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Review

Add-ons in IVF programme – Hype or Hope?

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Abstract

A series of new technologies and adjuvant therapies have been advocated in order to improve the success of IVF treatment. Dehydro-epiandrostenedione, growth hormones, Coenzyme Q 10, calcium ionosphores, immune therapy, heparin, low-dose aspirin, and vasodilators are among commonly prescribed pharmacological adjuvants. New technologies that are proposed to improve IVF outcomes include advanced sperm selection procedures, time-lapse embryo monitoring, preimplantation genetic screening, assisted hatching endometrial injury or embryo-glue. This review looked into current evidence to justify the use of these co-interventions and whether some of them can still be offered while awaiting more robust evidence to confirm or refute their role.

Key words: **Adjuvants, assisted conception, assisted reproduction, IVF, over-treatment, malpractice, technology.**

Introduction

Assisted reproduction (AR) is a relatively new specialty in medicine. Like with any rapidly growing scientific field, novel ideas and new technologies designed to improve the result of AR have inundated the literature. However, only a handful of them have demonstrated evidence-based effectiveness. The majority of the co-interventions still being practiced can be categorised as of equivocal, uncertain or no usefulness. Interventions that have a theoretical basis from their mechanism of action, yet lack the evidence of real benefit in AR, remain in practice as so called 'empirical treatments'. The overall final outcome of *in-vitro* fertilisation/ intracytoplasmic sperm injection (IVF/ ICSI) is still more often a failure than a success. It is therefore a natural inclination for both the patients and clinicians to try any new idea to achieve a success, especially after failed attempt(s). This often results in over-treatment, sometimes health hazards or even malpractice. Additional interventions also make IVF treatment more expensive with questionable actual advantage. This review describes certain interventions, which are being practiced with little justifiable evidence to support their use within the realm of IVF/ICSI programmes.

Adjuvants for ovarian response

An array of pharmacological agents is being employed to improve the outcome of IVF treatment. Some of the adjuvants are used before commencing IVF cycles, some are used throughout the course of treatment and a bulk of medications are prescribed around the 'implantation window' to improve the implantation rate (IR) and pregnancy outcomes.

- *Dehydro-epiandrostenedione (DHEA)*

DHEA is a dietary supplement widely available online and is unlicensed in Europe. It is thought to enhance follicular function in older women with diminished follicular reserve by increasing the production of insulin-like growth factor-1 (IGF-1) and augmenting estradiol production in granulosa cells, acting as a precursor of androstenedione and testosterone in the theca cells. There is a large number of publications demonstrating the effects of DHEA on hormone profile, ovarian reserve and IVF outcomes. No benefit was found when women with normal ovarian reserve had 12 weeks of DHEA pre-treatment (Yeung et al., 2015). To date, 2 meta-analyses of 8 randomised controlled trials (RCTs) have been published. The first one found a significant

improvement of the clinical pregnancy rate (CPR) in women with diminished ovarian reserve (RR 2.13; 95% CI 1.12-4.08); the finding was similar to those observed in case-control studies (Li et al., 2015). The second study was a Cochrane review that revealed higher on-going pregnancy rates (OPRs) or live birth rates (LBRs) following the use of DHEA (OR 1.88, 95% CI 1.30 to 2.71). However, no benefit was apparent when studies with high risk of performance bias were excluded (Nagels et al., 2015). The included RCTs in this review were of moderate quality and the safety data were insufficient. Although minor androgenic side-effects have been reported, long term risk of DHEA administration remains unknown. In the absence of good quality evidence on risks and benefit, DHEA supplementation cannot be recommended at this time.

- *Growth hormone (GH)*

One comprehensive review on the management of poor responders in IVF treatment found GH to be one of the two interventions that might improve LBR in this group of patients (Kyrou et al., 2009). It acts by increasing the IGF-1 in the follicles, which in turn, has been shown to potentiate the action of follicle stimulating hormone (FSH) on the granulosa cells, and thereby enhance estradiol production and oocyte maturation. GH is usually started on the first day of ovarian stimulation and is administered either daily or on alternate days. The doses used in the studies have varied from 8 to 24 iu/day. More recently, a prospective study reported a low-dose (0.5 iu/ day) of GH to be sufficient to improve the CPR in poor responders (Lattes et al., 2015). A Cochrane review of 10 RCTs demonstrated significantly higher CPRs and LBRs when GH was added in women suspected of having a low ovarian response (Duffy et al., 2010). However this review also put a caution in interpreting the result as the RCTs included in the meta-analyses were too few in number and too small in sample size to draw a definitive conclusion. Only the CPRs were significantly better, but not LBRs, in the sub-group of women who showed a low response in the previous treatment cycle (Duffy et al., 2010). No benefit was apparent when GH adjuvant was used in normal responders. The advantage of adding GH seemed to be limited to treatment cycles with GnRH agonist protocol only, mainly with long down-regulation (Duffy et al., 2010; Dakhly et al., 2015). No significant benefit was found by adding GH in an antagonist cycle (Eftekhar et al. 2013a; Dakhly et al. 2015). The usefulness of GH as an adjuvant therefore remains inconclusive.

- *Anti-oxidants including Coenzyme Q 10 (CQ-10)*

Antioxidants have been used to improve natural reproductive potential and IVF outcomes. Unlike their recognised role in the management of male infertility, antioxidant co-treatment has not been shown to be beneficial in women (Showell et al., 2013). However, one of the antioxidants, CQ-10, has recently generated interest due to its role in rejuvenating mitochondrial energy store in the granulosa cells (Bentov and Casper, 2013). Mitochondrial dysfunctions have been associated with ovarian aging both in animal and human studies. Preliminary works suggest that supplementing CQ-10 may defer ovarian aging (Ben-Meir et al., 2015). Presently, clinical trials on the application of CQ-10 in AR are very few. A recent RCT found no improvement of CPRs and the availability of euploid embryos when CQ-10 (600 mg daily dose) was added to the IVF/ICSI protocol among women between 35-43 years (Bentov et al., 2014). Further evidence is required before CQ 10 can be recommended for all low responders.

- *Artificial oocyte activation (AOA)*

The use of Calcium Ionophore to artificially induce oocyte activation at the time of fertilisation in an IVF/ ICSI has recently attracted attention. It increases calcium ion concentration around the ooplasm immediately following sperm-oocyte fusion and thereby has been shown to increase fertilisation rates (FR), subsequent to a low or total failed fertilisation in previous ICSI cycle(s). Initial publications on AOA were in forms of case-reports, case series or retrospective analyses showing inconsistent results. Recent RCTs did not find any advantage of calcium ionophore for oocyte activation in women with diminished ovarian reserve (Caglar Aytac et al., 2015) or male-factor infertility (Eftekhar et al., 2013b). A recent systematic review concluded that there was insufficient evidence to prove effectiveness of AOA (Sfontouris et al., 2015). The safety of this intervention is also uncertain at present. Until conclusive data in support of AOA become available, it should not be offered.

Adjuvants for implantation success

- *Immune therapy*

Maternal immune modulation in the peri-implantation window has been postulated to play an important role in acceptance of the embryo as a semi-allograft. Couples seeking a reason for IVF

failure find the rationale of immune rejection very appealing. Natural Killer (NK) cells, cytokines, growth factors, tissue necrosis factor- α (TNF- α), macrophages and a balance between T-helper 1 (Th-1) and T-helper 2 (Th-2) cells play an important role in the implantation process. A plethora of expensive tests are available to identify such immune dysfunctions, including peripheral blood NK (pNK) cell number and activity and uterine NK (uNK) cell number and activity. However, there is no consensus on what constitutes a definitive normal range for either of these tests although < 12% to < 16% pNK cells and < 5% to < 13.8% uNK cells are considered to be normal values in various studies (Moffett and Shreeve, 2015). Although the role of uNK cells in aiding implantation seems to be biologically plausible, testing for their levels requires immunohistochemistry of frozen paraffin sections or enzymatic digestion of endometrial tissue followed by flow cytometry. Such tests are neither standardized nor routinely available. The evidence for an association between elevated NK cell levels and implantation failure comes from retrospective and observational studies, which have yielded conflicting results. A systematic review found no significant difference in the pNK cell number and activity in women with or without implantation failure (OR 1.35, 95% CI 0.28 –6.46) or miscarriage after ART (OR 2.48, 95% CI 0.50 –12.32) and thus concluded that routine testing for pNK cells is of uncertain prognostic significance (Tang et al., 2011). No difference in the IRs for women with or without elevated uNK cells was found in the two studies included in the review. Other than NK cell over-activity, anti-phospholipid antibodies were also implicated for recurrent miscarriage (RM) (Coulam and Acacio, 2012). The role of other inherited thrombophilia conditions in causing recurrent implantation failure (RIF) or RM is even more controversial.

Various strategies have been proposed to reduce the pNK cell levels including intravenous immunoglobulins (IVIg) (Winger et al., 2009; Coulam and Acacio 2012), TNF-α inhibitors (Winger et al., 2009), granulocyte-colony stimulating factor (Wurfel et al., 2010), preimplantation factor (Roussev et al., 2013), lymphocyte immune therapy, leukaemia inhibitory factor (Ledee-Bataille et al., 2002), peripheral blood mononuclear cells, intralipids (Roussev et al., 2007), glucocorticoids and vitamin D supplementation (Bubanovic, 2004). The role of steroids is discussed separately. The common goal of all these interventions is to improve LBRs in IVF cycles when associated with RIF or RM, with or without any autoimmune disorders. A systematic review by Polanski et al. (2014) found increased odds of CPRs after the use of IVIg (3.41, 95% CI 1.90-6.11) and prednisolone (1.63; 95% CI 1.00-2.66). However, there was considerable heterogeneity in the normal values and the test methodology for evaluation of pNK cells in different studies (Polanski et al. 2014). There is also no good correlation between pNK and uNK cell levels. IVIg has also been shown to improve the outcome of IVF/ICSI when associated with RIF or RM due to the presence of anti-phospholipid syndrome and or elevated NK cells (Coulam and Acacio, 2012). However, IVIg use was associated with high incidence of side effects (15%) in the only study reporting on side effects. Being a blood product, IVIg could potentially give rise to serious complications including anaphylactic reaction or infection. Therefore the benefit-risk ratio for such treatment is low, considering the paucity of evidence, which is mostly retrospective or observational in nature. TNF-α inhibitors work in a similar way. The evidence of its benefit has been restricted to small non-randomised studies (Winger et al., 2009). Long-term use of TNF-α inhibitors had a number of side effects including lymphoma, cognitive heart failure, infections and induction of autoantibodies. The short-term complications have not been established yet. The use of intralipids (20% lipid solution) is mainly for parenteral nutrition. It has been claimed to improve implantation by increasing Th-1 response and reduce NK cell cytotoxicity (Roussev et al., 2007). Again, there is no RCT evidence on its effectiveness.

Although there is rational basis for immunotherapy in certain circumstances, none of the interventions has been assessed through RCTs. All these various treatments possess some risks, which could be serious and most treatments are expensive. Unless further evidence in their support is established, immunotherapies should not be offered.

- *Corticosteroids*

Prednisolone and dexamethasone have been widely used during the implantation period as immune-modulators. Corticosteroids work by suppressing NK cells and maintaining a normal profile on related cytokines and growth factors in the endometrium. A Cochrane review of 14 RCTs found no effect of glucocorticoids on LBRs, CPRs and miscarriage rates when used in an unselected IVF/ICSI population (Boomsma et al., 2012). The finding of a beneficial role of steroids in the subgroup of only IVF (not ICSI) cycles needs to be noted with caution, as apparently there is no plausible explanation for this. The previously described

systematic review by Polanski et al. (2014) found only one RCT that used prednisolone as the sole immunotherapeutic agent in women with elevated pNK and considered the evidence of low quality to indicate improved IVF outcomes. In contrast, more recent non-randomised or quasi-randomised studies reported a significant benefit of prednisolone, when prescribed along with low-molecular weight heparin in women with unexplained RIF (Siristatidis et al., 2013; Fawzy and El-Refaeey, 2014). A recent meta-analysis found significant improvement in LBR and reduction of miscarriage rates when prednisolone was co-treated for women with idiopathic RM (Dan et al., 2015). The effectiveness of corticosteroids in combination with low-dose aspirin has not been assessed by a well-designed prospective study. Large RCTs including corticosteroids alone or in combination in different clinical scenarios may ascertain its role in AR. The risks being low in short-term use, prednisolone may be offered in selected cases unless future studies confirm otherwise.

- *Aspirin*

Low-dose aspirin, as antiplatelet-aggregation agent, is widely prescribed for prevention of cardiovascular diseases. Evidence on the prophylactic role of aspirin against pre-eclampsia and intrauterine growth restriction by improving trophoblastic invasion led to extension of this role in treating implantation failure. A Cochrane review found that aspirin in combination with unfractionated heparin may prevent recurrent miscarriage (RM) in women with anti-phospholipid syndrome (Empson et al., 2005). On the contrary, a more recent Cochrane review of studies on women with RM (whether associated with thrombophilia or not) did not find beneficial effect of aspirin alone or in combination with low-molecular weight heparin on LBRs when trials with high-risk for bias were excluded (de Jong et al., 2014). In a meta-analysis of 4 trials, LBRs did not improve and early miscarriage rates did not decline by the addition of heparin to low-dose aspirin in women with congenital thrombophilia (Areia et al., 2015). The place of aspirin alone as a routine adjuvant or in cases of RIF is more controversial. Another Cochrane review of 13 RCTs found no improvement of CPRs, LBRs and miscarriage rates by the addition of aspirin in the peri-implantation period in unselected cases (Siristatidis et al., 2011). Similar findings were observed in an individual patient data meta-analysis (Groeneveld et al., 2011). Moreover, combined heparin and aspirin administration may increase the risk of vaginal bleeding (Kaandorp et al., 2010). Based on current evidence, aspirin should therefore be offered only in selected cases.

- *Heparin*

Heparin is thought to prevent micro-thrombi formation at the implantation site by inhibiting clotting factor Xa. Both unfractionated and low-molecular weight heparin with or without low-dose aspirin have been used to promote successful invasion of trophoblasts in the presence of anti-phospholipid syndrome. Three systematic reviews, including a Cochrane review, were published in the clinical setting of RIF or RM on an unselected population (Seshadri et al. 2012; Potdar et al., 2013; Akhtar et al., 2015). One meta-analysis that included RCTs of women with ≥ 3 RIFs and another review that also included a RCT of women in a first IVF cycle found a significant improvement of LBRs (77-79% increase) with heparin co-treatment (Potdar et al., 2013; Akhtar et al., 2015). In contrast, the third meta-analysis demonstrated significantly higher CPRs and LBRs in the observational studies but not when the RCTs data were pooled (Seshadri and Sunkara, 2011). As stated above, the evidence on the effectiveness of combined heparin and aspirin therapy remains conflicting. In addition, heparin treatment is not without risk as bleeding (especially when co-administered with aspirin) and thrombocytopenia are not uncommon. At present there is insufficient data to establish the risk-effectiveness of heparin (alone or in comparison to aspirin) in women with RM without thrombophilia (Kaandorp et al., 2009). Therefore it could be prescribed only when clinically indicated after proper counselling.

- *Uterine artery vasodilators*

Nitric oxide/ nitroglycerine (NTG) frequently given in the form of sildenafil is a vasodilator that has been shown to increase uterine and endometrial blood flow and thus increase endometrial thickness. A RCT did not find improvement in implantation rates, CPRs or uterine artery indices when NTG was added on the day before embryo transfer (Ohl et al., 2002). Studies on the role of sildenafil in improving endometrial thickness reported conflicting results (Sher and Fisch, 2002). Current evidence thus does not justify use of vasodilators for improving implantation.

ICSI for non-male factors

Other than abnormal semen parameters, the application of ICSI has been widely accepted in

treatment cycles following total failed fertilisation with standard insemination, for fertilisation of cryopreserved-thawed oocytes, fertilisation of oocytes matured in-vitro and in cycles where pre-implantation genetic screening (PGS) or diagnosis (PGD) is planned (ASRM, 2012). Even though the level of evidence is not high in most of these conditions, there are good theoretical reasons behind the consensus. More controversial is opting for ICSI in the following situations:

Unexplained infertility: Although initial studies found a significant improvement of fertilisation and reduction of the risk of total failed fertilisation (Hershlag et al., 2002), RCTs failed to find any difference in FR, IR or LBRs (Foong et al., 2006).

Fewer number of oocytes: It is tempting to use ICSI to maximise the number of available embryos when the oocyte yield is low. However, large retrospective studies (Luna et al., 2011) and a RCT failed to demonstrate benefit of ICSI in this circumstance (Moreno et al., 1998).

Advanced maternal age: Evidence is limited to support ICSI in women with advanced age (ASRM, 2012). A theoretical advantage of ICSI in preventing polyspermia in aging oocytes exists.

ICSI for all: Even ICSI for all cases has been proposed to optimise the fertilisation rate and number of embryos available for transfer. However, it has been demonstrated in one well-conducted RCT that FRs (58% vs. 47%, P< .0001) and IRs (30% vs 22%; 1.35, 95% CI 1.04-1.76) were in fact higher with conventional IVF than ICSI with no difference in CPRs when the two techniques were compared in the setting of non-male factor infertility (Bhattacharya et al., 2001). Total failed fertilisation was 5% with IVF and 2% with ICSI. According to this study 33 oocytes are needed to undergo ICSI to prevent 1 total fertilisation failure (Bhattacharya et al., 2001). As a procedure, ICSI is not without risk. The safety of this procedure has not been well evaluated when used for non-male factor indications. Not least, ICSI requires additional time (Bhattacharya et al., 2001), resources and costs (ASRM, 2012).

Advanced sperm selection techniques

It has been widely recognised that standard semen analysis is a crude assessment of male reproductive potential because it does not evaluate the functional capacity of sperm. Advanced sperm selection methods have been described to improve fertilisation rates, even when ICSI is performed. Fertilisation of oocytes by sperms of high DNA integrity or genetic competence is expected to improve embryo quality and thereby pregnancy outcomes. Advanced sperm selection techniques include sperm surface charge selection, non-apoptotic sperm selection, sperm birefringence, intracytoplasmic morphologically selected sperm injection, (IMSI), physiological intracytoplasmic sperm injection (pICSI) or Hyaluronic acid binding. Of these, only IMSI and pICSI have been assessed through RCTs. Cochrane reviews including 2 RCTs on pICSI and 9 RCTs on IMSI failed to find any improvement in CPRs when these methods are compared with standard ICSI (Teixeira et al., 2013; McDowell et al., 2014). However, the multi-centre RCT that compared pICSI with standard ICSI found a 12% rise in CPRs with pICSI, which is clinically significant (Worrilow et al., 2013). This RCT also found a significant reduction of pregnancy loss in the pICSI group. Evidence on pICSI is still in the formative stage and, with limited experience, no serious additional risk (over ICSI) has been reported yet. It may be sensible to recommend pICSI in indicated cases and after careful counselling.

Advanced embryo selection techniques

Conventional morphological selection of embryos has been found to have limited value in predicting the developmental competency of embryos (Alpha Scientists in Reproductive and Embryology, 2011) as it is affected by the timing of the assessment and is observer dependent (Arce et al., 2006). Apparently embryos with good morphology may not necessarily be genetically competent and vice versa (Alfarawati et al., 2011). A more dynamic assessment of embryos and selection of euploid embryos have raised the hope of transferring the most competent embryo to maximise IVF success.

- *Time-lapse monitoring (TLM) of embryo*

Multiple sequential imaging of the dividing embryos without bringing them out of the incubator and analysis of the time interval of certain developmental mile-stones ('morpho-kinetic assessment') provide us with more information about their developmental competence and may predict the clinical outcomes. TIM (embryoscope™) also limits repeated embryo exposures outside the incubator and thereby may avoid embryo damage due to temperature variation. It has added the advantage of reproducibility and flexibility of laboratory work. At the same time concern is raised on repeated light (ultra-violet ray) exposure while taking the images. This new technology has been tested as a predictor of blastocyst development, implantation success as well as aneuploidy detection. After publication of a series of encouraging retrospective studies, an RCT

reported a significantly higher OPR (OR 1.23, 95% CI 1.06-1.43) and IR (OR 1.43, 95% CI 1.05-1.39) with the use of embryoscope (Rubio et al., 2014). Another RCT also found OPR increasing from 40.4% to 68.9% (p= 0.02) following TLM (Yang et al., 2014). However the RCTs were alleged to be flawed by utilisation of different culture systems, timing of transfer (a mixture of day 3 and 5) and were not analysed on an intention-to-treat basis (Kirkegaard et al., 2015). No difference in LBRs, CPRs and miscarriages could be found in a recent Cochrane review of 2 published RCTs and 1 study presenting interim analysis (Armstrong et al., 2015). They concluded that there was insufficient evidence to choose between TLM and conventional morphological assessment.

Correlation has been found between time-lapse scoring of embryos and blastocyst development in currently available retrospective and few non-randomised prospective data. However, whether TLM is superior to conventional morphological assessment remains unclear. Similarly, the value of morpho-kinetic assessment in short listing euploid embryos for transfer has yet to be evaluated in large prospective trials. Until good quality RCT evidence is available TLM should be offered only in certain circumstances such as RIF after counselling as to its cost-effectiveness.

- *Pre-implantation genetic screening (PGS)*

Blastomere, polar body or trophectoderm biopsy at zygote/ cleavage stage or blastocyst stages have long been in clinical practice. In spite of its initial promising results and strong theoretical basis, PGS of blastomeres using the FISH technique revealed lower LBRs in the RCTs (Mastenbroek et al., 2011). No or negative effect was apparent in good prognosis women as well as those with advanced age (Twisk et al., 2006). Many theories have been postulated to explain such discrepancies: chromosomal mosaicism, particularly in blastomere biopsy and inefficiency in the FISH technology itself are the postulated reasons (Mastenbroek and Repping, 2014).

Comprehensive (24) chromosome screening (CCS) with comparative genomic hybridization (CGH)-array from a trophectoderm biopsy has raised renewed hope in PGS. Biopsy at the blastocyst stage rather than in cleavage stage very much reduces errors due to mosaicism. The RCTs published so far demonstrated a higher IRs, CPRs and even OPRs/ LBRs when CCS was employed to select single euploid blastocyst transfer (Scott et al., 2013; Yang et al. 2014). Another RCT found no difference in OPRs between double and single blastocyst transfers with a dramatic fall in multiple pregnancy when the single blastocyst was subjected to euploidy screening (Forman et al., 2013). More recently, a meta-analysis indicated significantly higher CPRs by addition of CCS (Dahdouh et al., 2015). The 'next-generation sequencing' (NGS) in PGS has shown to be very accurate (near 100% specificity and 100% sensitivity) and reliable (Fiorentino et al., 2014b). Initial clinical study findings have been encouraging: an uncontrolled observational study revealed 63.8% CPR per embryo transfer following NGS (Fiorentino et al., 2014a).

The NGS technique has yet to be assessed by RCTs.The RCTs of the array-based CCS technique mentioned above have been criticised because the studies dealt with mainly good-prognosis patients (Mastenbroek and Repping, 2014). It is to be borne in mind that biopsy at blastocyst stage means fewer available embryos for transfer, particularly so among women of advanced age who may actually benefit more from this procedure than good-prognosis ones. Also, procedural damage to the embryos, however rare, could be a significant loss for this category of patient. It has therefore been suggested that, at present, PGS may be used to decide in which order the embryos could be transferred, rather than rejecting likely aneuploidy embryos straight away (Mastenbroek and Repping, 2014).

Pro-implantation procedures

- *Endometrial injury (EI)*

Only a few interventions in the field of AR in recent years have drawn such a widespread attention and publicity as EI, popularly known as 'endometrial scratching'. This procedure, which is a simplified version of endometrial curettage, works by evoking pro-implantation chemical factors, including certain cytokines, interleukins, growth factors and macrophages in the endometrial layers. Induction of decidualisation has also been proposed. The concept developed from the former observation of pregnancy-enhancing property of hysteroscopy +/- curettage. When performed in the secretory phase of the cycles prior to the embryo-transfer cycle, EI has been shown to improve IRs and LBRs in women with repeated implantation failures. Even though there are now hundreds of publications on this matter, the number of RCTs is few. Three meta-analyses and systematic reviews have been published including combinations of 4 existing RCTs and other non-RCTs (El-Toukhy et al., 2012; Nastri et al., 2012a; Potdar et al., 2012). The

Cochrane review of 4 RCTs found significant OPRs and LBRs when EI was performed in women with 2 or more implantation failures (RR 1.96, 95% CI 1.21 to 3.16; P= 0.006) (Nastri et al., 2015b). However, no benefit of EI was observed when it was done before a first or following 1 failed IVF cycle. This gave rise to the dictum 'do not scratch everybody'. In spite of a moderate quality of evidence in favour of EI in all 3 meta-analyses, the primary RCTs have been criticized as being methodologically very heterogeneous (Simon and Bellver, 2014). The study population, number and timing of intervention (EI procedures) varied among the trials. The authors of all 3 reviews called for further well-designed RCTs before introducing EI in routine practice. Given that no serious risk is involved with this procedure (Pipelle endometrial sampling has been a long-established office-procedure) EI should be considered in cases of RIF unless and until future RCTs confirm no benefit.

- *Assisted hatching (AH)*

AH or artificial rupture of the zona pellucida is being practiced to improve implantation since the late 1980s, still there is no consensus on its use. Chemical or laser-assisted hatching has been proposed to help a hardened zona break and create channels for exchange of metabolites, growth factors and signals between the embryos and endometrium. AH has not shown to be beneficial in unselected or good prognosis patients (Martins et al., 2011). No advantage was observed in women with advanced age either. A systematic review of 28 studies found higher CPRs in the subgroup of patients who had previous IVF failure(s) (Martins et al., 2011). A Cochrane review of 31 RCTs found a significant but marginal improvement in CPRs (OR 1.13, 95% CI 1.01 to 1.27) with AH, but no difference in the LBRs (OR 1.03, 95% CI 0.85 to 1.26) (Carney et al., 2012). The included studies were significantly heterogeneous while analysing overall CPRs; the heterogeneity was low in the comparison of LBRs (where no difference was observed). Although higher CPRs have been noted in the sub-group of women who had AH due to previous IVF/ICSI failure, it was concluded that the evidence is insufficient to offer AH in the above group of patients (Carney et al., 2012). Both the above meta-analyses identified significantly higher multiple pregnancy rates with the AH technique. A retrospective study of a large data-base (422,949 fresh first ICSI cycles) from the United States found lower LBRs with AH when performed because of diminished ovarian reserve (Butts et al., 2014). Until a proven beneficial effect of AH on LBRs is established, AH should not be offered.

- *Embryo glue (adhesive compounds)*

Chemically, embryo glue is a culture medium with added hyaluronic acid, which is an adhesive compound that promotes implantation. A Cochrane review of 6 RCTs found higher LBRs after using embryo glue (OR 1.41, 95% CI- 1.17 to 1.69) (Bontekoe et al., 2014). However, the incidence of multiple pregnancies was also increased significantly (OR 1.86, 95% CI 1.49 to 2.31). No other serious complications were reported. The use of embryo glue needs proper counselling on the risk of multiple births before this procedure is undertaken. Further studies may reveal the role of adhesive compound in a single embryo transfer setting. The technique can also be of value in women with RIF.

Change practice or wait for the future?

It takes time for any new intervention to be adopted into the National Guidelines. For example, there was no mention of many of the above interventions in the updated guideline on the management of infertile couple from the National Institute for Health and Clinical Excellence (NICE), which was published in 2013. The remaining interventions were not recommended by the Guideline Development Committee, on the basis of insufficient evidence at present (NICE, 2013). The committee opinions from the American Society of Reproductive Medicine (ASRM) also reflect similar opinions. On the other hand, any new idea often generates widespread media publicity, it raises patient's expectation and consequently there is often pressure from the patients to try new options. It is also true that clinicians make their own clinical judgement. Perhaps it will be unfair to deny certain treatments, which have low risk profile and some emerging evidence of benefit. The following categorisation can be made:

1. **Significant risk and/ or costly with uncertain benefits:** Examples of this category are: immunotherapy, AOA, ICSI for all, AH and routine PGS without specific indications. *These should be used under research context only*.
2. **Low/ rare risk with uncertain or possible benefits:** Supplements including DHEA, GH and CQ-10 for poor responders; heparin with or without prednisolone or aspirin (in selected cases), pICSI, PGS when indicated (RIF/ RM), embryo glue in RIF, TLM in certain situation may fall in this category. *These may be offered*

after informed counselling on risks-benefits and cost, until and unless further evidence confirms no benefit.

3. **No apparent risk, low-cost with possible benefit:** The interventions in this category include EI, short-term prednisolone when some benefit is expected and in absence of contra-indications. *These co-treatments may be offered after counselling, until and unless further evidence shows no benefit*.

It is to be noted that many of the 'empirical' medications are not licensed for use in the context of AR. Patients should be made aware of the unlicensed use and informed consent is mandatory before deciding on these interventions.

Conclusion

While new techniques are the way to progress, proper assessment is a prerequisite before general use. They should not be marketed prematurely based on inconclusive trials. Many immune therapies, for instance, have been practised for decades without going through a single RCT. A well-designed and adequately powered RCT is worth more than a meta-analysis of several small, heterogeneous RCTs. In this way novel additions in assisted reproduction shall not perplex us for long as to whether to accept them or not.

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