvant therapy in patients undergoing assisted reproductive technology (ART) is a major dilemma for the treating clinician and the intending couple. Some are proposed to improve the success rates of In vitro Fertilization (IVF) in certain patient subgroups like recurrent implantation failure, recurrent pregnancy loss and low responders. But some of them have been routinely used for all patients in view of improving the success rates. This review is aimed at analyzing the physiological basis of using such add-ons and whether their usefulness in improving the pregnancy rates is evidence based.

Methods: We have analyzed various randomized controlled trials, meta analyses and systematic reviews for the available evidence and the quality of such evidence has been scrutinized. We have also looked into the guidelines and recommendations suggested by recognized fertility societies and the evidence available to support or refute the use of the adjuvant.

Results: Many of the adjuvants have proven not to be useful, while a few newer techniques like time lapse system, Pre implantation genetic testing for aneuploidy are still controversial and further studies are required to elucidate their effectiveness.

Conclusion: Patients with infertility who already experience a great level of stress and anxiety should not be subjected to unnecessary medications and procedures which do not contribute the success of the ART cycle. The clinician has to weigh the cost benefit and risk benefit ratio before opting for any adjuvant therapy.

Keywords: Adjuvant therapy; IVF; ART; Medical adjuvants; Procedural adjuvants

ABBREVIATIONS

Assisted Reproductive Technology- ART; In Vitro Fertilization- IVF; Pre Implantation Genetic Testing For Aneuploidy - PGT-A; Randomized Controlled Trials- RCT; Reactive Oxygen Species- ROS; Polycystic Ovarian Syndrome- PCOS; Ovarian Hyperstimulation Syndrome- OHSS; Granulocyte- Monocyte Colony Stimulating Factor- GM-CSF; Low Molecular Weight Heparin- LMWH, Recurrent Implantation Failure- RIF, Recurrent Pregnancy Loss- RPL; Endometrial Receptivity Array- ERA

INTRODUCTION

Assisted Reproductive Technology (ART) is a major breakthrough in the management of infertility since 1978. In 1978, the world's first ART baby "Louis Brown" was born in Oldham General Hospital, United Kingdom as a result of continued efforts from Robert Edwards, Patrick Steptoe and Jean Purdy. Since then the field of assisted reproductive technology has made rapid strides. In this first successful ART cycle, they did not use any drugs for stimulating the ovary and the oocyte retrieval was done laparoscopically, in a natural cycle [1]. But the success rate of natural cycle IVF was not good [2]. Hence various pharmacological agents, newer technologies and techniques have been introduced to increase the success rate of ART. Of these newly introduced adjuvants, some have become the norm, some have proved useless, and others still remain controversial.

An adjuvant is defined as "one that helps or facilitates: such as: a: an ingredient (as in a prescription or a solution) that modifies the action of the principal ingredient. b: something (such as a drug or method) that enhances the effectiveness of medical treatment used" [3].

In this review we have dealt with the various widely used adjuvant therapies to improve the success rate of In Vitro Fertilization (IVF) cycles, their advantages and disadvantages, whether their effectiveness has been proved by well powered randomized control trials done in large populations and the guidelines put forward by recognized fertility societies.

MEDICAL ADJUVANTS

Antibiotics

Empirical use of antibiotics has been advocated in both men and women prior to the starting of ART cycle or before embryo transfer.

Antibiotics usage in women undergoing ART

Rationale: In women chronic endometritis is a subtle pathology often asymptomatic or accompanied by mild symptoms. It is thought to hamper the endometrial receptivity due to alterations in the leucocyte subsets present in the endometrium. Lower percentage of CD56+, CD16, CD56 bright and CD16- and a higher percentage of CD3+ cells with cytolytic activity were found in the secretory endometrium of women with chronic endometritis [4]. Hence it was proposed that antibiotics given prior to embryo transfer would improve the endometrial receptivity.

Evidence: But the results of a prospective randomized control study done by Perishkivili et al, in 2004 showed that there was no significant improvement in pregnancy rates when patients were treated with antibiotics prior to embryo transfer [5]. Results of a meta-analysis showed that administration of amoxicillin-clavulanic acid prior to embryo transfer did not alter the clinical pregnancy rates [6]. An overview of Cochrane reviews published in 2018 confirmed that the evidence available is of moderate quality and no data was available from RCTs to support or refute antibiotic regimens in this setting [7].

Antibiotics in men prior to ART

Rationale: Seminal plasma usually contains insignificant quantities of microbes which are contaminants from the urine or genital skin. Studies done on seminal plasma showed that leucocytospermia was not associated with clinical symptoms or bacteriospermia [8,9].

Evidence: Both fertile and infertile men are found to have positive semen cultures and the same is not routinely indicated prior to IVF [10]. As per the NICE guidelines proposed in 2016, men with leucocytes in their semen should not be offered antibiotic treatment unless there is an identified infection because there is no evidence that this improves pregnancy rates [11].

Androgens

Androgenic agents like testosterone and Dehydroepiandrosterone (DHEA) have been used to augment the follicular response in women with low response to ovarian stimulation and low ovarian reserve.

Rationale: Androgens act directly via androgen receptors of

the pre antral and antral follicles and increase the expression of FSH receptors and thereby increase the sensitivity to FSH [12].

Evidence: A Cochrane review which included 17 randomized controlled trials done on a total of 1,496 low responder women showed that the evidence was of moderate quality that androgens improved the live birth rates. There was insufficient evidence regarding the safety of androgens [13]. Bosdou, et al did a randomized controlled trial on transdermal testosterone pretreatment in low responders which concluded that there was no significant increase in the number of cumulus-oocyte complexes retrieved, the fertilization and live birth rates in both the groups with and without testosterone pretreatment [14]. Many studies showing an increase in live birth rates with androgen usage in low responders are inadequately powered non-randomized trials done on a small number of subjects from a heterogenous population. The NICE guidelines put up in 2016 have clearly stated not to use androgens and DHEA as adjuvants in IVF [11].

Antioxidants

Antioxidants in men: Rationale: Reactive Oxygen Species (ROS) is a cause for the cellular damage and apoptosis [15]. The spermatozoa are found to be vulnerable to ROS. There is a reduction in the sperm motility index and thereby affect the fertilizing ability when there is excess ROS [16]. Many antioxidants like carnitine, glutathione, selenium, coenzyme Q10, zinc and few more drugs have been studied with regard to improvement in success rate of the ART cycle.

Evidence: In a Cochrane review of 48 RCTs, including 4179 sub fertile men, there was low quality evidence that antioxidants for male partners increased the clinical pregnancy and live birth rates [17]. The effectiveness of antioxidants to improve sperm parameters and their safety profile are inconclusive [18,19]. Anne Steiner made an oral presentation in ESHRE 2018 on a large US clinical trial of 174 couples which found that an antioxidant formulation taken daily by the male partner for a minimum of three months made no difference to sperm concentration, motility or morphology, nor to the rate of DNA fragmentation.

Antioxidants in women: There was very low quality evidence to show that antioxidants may provide benefit for sub fertile women. Regarding the adverse events of antioxidants there is insufficient evidence [20].

Aspirin

Rationale: Aspirin is used in IVF patients to improve the endometrial blood flow by reducing platelet aggregation and vasoconstriction and thereby proposed to improve the endometrial receptivity [21]. The correlation between the endometrial and sub endometrial blood flow and pregnancy rates are controversial.

Evidence: The results of various studies done on routine use of low dose aspirin show that there is no improvement in pregnancy and live birth rates [21,22]. A Cochrane review done in 2016 including 13 trials and a total of 2,653 patients concluded that there is no evidence in favour of routine use of aspirin in order to improve pregnancy rates and evidence does not exclude the adverse effects of aspirin due to its antiplatelet effect [23]. There has been an increase in the risk of developing sub chorionic hemorrhage during the first trimester in patients who use aspirin [24]. Usage of aspirin as empirical therapy to improve the pregnancy rates should not be encouraged as per the Practice committee meeting of American Society for

Reproductive Medicine(ASRM) 2018 [25].The ESHRE has also proposed a conditional recommendation that aspirin may be used in combination with heparin only in patients with anti-phospholipid antibodies [26].

Cabergoline

Rationale: Cabergoline is a dopamine agonist, which inhibits Vascular-Endothelial Growth Factor Receptor-2 (VEGFR-2) phosphorylation, used in patients with Ovarian Hyper Stimulation Syndrome (OHSS) and in patients with risk factors to develop OHSS. Other dopamine agonists like quinagolide and bromocriptine have also been tried. These drugs may reduce the vascular permeability and prevent third space fluid loss.

Evidence: A meta-analysis by Leitao et al, in 2014 showed a decreased risk of OHSS in women receiving cabergoline as compared to those who did not with no effect on live birth rate [27]. In an updated Cochrane review by Tang et al, 2016, an analysis of 16 RCTs, there was moderate quality evidence that cabergoline improved the clinical pregnancy rates. However there is increase in adverse events such as gastrointestinal symptoms [28]. These results are further reinforced in the narrative evidence provided for WHO guidance [29] supporting the use of cabergoline starting from the day of trigger for atleast 6 days thereby decreasing the incidence of moderate and severe OHSS in potential hyper responders.

Combined Oral Contraceptives (COC) and estrogen pretreatment

Rationale: Pretreatment with oral contraceptive pills suppress the natural hormone production and help the synchronous development of the cohort of follicles [30]. Hence COC has been tried in low responder patients in order to improve the ovarian response.

Evidence: A Cochrane systematic review by Farquhar et al showed that there was no significant difference in the pregnancy rates with and without steroid hormone usage [31]. The WHO guidance 2017 states that the evidence for and against the usage of oral contraceptives as pretreatment for ART cycles is insufficient [29].

Growth hormone

Rationale: Growth hormone is reported to up regulate the synthesis of insulin like growth factor -1. IGF -1 plays an important role in granulosa cell stimulation, estrogen production and oocyte maturation [32]. Growth hormone has been used in low responders and patients with low reserve to increase the follicular response to gonadotropin stimulation.

Evidence: Several studies have been performed to evaluate the effectiveness of growth hormone as an adjuvant to increase the live birth rates in low responders. Initial studies demonstrated an increase in the live birth rates in poor responders but the studies evaluated in the meta-analysis were few and had a small sample size [33]. The results of recent RCTs and meta-analysis showed an increase in the number of oocytes retrieved, number of m2 oocytes and the embryo quality but no difference in clinical pregnancy rates [34,35]. NICE guidelines issued in 2013 has clearly stated that growth hormone should not be used as adjuvant for ART cycles [11].

Granulocyte - Monocyte Colony Stimulating Factor (GM-CSF)

It is a cytokine secreted by fibroblasts, monocytes, macrophages, endothelial cells, stromal cells, and bone marrow cells. Apart from



hematopoietic cells, receptors of GM-CSF are present in endothelial cells, placental cells, trophoblast cells, feto maternal interface and luteinized granulosa cells [36].

Rationale: GM-CSF leads to accumulation of leucocytes in the follicle and thereby causing ovulation [36]. A positive correlation has been identified between the follicular fluid GM-CSF level and IVF outcomes [37]. Adding GM-CSF to the culture media is also found to increase the survival of embryos protecting them from the culture induced stress [38]. It is found to play a role in endometrial remodeling, probably by suppressing the immune response towards the embryo and thereby improving the endometrial receptivity [39]. It is proposed to improve the aneuploidy rates and IVF success rates [40].

Evidence: A randomized controlled trial performed in a total of 62 women with thin endometrium (< 7mm) showed that local infusion of GM-CSF improved the endometrial thickness but did not improve live birth rates [41]. Another non randomised controlled trial performed in 68 patients showed that there was no increase in endometrial thickness with intrauterine infusion of GM-CSF, but there was a slight increase in clinical pregnancy rates which did not reach statistical significance [42]. A meta-analysis done in Asian population which included six studies found that there was an increase in the endometrial thickness, clinical pregnancy rates and live birth rates with trans vaginal infusion of GM-CSF in patients with thin endometrium and recurrent implantation failure [43]. Another meta-analysis of seven studies concluded that there is insufficient evidence regarding the usage of GM-CSF in patients with recurrent implantation failure and further well powered double blind controlled studies are needed. Further there are still many unanswered questions such as the dosage, route of administration and the patient population that benefits from such treatment [44]. The ASRM Practice committee 2018 also recommends that there is insufficient evidence that GM-CSF increases the endometrial thickness or the clinical pregnancy rates [25].

Corticosteroids

Rationale: Corticosteroids are tried in patients undergoing IVF in view of increasing the follicular response as it acts as a substrate for 11- β hydroxyl steroid dehydrogenase present in the oocytes. It also sensitises the ovary to the exogenous gonadotropins [45]. As immunological causes are attributed for recurrent implantation failure and recurrent pregnancy loss; corticosteroids have been tried in the peri implantation period for producing immunosuppression.

Evidence: In a meta-analysis by Boosma, et al total of 14 studies done in 1879 couples concluded that there was no clear evidence to support the use of corticosteroids during the peri implantation period. In a small group of patients who underwent IVF instead of ICSI had a slightly improved pregnancy rates and these studies did not include women with autoimmunity [46]. The British Fertility Society Policy and Practise committee held in 2015 recommended not to use corticosteroids as adjuvant in ART cycles as there is limited evidence that they may improve the clinical pregnancy rates even if they are auto immune [47]. Corticosteroids have been tried in combination with heparin in a few studies which seem to support the use of these adjuvants, but these are not adequately powered trials and cause effect association could not be clearly made out [48,49]. A Cochrane review on four studies involving 416 women who underwent ART, glucocorticoid administration during the ovarian stimulation was compared with placebo. The review concluded that there was no

significant difference in live birth rate between the glucocorticoid group and the placebo group [50]. An overview of reviews published in the Cochrane database recently has listed glucocorticoids both during controlled ovarian stimulation and peri implantation period as a possibly ineffective intervention [7]. The practise committee of ASRM has issued a guideline in 2018 which recommends against using glucocorticoids during Controlled Ovarian Stimulation (COS) and peri implantation period [25]. There has been an improvement in live birth rates with steroid use only in patients with antithyroid antibodies [51] which suggests that it may be of use in patients with autoantibodies but further studies are required to elucidate if there are clear benefits. The adverse effects of corticosteroids such as immunosuppression, fluid retention, gastritis have not been adequately reported in these studies.

Metformin

Rationale: Metformin is an insulin sensitizing drug. In a study by Tang et al metformin was found to reduce the androgen production and also decrease the levels of insulin and insulin like growth factor which in turn stimulates FSH action on estrogen production in PCOS patients [52]. High androgen levels may also affect endometrial receptivity. High estrogen levels from multifollicular development in PCOS patients predispose to the development of OHSS. It is proposed that both these effects are prevented by metformin [52].

Evidence: Several randomized controlled trials done on women with PCOS with metformin co treatment vs placebo showed that even though there was a slight increase in clinical pregnancy rates, there was no difference in live birth rates and a reduction in OHSS risk was evident. Also there was a decrease in the number of oocytes retrieved and the fertilization rate in the metformin group [53]. In a systematic review of nine RCTs published in the Cochrane database by Leopoldo et al, it was noted that metformin reduced the OHSS risk but there was no conclusive evidence that it improved live birth rates [54]. All these trials were done in agonist cycles. A randomized controlled trial done in antagonist cycles showed that metformin does not reduce the OHSS risk too [55]. The International PCOS network has issued an evidence based recommendation that adjuvant therapy with metformin for PCOS patients undergoing IVF increases clinical pregnancy rates and decreases the risk of OHSS [56].

Melatonin

Rationale: Melatonin, structurally called N-acetyl-5-methoxy tryptamine is secreted by the pineal gland at night. In addition to maintaining the circadian rhythm it is also believed to regulate the ovarian function by its specific receptors which mediate gonadotropin release from hypothalamic-pituitary axis. It also acts as a free radical scavenger, thereby reducing the oxidative stress thereby increasing the oocyte quality [57]. This is supported by the presence of higher levels of melatonin in the pre ovulatory follicular fluid compared to the serum [58]. There is an inverse correlation between the intra follicular concentrations of melatonin and 8-OH deoxy guanine which is a DNA related stress marker suggesting that melatonin prevents oocyte damage by free radicals [57].

Evidence: In a recently performed double blind randomized control trial which compared the clinical pregnancy rates, oocyte and embryo parameters between the patients who took 2,4 and 8 mg of melatonin with those who were given placebo, there was no significant difference in the above mentioned parameters in all the four groups [59]. Another prospective randomized control trial in

which melatonin along with myoinositol administered in patients with prior history of poor oocyte quality improved the oocyte quality and the pregnancy rates [60]. But the effect is due to melatonin or myoinositol or combination of both is not elucidated in the study. Similarly in another RCT done in women with decreased ovarian reserve, there was an increase in the number of oocytes retrieved, number of good quality oocytes and embryos in melatonin group, but there was no difference in the ART outcome between the melatonin and placebo group [61].

Intra lipids

Rationale: Intra lipid is a fat emulsion (20%) consisting of soybean oil, egg yolk phospholipids, glycerin, and water, which is used intravenously during and after embryo transfer. It is hypothesised to reduce the in vivo abnormal NK cell activity in patients with recurrent implantation failure. The fatty acids within the intralipid emulsion activate the peroxisome proliferator activated receptors of the NK cells thereby reducing the NK cell activity improving implantation [62].

Evidence: A double blind randomised controlled study done in patients with recurrent pregnancy loss with elevated levels of NK cells, intralipid infusion did not increase the clinical pregnancy rates [63]. The Practice committee of ASRM 2018 also concluded there is insufficient evidence to support the use of intralipids as an adjuvant to improve the pregnancy rates [25]. The dosage, duration and frequency of administration, adverse effects of intralipids have not been adequately studied. The British fertility society also suggests that due to lack of evidence, intralipid infusion therapy cannot be recommended as an adjuvant in IVF cycles [47].

Intravenous Immunoglobulins (IV Ig)

Rationale: Animal studies showed that the shift in the cytokine profile from Th2 to Th1 predominance has been proposed to play a role in recurrent implantation failure and recurrent pregnancy loss [64]. Hence the use of IV Ig has been advocated in patients with abnormal immune activation.

Evidence: A prospective controlled trial done in a small group of 75 patients with recurrent implantation failure with elevated Th1/Th2 cytokine ratio, found that there was no difference in pregnancy rates with IV immunoglobulin, adalimumab and heparin [65]. Another prospective randomized controlled trial done in fifty one couples with unexplained IVF failure, for whom iv immunoglobulins were administered during embryo transfer and after confirmation of pregnancy results showed that there was no benefit from immunoglobulins [66]. A meta-analysis of ten trials in which the use of immunoglobulins were evaluated in patients with recurrent pregnancy loss, four of the trials showed improvement in live birth rates while the rest of them did not show any benefit [67]. Another recently published systematic review of 30 trials in which the efficacy of commonly used immunomodulators concluded that immunotherapy should not be used in routine clinical practice in view of improving the ART outcomes [68]. As per the British Fertility society, there is no convincing evidence for the use and safety of IVIg as adjuvants in women with recurrent implantation failure embarking on IVF [47].

Heparin

Rationale: Heparin is a polysulphated glycosaminoglycan which acts as an anticoagulant by inhibiting the factor Xa and thrombin [69]. The prevention of placental thrombosis and infarction by heparin

is the proposed hypothesis of its effect in Recurrent Implantation Failure (RIF) and Recurrent Pregnancy Loss (RPL) [70]. Recent studies on the human endometrial decidual stromal cells show that administration of heparin makes them resistant to oxidative stress thereby favouring implantation [71].

Evidence: On 150 women with two or more IVF failures showed a mild increase in the live birth rates with luteal phase heparin but the outcomes were not statistically significant [73]. Another meta-analysis of 3 randomised trials involving 386 women in whom peri implantation LMWH was given. There was low quality evidence that LMWH improved the clinical pregnancy rates. The main drawback of the meta-analysis was that there was heterogeneity among the patient population, inclusion and exclusion criteria. Hence the study concluded that there was no justification for the use of heparin in sub fertile women [74]. Another meta-analysis of 10 trials including 1217 cycles of observational studies and 732 cycles of randomised controlled studies showed no improvement in clinical pregnancy and live birth rates [75]. Another recently published multicentre cohort study of 230 women with RIF showed no increase in IVF outcomes with adjuvant heparin therapy [76]. Same findings are emphasised by the study by Yang et al, 2018 [77]. Most of these studies did not report on the adverse events with the usage of heparin. British fertility society has proposed that the routine administration of heparin is not supported by good evidence. However, it has some role in women with thrombophilia [47]. The ASRM also does not support the usage of heparin routinely in all IVF patients [78].

Myoinositol (MI) and D-Chiro Inositol (DCI)

Rationale: Myo and d-chiro inositol bind to nuclear receptors and through signal transduction of second messengers such as Diacyl Glycerol (DAG) and Inositol Triphosphate (IP3) pathways. DAG activates protein kinase C and IP3 activates calcium release. Both effects facilitate oocyte maturation. Hence it is suggested that usage of the inositols might increase the oocyte quality [79]. These drugs are thought to decrease the fasting insulin levels and homeostasis model assessment index, thereby positively affecting the metabolic status and decreasing the level of hyperandrogenism in PCOS patients [80].

Evidence: In a study by Antonio et al, there was a reduction in the length of stimulation and dosage of gonadotropins with co administration of myo inositol [81]. In a prospective randomized controlled trial by Pacchiaroti et al, myoinositol in synergy with melatonin improved the oocytes and embryo quality [82]. Results of Mendoza et al's study contradicted the above mentioned study. It showed that there was neither an improvement in the oocytes and embryo quality, nor in the pregnancy rates with MI and the role of DCI was inconclusive [83]. A Cochrane review of 13 RCTs involving 1472 women, there was a low to very low quality evidence that there was any benefit of using MI. There was no clear evidence of benefit or harm from using MI in PCOS patients undergoing IVF [84].

TNF alpha inhibitors

Rationale: Tumour necrosis factor -alpha is a cytokine secreted by the Th1 cells and high values of TNF- alpha are noted in patients with recurrent implantation failure, recurrent pregnancy loss and pre-eclampsia. Hence therapy targeted against TNF- alpha has been tried in such patients to improve the pregnancy rates [85].

Evidence: A systematic review by Chiara et al in 2018 which analyzed the efficacy of various immunomodulators in IVF patients did not find any supporting evidence [68]. Considering the various



adverse effects of immunotherapy like anaphylactic reactions and risks of immunosuppression, the ASRM practice committee suggested not to use any form of immunotherapy as adjuvant treatment in IVF patients [25]. The British fertility society also does not support the use of anti TNF-alpha agents as adjuvant therapy since there is no evidence that it is safe and effective [47].

Vasodilators

Rationale: Thin endometrium has been attributed to cause recurrent implantation failure and is associated with high blood flow impedance of uterine artery, decreased VEGF expression and poor vascular development apart from infections and iatrogenic causes [86]. The most commonly used vasodilators are sildenafil and L-arginine. Sildenafil is a phosphodiesterase -5 inhibitor, which causes, nitric oxide mediated vasodilatation by inhibiting the breakdown of cGMP. L-arginine is a precursor amino acid for the production of nitric oxide.

Evidence: A meta-analysis of 15 studies involving 1326 women in whom vasodilators were compared with placebo and no treatment found low to moderate quality evidence of increase in endometrial thickness and clinical pregnancy rates but no improvement in live birth rates [87]. As per the British fertility society, routine use of sildenafil and nitroglycerine as adjuvants in IVF cycles is not recommended [47].

PROCEDURAL ADJUVANTS

Acupuncture

Rationale: Acupuncture is an ancient Chinese practice which involves stimulation of pressure points. Acupuncture has been proposed to decrease the uterine artery impedance and thereby increase the uterine blood flow [88]. Also uterus is rendered quiescent with the final effect of improving the endometrial receptivity [89]. Additionally it is suggested that acupuncture reduces the stress and anxiety and thereby contributing to the success of an ART cycle [90]. Hence acupuncture has been used as an adjuvant therapy in many ART centers either after oocyte retrieval or embryo transfer to increase the success rates.

Evidence: Nearly 3000 women were studied in 14 randomized controlled trials which yielded high quality evidence that acupuncture did not improve the IVF outcome [91]. Similar results were obtained in another meta-analysis by Toukhy et al, in which thirteen trials performed in a population of 2500 women showed that acupuncture done around the time of oocyte pickup or embryo transfer did not improve the pregnancy rates [92]. Recent studies comparing the efficacy of acupuncture and sham acupuncture also yielded similar results [93]. The ASRM practice committee meeting held in 2017 at Birmingham concluded that there is grade B evidence that acupuncture performed around the time of embryo transfer does not improve live birth rates in IVF [94].

Routine pre- IVF hysteroscopy

Rationale: The prevalence of unsuspected uterine abnormalities in patients undergoing their first ART cycle is estimated to be 11% in a study by Fatemi et al, in 2010 as compared to prevalence of 20-45% in older studies [95]. Many ART centers use routine hysteroscopy as a tool to detect intrauterine abnormalities.

Evidence: But studies have shown that there is no improvement of live birth rates with routine use of hysteroscopy in women with a

normal uterine cavity as diagnosed by the transvaginal ultrasound. The same findings were emphasized by the 'INSIGHT' trial and the 'TROPHY' trial [96,97]. The TROPHY trial is a multicentric randomized trial, involving 702 women less than 38 years of age and with previous two or more IVF failures. Performing a routine hysteroscopy in these patients did not improve the IVF outcomes. Since the procedure involves the risk of pelvic infection and anaesthetic complications it should be performed only in cases where there is a clear benefit such as excision of a large polyp or resection of a septum, submucous myoma or intrauterine adhesions. Another study by Negm, et al compared the efficacy of three dimensional sonohysteroscopy and hysteroscopy and found good concordance between the two with 3-D sonohysterogram being significantly short and less painful procedure [98]. The NICE guidelines issued in 2013 has clearly stated that women should not be offered hysteroscopy on its own as part of the initial investigation unless clinically indicated because the effectiveness of surgical treatment of uterine abnormalities on improving pregnancy rates has not been established" [11].

Endometrial scratching

Rationale: Endometrial scratching, the procedure of causing endometrial injury purposefully prior to IVF has been followed widely among ART clinicians. A worldwide survey by Lensen, et al in 2016 found that 92% clinicians recommended endometrial scratching for patients with previous IVF failures and 6% recommended the procedure for all IVF patients [99]. The amount of HLA-DR-CD11c cells and various pro inflammatory cytokines such as TNF-alpha, Interleukin -5, Macrophage Inflammatory Protein (MIP) were found to be high in day 21 endometrium and positively correlated with pregnancy rates, hence considered essential for implantation [100]. There are various hypothesis regarding the effect of endometrial injury on implantation. Injury to the endometrium is thought to increase the decidualisation and the process of wound healing increases the secretion of cytokines, interleukins, growth factors, and macrophages and dendritic cells. It is also proposed that the injury retards the growth of the endometrium which is thought to be abnormally advanced due to controlled ovarian stimulation [101]. All these factors remain as hypothesis and are not yet proved.

Evidence: The studies done in the initial period demonstrated an increase in the pregnancy rates in patients whom endometrial scratching in the previous cycle. In a study by Bakshi et al, there was improvement in ART outcome in patients undergoing first cycle of IVF but not in patients with previous IVF failures [102]. Several underpowered non randomized trials done supported the procedure and was used routinely in most of the centers. But later when well organized and well powered randomized trials and meta analyses were done the results showed that there was no improvement in pregnancy rates; some even showed a decrease in pregnancy rates when scratching was done on the day of oocyte pickup [103,104]. A recently performed multicentre RCT on 1364 women with 1:1 randomisation showed no evidence of improvement in ART outcome with scratching [105]. Similar results were obtained in oocytes donation cycles also which further reinforced that endometrial scratching was of no benefit [106]. We should also keep in mind the complications of the procedure such as risk of infection, pain experienced by the patient, cost incurred and the risk of developing Ashermans syndrome. RCOG guidelines, 2016 says "Further prospective randomized studies of sufficient power are required to confirm or rule out the clinical value of local endometrial trauma" [107]. A well powered RCT done by Yueng et al in 2014 revealed that there was no increase



in pregnancy rates with endometrial scratching [108]. With regard to this study Hans Evers commented on endometrial scratching-“If you read this in 2028, you might say ‘Huh? Scratching? The intellectual bankruptcy of reproductive medicine?’” in his article on top 10 in the past 6 years as editor of Human reproduction [109].

Assisted hatching

Rationale: Hatching is process by which the zona is lysed by the production of an embryonic lysin and the blastocyst expands following this. When the zona pellucida thickness is more than 15 μm , it is considered as thick zona which may occur intrinsically or as a result of in vitro culture or cryopreservation and it is thought to prevent the blastocyst from hatching [110]. Assisted hatching is a technique wherein a deficiency is made in the zona pellucida either mechanically, chemically or by using LASER. Assisted hatching is proposed to aid in implantation and improve the pregnancy rates especially in patients with RIF.

Evidence: A Cochrane review which included 28 trials involving 3646 women concluded that the data was insufficient to assess the effect of assisted hatching [111]. Another Cochrane review published in 2012 analysed 31 trials which showed a slight improvement in clinical pregnancy rates but no improvement in live birth rates [112]. Also a higher risk of monozygotic twinning is associated with assisted hatching [113]. Another retrospective analysis of 623 pregnancies following assisted hatching, it was observed that there was a higher prevalence (5.4%) of ectopic pregnancy as compared to control group (2.2%) [114]. The Practice committee of ASRM, 2014 has put forward that assisted hatching should not be recommended for all patients [115]. NICE guidelines issued in 2013 recommends against assisted hatching since it does not improve the pregnancy rates [11].

Endometrial Receptivity Array (ERA)

Rationale: The transcriptomic signature of the endometrium including 238 genes, is assessed by the endometrial receptivity array and there by identifying the implantation window for planning personalized embryo transfer. A prospective study of patients with RIF as compared to the control group, the authors proposed a “displacement of window of implantation” supporting personalized embryo transfer [116].

Evidence: In a study by Diaz Gimano, et al. the sensitivity and specificity of ERA was found to be 0.99 and 0.88 respectively [117]. The same author had tested the reproducibility of ERA in a study published in 2013 involving 86 oocyte donors divided into two cohorts, found that ERA was more accurate in dating as compared to histologic dating and reproducibility was 100% consistent. But reproducibility in same patient was assessed only in 7 patients in a time gap of 29-40 months. The rationale of doing so was not explained in the study [118]. In a recent retrospective analysis by Tan et al, there was an improvement in pregnancy rates with personalized embryo transfer guided by ERA in patients who previously failed to conceive with euploid blastocyst transfer. But the differences were not statistically significant [119]. Large scale randomized studies have not been conducted to test the effectiveness of ERA.

Physiological Intra-Cytoplasmic Sperm Injection (PICSI)

Rationale: It has been proposed that there is an increase in the rate of aneuploidy in immature spermatozoa. Hyaluronic acid binding ability is considered to be an indicator of sperm maturity. The hyaluronic acid receptor in mature spermatozoa identified by

the HA binding sites of the PICSI dish helps to identify the mature spermatozoa thereby reducing the chromosomal disomy and diploidy [120].

Evidence: Though the rationale of the hypothesis seems relevant studies reporting pregnancy rates did not confirm the same. Randomized controlled trials done to evaluate the effectiveness of PICSI did not find any improvement in pregnancy rates but there was a slight decrease in the pregnancy loss rates which was not statistically significant [121,122]. A recently done systematic review by Avalos et al did not find any significant difference in pregnancy rates between conventional ICSI and PICSI [123]. A Cochrane systematic review published in 2014, which included 2 RCTs, showed that evidence was insufficient that PICSI improves the pregnancy rates [124].

Intracytoplasmic Morphologically Selected Sperm Injection(IMSI)

Rationale: Intracytoplasmic Morphologically Selected Sperm Injection (IMSI) is an advanced sperm selection technique by which spermatozoa are selected for ICSI after examining them under high magnification (over 6600x), as compared to the routine magnification of 200-400x.

Evidence: A randomized trial which compared ICSI and IMSI supported the procedure as there was a higher clinical pregnancy rates in patients with severe male infertility especially with previous IVF failures [125]. Another study also found that there was a decrease in congenital malformations and miscarriages in IMSI group [126]. Later several RCTs and meta analyses yielded contradictory results wherein there was no improvement in pregnancy rates in IMSI sperm selected patients and there was no difference in the congenital anomaly rate [127,128]. This finding was also reinforced by a Cochrane review of 9 RCTs by Texeira et al, in 2013 where the authors found a very low quality evidence that IMSI for sperm selection improved the clinical pregnancy rates. However there was no difference in the miscarriage rates and none of the studies reported live birth rate or congenital abnormalities [129]. The embryo quality assessed on day 2 was also not significantly different in IMSI and conventional ICSI patients [130]. Considering the longer duration of exposure of the sperm under the microscope and the longer embryologist time required conventional ICSI is preferred to IMSI.

Time lapse imaging

Rationale: Time lapse imaging helps us to continuously monitor the embryo development and morphokinetic parameters based on which embryos can be selected for transfer. Embryo selection becomes an important issue while considering elective single embryo transfer.

Evidence: Chawla et al, made a comparative analysis of euploid and aneuploid embryos cultured in time lapse system and verifying the ploidy status by CGH microarray analysis for around 460 embryos and found that aneuploid embryos had abnormal morphokinetic parameters [131]. An aneuploidy risk assessment model was developed by Campbell et al and thereby embryos were selected with low risk of aneuploidy for transfer. Such embryos were found to have an improved pregnancy outcome in the same study [132]. But the same study was criticised by Ottolini et al. as having age unmatched sample which can obviously affect the pregnancy outcome [133]. Another study also tested retrospectively the blastocyst prediction model and found that there was a 30% increase in implantation rate with ‘usable’ embryos, however 50% of the embryos deemed as ‘low



chance of being usable' had implanted [134]. In a meta-analysis by Pribenzsky, et al on five RCTs involving 1637 patients, the authors reported an increase in clinical pregnancy and live birth rate and a decrease in early pregnancy loss rate, but the evidence was of low to moderate quality [135]. In a prospective cohort study by Cruz et al, 478 embryos cultured in embryoscope from 60 couples undergoing oocytes donation cycles were analysed. No significant differences were found between the blastocyst development rate, blastocyst viability and ongoing pregnancy rates after embryo transfer of the 3 group of embryos cultured in embryoscope, conventional incubator and a combination of both [136]. In another randomised control trial of 235 patients who were randomized to embryoscope selection and morphological selection of embryos found that there was no significant difference in the pregnancy rates between the 2 groups [137]. In a Cochrane overview of reviews by Farquhar et al in 2015 there was insufficient evidence that there was an improvement in ART outcome while using time lapse system as compared to the conventional incubator [7]. Recently there are studies with conflicting results. A retrospective analysis of 1064 cycles using time lapse and 818 cycles using conventional incubators found that there was an increased pregnancy rate in patients who underwent fresh transfers but not in frozen transfers. The perinatal outcomes were found to be better with time lapse system [138]. A prospective RCT done by Kovacs et al, 2019 concluded that even though there is a trend favouring time lapse selection there were no significant differences in pregnancy rates when time lapse selection and morphological selection were compared [139]. Though time lapse system helps us to study the morphokinetics and several changes occurring in the embryo which can be missed out in routine morphological assessment, few studies are supportive of its use and there is no strong evidence in favour of time lapse system in improving ART success.

Preimplantation Genetic Testing for Aneuploidy (PGT- A)

Rationale: Preimplantation genetic testing was initially done for couples who were at risk for genetic disorders. Later it was suggested that by applying Preimplantation Genetic Screening (PGS), euploid embryos can be selected for transfer which might improve the ART outcome. Earlier Fluorescent In Situ hybridisation (FISH) technique was used for PGS which could analyse only few chromosomes. Later array- Comparative Genomic Hybridisation (a-CGH) was used in which all 24 chromosomes could be analysed. The newer technique that is coming into play is Next Generation Sequencing (NGS) which analyses major part of the genome. Initial studies employing FISH and array-CGH did not find any improvement in IVF success [140].

Evidence: But recent studies applying NGS has contradictory findings. In a recently conducted randomized controlled trial by Yang et al in April, 2019, has found increased clinical pregnancy and ongoing pregnancy rates when NGS is combined with morphological assessment of embryos when compared to morphological assessment alone [141]. Another retrospective study by Simon et al also concludes that single nucleotide polymorphism based PGT- A is helpful in older maternal age patients and while using this technique single and double embryo transfer yield the same results, hence double embryo transfer can be avoided to minimise the risk of multiple pregnancy [142]. In contrast the study by Neal et al on 8998 patients from 74 IVF centres concluded that the cumulative live birth rate was similar in both the group of patients undergoing IVF with and without PGT-A [143]. Similar results were obtained by Murphy et al where the live birth rate per transfer was high in the group of patients for whom PGT-A was done but cumulative live birth rate per oocyte retrieval was the same

even when PGT-A was not done [144]. These findings were reinforced by donor oocytes studies also [145]. While considering PGT-A, the problem of mosaicism is encountered. When 20-80% of the biopsied trophoblast cells are abnormal, it is called mosaicism. The mosaic embryos are found to have a decreased implantation potential but still implantable and are potential healthy babies. This creates a dilemma for the clinician whether to transfer or discard the mosaic embryos. The pregnancy potential of these embryos are not dependent on the degree of mosaicism [146]. Mosaic blastocysts from female patients with age < 34 years and single mosaic segmental aneuploidies are found to have better pregnancy potential [147]. Amplification of the genome can affect the degree of mosaicism due to the presence of artifacts and mosaic embryos are capable of autocorrecting. All these facts increase the confusion and dilemma in interpreting the results of PGT-A. The intending couples have to be counseled adequately before transferring the mosaic embryos which might as well increase the anxiety of the couple. ASRM practice committee held in 2018 suggested that "as of now PGT-A should not be used as embryo selection tool and large scale randomised trials are to be performed and PGT-A might prove beneficial in future" [148].

Coculture

Rationale: Autologous endometrial coculture has been suggested to improve the embryo quality by providing increased levels of IGF-I, IGF-II, VEGF-A and VEGF-C in the culture system thereby providing the natural uterine environment [149].

Evidence: Some studies demonstrated improvement in embryo quality as well as pregnancy rates [150,151]. A double blind RCT in which endometrial biopsy was done on Day 5-7 of the previous cycle and autologous endometrial coculture was compared with control group who underwent conventional embryo culture, though there was an improvement in the embryo quality there were no significant differences in the ART outcome [152]. Further randomized controlled studies are required to support or refute the hypothesis.

Artificial Oocytes Activation (AOA)

Rationale: Fertilization failure is often encountered in patients undergoing IVF. Though its occurrence is less in ICSI cycles, it is not rare. Artificial oocytes activation is proposed to overcome the fertilization failure by using calcium inophore and few other substances like strontium chloride and calcimycin.

Evidence: Results from various studies are contradictory. In a meta-analysis by Murugesu et al involving 14 studies, there was a significant increase in clinical pregnancy and live birth rate with calcium inophore apart from an improvement in fertilization, cleavage, blastocyst and implantation rates [153]. Another study which used Strontium chloride and calcimycin also found an improved pregnancy rates but the study was an open label trial which decreased the power of the study [154]. When artificial oocytes activation was tried for patients with previous history of fertilization failure there was an increase in the quality and number of cleavage stage embryos, but no effect on fertilization rate. However there was an increase in the fertilization rate with ICSI- AOA as rescue for unfertilized oocytes. But there was insufficient evidence as to any improvement in pregnancy rates with AOA [155]. Another randomized controlled trial done on low reserve patients with normozoospermic partners, AOA did not improve the clinical and ongoing pregnancy rates [156].

Adherence compounds

Rationale: Adherence compounds are added to the embryo



transfer medium to improve the pregnancy rates in many ART centres. Mostly hyaluronan and in some studies fibrin sealant and protein supplements have been tried.

Evidence: In a study by Hazlett et al, routine use of embryo glue in all patients was found to have no difference in ART success [157]. In another RCT involving 581 IVF-ET cycles in four group of patients with advanced maternal age, poor embryo quality, previously failed IVF cycles and low responder patients, there was no increase in clinical pregnancy rates, implantation rates and delivery rates with addition of hyaluronan to the transfer medium [158]. Another prospective case control study involving 42 patients, where 50 µL of embryo glue was instilled into the uterine cavity 10 minutes prior to embryo transfer, no difference was found in pregnancy rates between the cases and controls [159]. Similar results were obtained in a study on synthetic serum substitute [160]. Different concentrations of hyaluronan also did not cause any difference in pregnancy rates [161]. A Cochrane review on sixteen randomized controlled trials involving a total of 3698 patients showed moderate quality evidence that adherence compounds improved the pregnancy rates. There was also an increase in multiple pregnancy rates, but the adherence compounds could not be attributed as the cause since single embryo transfer was not followed. The review concluded that further studies are required to confirm the effect of adherence compounds [162].

CONCLUSION

Many adjuvant treatment strategies have been practiced without any convincing evidence from RCTs. Even retrospective analysis of such practices does not provide strong evidence. The usage of growth hormone, androgens, OCP and estrogen pretreatment in low responder patients, routine use of aspirin, heparin, immunotherapy in patients with recurrent implantation failure have been evaluated by several randomized controlled trials and meta-analysis and are not found to be effective. Techniques like assisted hatching and endometrial injury are not found to be effective and hence advised not to be followed by the recognized fertility organizations.

Newer techniques like PGS, embryo glue and time lapse system are under consideration and it is still premature to support or refute these techniques. Initial studies showed benefit with these techniques, but recent trials are contradictory. Further randomized controlled trials are required to confirm their role in ART. Until then they should not be used in patients.

However before starting any adjuvant therapy or before applying any new technique, it is the responsibility of the clinician to analyze whether there is any proven advantage of the adjuvant. Also the risk benefit ratio and the cost benefit ratio have to be taken into account. The effect of the adjuvants on the growth and development of the IVF babies have to be followed up and monitored. Follow up studies on the physical, neurological, psychological and sexual development of the babies born out of ART are needed to come to a conclusion.

It is evident that many of the adjuvants increase the dilemma of the treating physician. There is an increase in the cost incurred, exposure to the adverse effects of drugs, unnecessary procedures with risk of anaesthesia and infection to the patient. Some complications like infection and adhesions might further affect the fertility. There is also an increase in the anxiety level of the intending couple without any proven benefit.

At present, there is not enough evidence to encourage the usage of any adjuvant in the IVF population except for few which are

indicated in certain patient subgroups, such as heparin and aspirin for patients with APLA syndrome.

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