Adjuvant therapy, also known as adjunct therapy or add-on therapy, is therapy given in addition to the primary or initial therapy to maximize its effectiveness. Add-ons have become ubiquitous with the process of assisted reproduction (ART) which is markedly more complex than it was at its inception. This billion-dollar industry nurtured by the desperate and vulnerable couples it serves fuels the proliferation of adjuvants therapies in the hope of improving its success. In many cases they have been introduced without evidence of benefit and have resulted in a substantially more expensive offering without an equivalent efficiency gain posing a risk to the economic health of those affected and potentially decreasing access to care. In this commentary the case for adjuvants is examined in 3 areas of assisted conception namely periconceptual therapies, laboratory procedures and treatments designed to improve implantation.

Periconceptual adjuvants

Chinese Herbal Medicine (CHM) is commonly used by patients trying to conceive or during assisted conception. Whole system CHM is a combination of herbal treatments and acupuncture and has been shown to be of benefit in retrospective studies and active ingredients have plausible biological mechanisms in oogenesis and implantation. Cao et al has recently reviewed CHM in ART and included 20 studies with clinical pregnancy and on-going pregnancy rates as the primary outcome. Live birth rates were not reported. The analysis showed that combination of in vitro fertilization (IVF) and CHM improved IVF success; ongoing pregnancy rate (odds ratio (OR), 1.91; 95% confidence interval (CI), 1.17–3.10). However, there was a high risk of bias reported by the authors who conclude that the significant differences found are unlikely to be accurate. The evidence for or against CHM is fraught with poorly conducted studies with significant risk of bias and to date their role is unproven. They are unlikely to pose risk but with acupuncture can be costly.

Oxidative stress has a significant negative impact on important cellular and molecular reproductive processes through the production of oxygen free radicals related to inflammatory conditions. Antioxidants including the adjuvants vitamin E and C, Omega 3 polyunsaturated fatty acids, and pentoxifylline have been evaluated for their impact on infertility and its treatment. The recently updated Cochrane review showed an increased live birth rate compared with placebo or no treatment (OR, 2.13; 95% CI, 1.45–3.12; P = 0.001) [8 randomized controlled trials (RCTs), 651 women] for couples with female infertility. The authors concluded the evidence to be of very low-quality. The same author undertook a systematic review of antioxidants given to men whose partner was undergoing IVF for male factor infertility and found a significant improvement in live birth rates (OR, 4.85; 95% CI, 1.92–12.24; P = 0.0008[7]). Because of the heterogeneity between the studies, the small number of participants and live births the findings should be interpreted with caution.

Folic acid is generally accepted as a necessary periconceptual supplement to reduce the risk spina bifida, but recent reviews have also drawn attention to the importance of vitamin D in reproduction, in particular ovarian physiology and endometrial function. Observational studies have shown no correlation between vitamin D and IVF outcome, whereas Polyzos et al[14] found vitamin D deficiency was independently associated
with lower clinical pregnancy rates (OR, 0.61; 95% CI, 0.39–0.95) and Garbedian et al. found levels of vitamin D < 75 nmol/L may be an independent predictor of IVF outcome (OR, 1.01; 95% CI, 1.00–1.03). In a prospective RCT, Polak-de-Fried[16] found no improvement in ART outcome with vitamin D supplements. By contrast in a recent systematic review live birth was found to be more likely in women replete in vitamin D when compared with women with deficient or insufficient vitamin D status (OR, 1.33; 95% CI, 1.08–1.65)[17], and recommendations for dietary intake have been established[18,19].

Aspirin is generally regarded as a medical panacea and its use as an adjuvant to improve ART outcome has been extensively investigated. The plausible mechanisms are improved blood flow with possible impact on ovarian response and implantation. In a multicenter randomized controlled trial of low-dose aspirin (LDA) with prior pregnancy loss live birth rates were 58% (309/535) compared with 53% given placebo (286/543) (risk difference, 5%; 95% CI, 0.8 to 11)[20]. In a secondary analysis of the same study LDA increased live birth among women with raised inflammatory markers (C-reactive protein) to 59% [relative risk (RR), 1.35; 95% CI, 1.08–1.67], similar to rates in the lower and mid C-reactive protein tertiles raising the prospect that in a subset of infertile women with an inflammatory process LDA may be beneficial. However, to date there has been insufficient evidence to support its routine use[21,22]. Dentali et al[23] reviewed 17 studies with live birth rate as the outcome and found no benefit (OR, 1.08; 95% CI, 0.90–1.29). Currently the evidence does not support the routine use of Aspirin as an adjuvant to ART.

Androgens play an important role in reproductive processes and intrafetal physiology and their use as adjuvants in ART, especially in poor ovarian responders has been advocated. Dihydroepiandrosteredione (DHEA) metabolically converted to estrogen and testosterone is the most widely used most commonly in poor ovarian responders. Three reviews[24–26] have concluded there was insufficient evidence to recommend DHEA as routine supplementation in poor responders. In a small RCT involving patients not known to be poor responders Tartagni et al[27] found a significantly higher live birth rate in the DHEA supplemented group (P < 0.05). Nagels et al[28] has most recently reviewed the evidence in Cochrane. The 8 trials included both treatment naive and known poor responders. Overall pretreatment with DHEA was associated with higher live birth rates (OR, 1.88; 95% CI, 1.30–2.71); 8 RCTs, N = 878. Testosterone similarly found higher live birth rates compared with placebo or no treatment (OR, 2.60; 95% CI, 1.30–5.20); 4 RCTs, N = 345. However, following a sensitivity analysis the significance for both DHEA and Testosterone disappeared. A subgroup analysis of 2 trials involving treatment naive women not known to have diminished ovarian reserve showed no evidence of treatment effect (OR, 1.18; 95% CI, 0.53–2.60). To date there is insufficient evidence to recommend androgens routinely to supplement ART.

**Laboratory adjuvants**

Time lapse imaging (TLI) of embryo development seems intuitively to be a sensible approach to support decision making in embryo selection for transfer. Like many adjuvants to standard IVF treatment TLI has been introduced into routine practice without evidence for benefit[29–33]. In a large prospective study 843 patients were randomized to either TLI or conventional culture[34]. Those receiving TLI had a significantly higher ongoing pregnancy rate 51.4% (95% CI, 46.7–56.0) compared with 41.7% (95% CI, 36.9–46.5) for the standard incubation group. Three reviews have concluded there is insufficient evidence to support the use of TLI in routine practice[35–37]. A Cochrane review including 8 studies and 2303 women showed no evidence of difference in live birth rates in those patients whose embryos were cultured with TLI compared with conventional culture regardless of whether a software algorithm was used for decision making (OR, 0.73; 95% CI, 0.47–1.13)[38]. The possible benefits derived from TLI culture systems may lie more in the increased stability of the system and decreased disturbance to the environmental conditions of the embryo. To date there is no generalizability of the currently available decision-making algorithms which require further evaluation.

Assisted hatching (AH) has been widely used as an adjunct to standard ART[39] without evidence to support its use and in some cases with a negative consequence[40–43]. A series of systematic reviews have been undertaken to investigate the impact of AH. Martins et al[44] reviewed 28 studies including 5507 participants and found no increase in live birth rate (RR, 1.03; 95% CI, 0.91–1.16); although in a subgroup analysis, a significant difference was observed in women with previous repeated failure (RR, 2.51; 95% CI, 1.06–5.96). In the Cochrane review, Carney et al[45] found no significant difference in the odds of live birth in the AH group compared with the control group (9 RCTs; OR, 1.03; 95% CI, 0.85–1.26). Li et al[46] reviewed 36 RCTs including 6459 participants. Compared with those women in the control group, women who underwent AH had a nonsignificant OR of live birth (OR, 1.09; 95% CI, 0.92–1.30). Zeng et al[47] reported a review of 12 RCTs with > 2574 participants undergoing frozen embryo transfer and randomized to receive laser AH or no intervention and found no difference in live birth (OR, 1.09; 95% CI, 0.77–1.54). Despite a large number of trials and several reviews there is no evidence to support the routine use of AH as an adjuvant in assisted conception. It remains to be proven whether AH has a place in a subgroup of patients who have had repeated implantation failure.

In vitro maturation has developed to the extent that in some centers it now results in pregnancy rates comparable to those achieved with conventional treatment. Its place in IVF practice is confounded to mitigation strategies for severe ovarian hyperstimulation syndrome risk and as part of fertility preservation protocols. Its efficacy compared with other interventions and to normal treatment has yet to be demonstrated in prospective RCTs.

Blastocyst culture and transfer is now embedded into ART practice[48] but should still be regarded as an adjuvant, to the extent that it IVF/intactyploicat sperm injection (ICSI) can be successfully carried out without this addition. Blastocyst culture was driven initially by the need to compensate for reduced pregnancy rates consequent upon single embryo transfer policies adopted to reduce multiple pregnancy rates. More recently it has been used to support trophectoderm biopsy and preimplantation genetic screening (PGS). There are advantages and disadvantages to blastocyst culture[49]. The impact of additional laboratory costs, reduced availability of spare embryos for cryopreservation and the possibility of failure to transfer versus the potential advantage gained through increased implantation requires further evaluation. Aside the need for trophectoderm biopsy, the evidence to support blastocyst over cleavage stage transfer is currently lacking[50–52] but it may now be too late to undertake
the definitive trial with economic evaluation. In resource-constrained environments practitioners should consider the option of simplified culture and transfer at cleavage stage as it can produce equivalent pregnancy rates.

Preimplantation genetic screening of embryos (PGS/PGTa) in routine IVF/ICSI practice is controversial. Unquestionably aneuploidy is a common occurrence in human embryos and is associated with poor reproductive outcome. PGS/PGTa is driven principally by the need to select the embryo most likely to implant and interpretation of outcome data should be seen in the context of rapidly evolving science, not reflected in current guidance, including array comparative genomic hybridization, single nucleotide polymorphism microarrays, quantitative real-time PCR, and whole-genome next-generation sequencing. Recent reviews confirm the potential benefit of PGS in good prognosis groups using comprehensive chromosome screening on trophectoderm biopsy but underline the lack of good quality evidence for its general application in standard IVF and use in poor prognosis groups. The impact of reduced availability on numbers of embryos available for cryopreservation in PGS/PGTa cycles, the presence of mosaicism confounding analysis and the cost-effectiveness of PGS/PGTa compared with alternate approaches all require further evaluation before PGS/PGTa can be considered as a routine adjuvant.

Interventions to support implantation

Adherence compounds have been used for some time in ART as an adjuvant to aide embryo implantation. The main candidate is hyaluronic acid (HA), a molecule naturally occurring in the genital track and utero-tubal fluid, whose mode of action is uncertain but whose properties include promotion of cell to cell adhesion. Fibrin, a naturally occurring protein involved in clotting has also been proposed but has limited data on efficacy. Bontekoe et al undertook a systematic review which included 17 eligible studies. Six RCTs including 1950 subjects had HA added to the embryo transfer media and reported live birth rates. When compared with no treatment there was a trend toward benefit which was not significant (OR, 1.35; 95% CI, 0.86–2.12). However, when compared with no and low HA levels and high to low levels the results reached significance: OR, 1.41; 95% CI, 1.17–1.69 and 1.42; 95% CI, 1.16–1.73, respectively. Only one trial investigating Fibrin was eligible for inclusion and showed no benefit (OR, 0.98; 95% CI, 0.54–1.78). There is evidence of benefit for the addition of HA to embryo transfer medium, however, there is no agreement about the optimal concentration, timing and duration of exposure and there is limited evidence for the safety of embryos exposed to supraphysiological concentrations of HA.

Granulocyte colony–stimulating factor (G-CSF) is a cytokine that stimulates the production of a range of inflammatory cells and is thought as a result to have a role in several aspect of reproduction including implantation although the exact mechanism is not understood. The evidence for the intervention has been reviewed in 2 metaanalyses. Compared with placebo or no treatment, Xie et al found the use of G-CSF was associated with a higher clinical pregnancy rate (RR, 1.563; 95% CI, 1.122–2.176). Moreover, more significant differences were detected in the subgroup of patients with thin endometrium or repeated IVF failure (RR, 2.312; 95% CI, 1.444–3.701). Kamath et al confined the analysis to those patients with a thin endometrium (mean difference, 0.47; 95% CI, −1.36 to 2.31); and recurrent implantation failure; clinical pregnancy rate (RR, 2.51; 95% CI, 1.36–4.63) but they describe the quality of evidence as low to very low.

Endometrial injury produced either as an isolated procedure or as part of an investigative procedure such as hysteroscopy has become part of routine practice in some centers. Its action is unclear but thought to be modification of the endometrial inflammatory response to implantation or timing of decidualization. Evidence for benefit has been inconsistent. In contrast to first cycle, increased benefit has been found in women with repeated implantation failure reviewed the evidence from 7 studies (3 nonrandomized) including 2062 subjects and found a significant benefit when uterine instrumentation was undertaken in the cycle preceding IVF in cases of recurrent implantation failure (RR, 2.32; 95% CI, 1.72–3.13); following endometrial scratch and (RR, 1.31; 95% CI, 1.30–1.75) following hysteroscopy. Nastri et al, 14 trials involving 2128 subjects, found no difference in the live birth rate or on-going pregnancy rate in women who had no more than one previous embryo transfer (RR, 1.11; 95% CI, 0.94–1.32). In contrast subgroup analysis of those women who had 2 or more prior embryo transfers showed modest benefit with intervention (RR, 1.63; 95% CI, 1.12–2.38). Wadhwa and Mishra reported increased pregnancy rates following endometrial injury in a single center RCT including cases of repeated implantation failure but this did not reach significance. Two recently reported multicenter RCTs reported no improvement in live births in women undergoing hysteroscopy in the cycle before IVF. The evidence suggests possible benefit in women with previous implantation failure but not in those who are treatment naive. Further evidence is required before this intervention can be recommended and the results of a series of RCTs currently underway are eagerly awaited.

Adjuvant drug therapies designed to enhance uterine blood flow through vasodilatation have been used and include nifedipine, nimodipine, pentoxifylline; nitric oxide donors such as glyceryl trinitrate and isosorbide mononitrate; and sildenafil. Observational studies have shown benefit with the use of Sildenafil in patients with a thin endometrium undergoing assisted conception reported improved on-going pregnancy rates in patients with thin endometrium following treatment with Sildenafil. In the only review of this intervention, Gutarra-Vilchez et al found no benefit in live birth rates following glyceryl trinitrate or pentoxifylline (RR, 1.18; 95% CI, 0.82–1.69) or in clinical pregnancy rate following Sildenafil (OR, 1.59; 95% CI, 0.63–4.03). There is insufficient evidence to recommend these adjuvants.

Heparin has been considered potentially beneficial as an adjuvant therapy, the putative mechanism being improved blood flow at the implantation site. Akhtar et al published a Cochrane systematic review including three studies. This showed significant improvement in live birth rate although this disappeared when a random effects model was applied. The authors comment on the small numbers in each study and the significant heterogeneity between each and advise caution in interpretation. In a further systematic review of three studies, including only cases of recurrent implantation failure, reported significant improvement in live birth rate (RR, 1.79; 95% CI, 1.10–2.70) in this subgroup. These authors also caution interpretation because of the small number of subjects included.
The unique immunologic response of mother to allogenic fetus has been a biological anomaly which has taxed researchers. The balance of inflammatory cells, cytokines and other immunomodulatory cells which interact at the feto-maternal interface to permit tolerated invasion of the decidua by syncytiotrophoblast is finely controlled and its disturbance potentially detrimental to implantation. Natural killer cell cells and their control are thought to be key to this interaction. However, to date, the precise interaction between the various inflammatory cells, their impact on reproduction, the diagnostic measures of disturbance and treatments which may bring about positive benefit remain to be demonstrated. Despite uncertainty a range of immunomodulatory treatments have been used in practice to improve implantation and reduce the risk of early miscarriage.

Injection of paternal or third-party lymphocytes has been investigated for several decades in the treatment both in recurrent miscarriage and implantation failure. There have been no RCTs demonstrating benefit[79,80]. Intralipid therapy has been proposed as an immunomodulatory treatment in patients with implantation failure or recurrent miscarriage with raised natural killer cells based on observational studies[81–83]. There have been no randomized controlled studies undertaken and its use as an adjuvant as part of normal IVF has not been assessed[84].

Steroids have also been proposed as an adjuvant because of their immunomodulatory activity. A recent systematic review of the use of glucocorticoids in ART concluded that they did not improve the overall chance of live birth[85]. However, the use of glucocorticoids in women undergoing IVF, rather than ICSI, was associated with an improvement in pregnancy rates of borderline significance (OR, 1.53; 95% CI, 1.07–2.19). The evidence does not currently support the routine use of steroids but there is sufficient evidence to support further research[86].

Intravenous immunoglobulin therapy (IVIg), another drug with immunomodulatory effect has been investigated for its efficacy in improving embryo implantation. The likely mode of action is its ability to suppress an abnormal host immune response to the developing pregnancy. De Placido et al[87] randomized 39 patients who had either failed IVF or had 2 or more miscarriages to receive IVIg or placebo and found no benefit. Sher et al[88] compared groups undergoing IVF receiving IVIg and heparin/aspirin with heparin and aspirin alone and found a higher live birth rate with IVIg, total births 23 (P = 0.027). In an RCT Stephenson and Fluker[89] found no improvement in live birth rate with IVIg given to women with repeated implantation failure, births 4 (P = 0.52). Several reviews have been undertaken, each with significant methodological flaws.Clark et al[90] undertook a meta-analysis of the 3 trials[87–89] and found that the pooled OR, 2.52; 95% CI, 1.2–5, favouring IVIg with live birth as the outcome. Li et al[91] undertook a review of 10 studies only one of which was a randomized trial and reported live birth to be significantly improved with the intervention (RR, 1.616; 95% CI, 1.243–2.101). Polanski et al[84] included 2 observational studies in their review and concluded there is insufficient evidence to recommend IVIg treatment. There have been no trials investigating the use of IVIg in patients undergoing IVF without evidence of implantation failure or recurrent miscarriage. The quality of evidence is poor with low subject numbers, heterogeneity between the studies and a plethora of observational retrospective studies and therefore insufficient evidence to support the use of IVIg.

Anti TNFα is an immunomodulatory agent and is used as an adjunct to assisted conception to improve implantation. Its use is based on hypothesis and observational data. To date there have been no prospective RCTs conducted to investigate its efficacy.

Conclusions

A common theme runs through the use of adjuvant therapies in assisted conception. They have been introduced, typically with some plausible pathophysiologic or molecular basis but without the scientific rigor of evaluation normally expected before new therapies are introduced into clinical practice. In this paper the evidence for a range of 18 adjuvants in use in many parts of the world has been examined. In Figure 1 adjuvants are listed according to the strength of evidence and in only two cases is there evidence to support their use. In the case of blastocyst transfer pregnancy is significantly more likely compared to that of a cleavage stage embryo transfer however when sequential replacement of embryos arising from the same stimulation cycle is considered the difference disappears. The evidence in support of adherence compounds.

In the recent review of adjuvants only those adjuvants used in the laboratory were reviewed but drew similar conclusions finding limited evidence for TLI and preimplantation genetic screening and no evidence for the use of AH[92]. They found some evidence for the use of adherence compounds but that further evaluation was necessary before adoption. Shirlow et al[93] reported a retrospective analysis of > 13,000 embryo transfers from a single IVF service and applied a logistic regression analysis to estimate the impact of 13 pharmacological interventions on outcome. Approximately half of the 1904 patients received 2 or more adjuvants. Three showed significant improvement in live birth rate namely aspirin, steroids, and melatonin. All others showed no benefit. This contrasts with the present study which showed no benefit for the routine use of aspirin or steroids.

Couples undergoing assisted conception treatment naturally want to do the very best they can to improve the chances of pregnancy and are susceptible to the suggestion that adjuvant therapies will improve that chance. The cost of assisted conception is materially increased by the addition of adjuvants to the extent in some cases that the basic cost may double. Worldwide, treatment costs are often born by the patient and the consequence of escalation of costs can be a crippling burden of debt, increased psychological stress and ultimately reduced access to care, most acutely felt in economically challenged situations[94]. Furthermore, the fact that practitioners promulgate adjuvant therapies despite limited or absent evidence, some not without risk raises the question of ethical practice.

In conclusion many of the “add-ons” in common use are without proof of benefit. There are interventions which have promise, for example, TLI and endometrial injury but evidence to date is lacking and well conducted RCTs are required. Until good quality evidence is available clinicians should avoid advising unproven and costly add-ons or use them only in the context of trials. Patients should be provided with accurate and unbiased information and funders and health departments should be advised of the effectiveness of all therapies in use.
Conflict of interest statement

The author declares that there is no financial conflict of interest with regard to the content of this report.

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