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ARTICLE

Live birth rates after combined adjuvant therapy in IVF–ICSI cycles: a matched case-control study

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Abstract The effectiveness of combined co-treatment with aspirin, doxycycline, prednisolone, with or without oestradiol patches, was investigated on live birth (LBR) rates after fresh and frozen embryo transfers (FET) in IVF and intracytoplasmic sperm injection cycles. Cases (n = 485) and controls (n = 485) were extensively matched in a one-to-one ratio on nine physical and clinical parameters: maternal age, body mass index, smoking status, stimulation cycle number, cumulative dose of FSH, stimulation protocol, insemination method, day of embryo transfer and number of embryos transferred. No significant differences were found in fresh cycles between cases and controls for the pregnancy outcomes analysed, but fewer surplus embryos were available for freezing in the combined adjuvant group. In FET cycles, LBR was lower in the treatment group (OR: 0.49, 95% CI 0.25 to 0.95). The lower LBR in FET cycles seemed to be clustered in patients receiving combined adjuvant treatment without luteal oestradiol (OR 0.37, 95% CI 0.17 to 0.80). No difference was found in LBR between cases and controls when stratified according to the number of previous cycles (<3 or ≥3). There is no benefit of this combined adjuvant strategy in fresh IVF cycles, and possible harm when used in frozen cycles.

KEYWORDS: antibiotics, aspirin, intracytoplasmic sperm injection, in-vitro fertilization, oestradiol, prednisolone

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Introduction

Over the past 25 years, IVF has seen significant improvements, leading to much improved outcomes for many infertile couples. Nevertheless, a significant proportion of couples still experience repeated failure to conceive despite IVF. A history of repeated IVF failure is particularly distressing for couples undergoing fertility treatment, and presents a formidable challenge to the fertility specialist offering clinical guidance.

Couples generally understand that a successful outcome may take more than one attempt. the high cost of treatment and the associated physical and mental burden, however, places considerable pressure on the couple and the clinical team to achieve a successful outcome, preferably defined as the birth of a singleton healthy baby at term (Min et al., 2004), in the shortest possible time.

Further adding to the patient's frustration is our restricted ability to diagnose underlying problems of endometrial receptivity or gamete biology (Lessey, 2011; Salamonsen et al., 2009). By the time patients are convinced something must be wrong, they are often armed with online information from internet-based support forums and chat groups and insist on alternative approaches as they become increasingly desperate to try something new. Such alternative approaches are often based on a standard protocol with the addition of adjuvantia such as aspirin, antibiotics, glucocorticoids or luteal phase estradiol supplementation, which have not been associated with a clinical benefit in a significant number of studies (Boomsma et al., 2012; Brook et al., 2006; Dentali et al., 2012; Gelbaya et al., 2007, 2008; Groeneveld et al., 2011; Jee et al., 2010; Kaandorp et al., 2010; Khairy et al., 2007; Peikrishvili et al., 2004; Siristatidis et al., 2011). In two other studies, however, a potential clinical benefit of adjuvantia for selected groups of IVF patients has been shown (Boomsma et al., 2012; Dentali et al., 2012). These considerations have led us to reflect on the clinical practice in our IVF unit where combinations of adjuvantia are used by some practitioners.

To the best of our knowledge, no studies exist on the combined use of aspirin, doxycycline and prednisolone, with or without luteal phase ostradiol during IVF. We therefore sought to investigate whether this combined adjuvant treatment improves live birth rates in fresh and frozen IVF and intracytoplasmic sperm injection (ICSI) cycles.

Materials and methods

Study design

This one to one matched case-control study includes patients presenting for a fresh or frozen IVF–ICSI cycle at a private assisted conception clinic between 2005 and 2008. A total of 20,962 cycles were analysed retrospectively. Cycles using donor oocytes, cycles with embryo transfer on days other than 2, 3 or 5 or those without a documented body mass index were excluded from the analysis, as were all cycles where no embryo transfer occurred. For cases only, the first cycle with the combined adjuvant protocol was included, even if the patient had had further cycles with the same protocol. An outline of the selection process is presented in **Figure 1**.

The use of combined adjuvant treatment is decided by the treating IVF specialist. The clinical indication often includes a history of repeated IVF failure, but some specialists prescribe this adjuvant protocol routinely in all patients. Many specialists in our unit, however, do not prescribe combined adjuvant therapy. As such, it is unlikely that unexposed controls would be subject to significant indication bias. Controls were carefully matched on physical and clinical parameters (±range); maternal age (±12 months), body mass index (BMI) (±4 kg/m^2), current smoking status (smoker/non-smoker), stimulation cycle number (±2), total dose of FSH (IU), type of stimulation protocol, insemination method (IVF or ICSI), day of embryo transfer (day 2, day 3 or day 5) and number of embryos transferred (1 or 2). For 90 cases (15.7%), a suitable match could not be achieved, and these cases were excluded from the final analysis, leaving a total of 485 women that were successfully matched with controls.

Ovarian stimulation and frozen embryo transfer protocols

Women undergoing a fresh IVF–ICSI cycle underwent either a gonadotrophin-releasing agonist (GnRH) (with or without oral contraceptive pill scheduling) (Synarel®; Pfizer Australia, West Ryde, Australia) or a GnRH antagonist cycle (without oral contraceptive pill scheduling) (Orgalutran®; Ganirelix; Merck Sharp & Dohme, Macquarie Park, Australia) with recombinant FSH (Gonal-F®; Merck Serono, Frenchs Forest, Australia; Puregon; Merck Sharp & Dohme, South Granville, Australia) for ovarian stimulation. Women undergoing a frozen embryo transfer (FET) cycle either had their embryo transfer in a natural cycle or a hormone replacement cycle (with or without oral contraceptive pill and GnRH agonist downregulation). In natural FET cycles, ovulation was detected with the use of a urinary LH kit (Clearblue®; Procter & Gamble, Cincinnati, US) with subsequent confirmation of the LH surge with blood tests. Women undergoing a hormone replacement cycle were prescribed oestradiol valerate 2 mg/day orally (Progynova®; Bayer Australia, Pymble Australia) and when the endometrial lining reached at least 6 mm, vaginal progesterone was added at a dose of 200 mg three times a day. Importantly, cases were carefully matched with controls undergoing exactly the same protocol.

Adjuvant treatment

The following combination of medical adjuvantia was prescribed. Acetylsalicylic acid (100 mg/day orally of Astrix®; Mayne Pharma International, Salisbury South, Australia) was administered from the day after oocyte retrieval (fresh cycles), the first day of the period (natural frozen cycles) or the day of commencement of hormone replacement therapy (HRT) (HRT frozen cycles). In fresh cycles, the male partner was prescribed doxycycline (100 mg/day orally of Doxycycline®; Sandoz, Australia) for 7 days to commence with the start of ovarian stimulation. The female partner was prophylactically administered amoxicillin 500 mg/clavulanate 125 mg bd orally (Augmentin®; GlaxoSmithKline, Boronia, Australia) for 5 days starting on the day after HCG trigger injection (fresh

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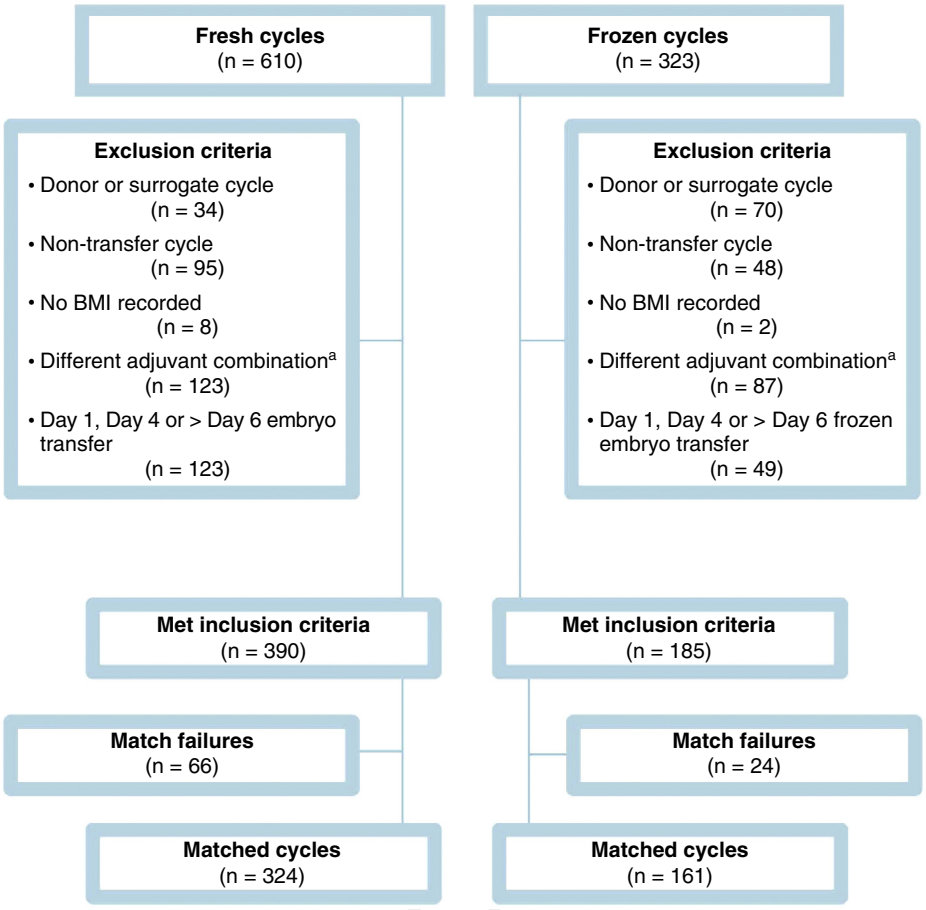

Figure 1 Selection process. [a]Only patients with a combination of prednisolone plus aspirin plus antibiotic with or without luteal phase oestradiol included. Exclusion factors are not mutually exclusive. BMI, body mass index, N, number of individual patients.

cycles), the day of ovulation (natural frozen cycles) or the day of commencement of progesterone (HRT frozen cycles). Prednisolone (25 mg/day orally of Solone®; iNova Pharmaceuticals, Chatswood, Australia) was administered from the start of each cycle. When given, luteal oestradiol was administered using oestradiol transdermal patches (100 µg/day of Estradot®; Novartis Pharmaceuticals, North Ryde, Australia) every 3 days from the day after oocyte retrieval (fresh cycles), the day of ovulation (natural frozen cycles) or the day of commencement of progesterone pessaries (HRT frozen cycles) (400 mg twice a day Compounded, Slade Pharmacy, Richmond, Australia). Except for the antibiotics, adjuvants were continued until the end of the first trimester if patients were pregnant.

Outcome measures

The primary outcome measure was live birth rate (LBR). Secondary outcome measures included the number of retrieved oocytes, fertilization rate, embryo implantation rate, clinical pregnancy rate, viable pregnancy rate, spontaneous abortion rate and rate of ovarian hyperstimulation syndrome (OHSS). The following pregnancy-related definitions were used. Implantation rate was defined as the number of viable sacs observed on ultrasound divided by the number of embryos transferred. A clinical pregnancy was defined as a pregnancy proven on ultrasonography at 7 weeks. A viable pregnancy was defined as a pregnancy with the presence of fetal cardiac activity on ultrasonography at 12 weeks. Spontaneous abortion was defined as a loss of a clinical pregnancy occurring before 20 weeks of gestation. The LBR rate was defined as the number of deliveries with at least one live born infant.

Statistical analysis

The study's overall null hypothesis predicted no difference in the live birth rate between women in the treatment group and the control group. *A priori*, two further subanalyses were planned for the following subgroups: cycles with or without luteal phase oestradiol patches, and stimulation cycle number less than three or three or more. Differences between groups were analysed using chi-squared tests for categorical variables and independent samples t-test for comparison of group means. Fresh and FET cycles were analysed independently. Cases and controls were first compared as a whole group and further sensitivity analyses were conducted as described for the primary outcome measure. Data are presented as means (+SD), frequency (%). PASW version 19 (SPSS Inc., Chicago, Illinois) was used for data analysis. $P < 0.05$ was considered statistically significant.

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Ethical approval

This retrospective study was approved by the Human Research and Ethics Committee of the Monash Surgical Private Hospital (P07078, 25 November 2013).

Results

Fresh embryo transfer cycles

Comparisons between 324 cases and 324 controls confirmed that matching yielded highly similar groups for maternal age (36.8 ± 4.0 years versus 36.9 ± 4.0 years), body mass index (24.7 ± 4.9 versus 24.7 ± 4.5 kg/m^2), stimulation cycle number (3.3 ± 2.1 vs 3.1 ± 2.2). This included 20 cases and controls on their first IVF cycle, stimulation protocol and insemination method used, total FSH dose, day of embryo transfer and the number of embryos transferred (Table 1). The number of self-reported smokers was low in both groups, but, compared with controls, the numbers were marginally higher in the treatment group (3.1% versus 0.9%, $P = 0.05$).

No significant differences were found between the two groups in any of the clinical outcome parameters (e.g. ovarian response, fertilization rate, number of discarded oocytes, implantation rate and spontaneous abortion rate). Significantly more embryos, however, were suitable for cryopreservation in the controls ($P = 0.01$) (Table 2). A total of 126 (38.9%) clinical pregnancies were achieved in the treatment group compared with 125 (38.6%) in the control group (OR 1.01, 95% CI 0.74 to 1.39). Similarly, no differences were observed in the viable pregnancy rate (OR 1.04, 95% CI 0.76 to 1.43) or live birth rate (OR 1.00, 95% CI 0.72 to 1.39) between the two groups. A sub-analysis for cycle number (<3 versus ≥3) and treatment with or without the luteal phase estradiol patches showed no difference in clinical pregnancy, viable pregnancy or live birth outcome (Table 2).

Frozen embryo transfer cycles

Comparisons between 161 cases and 161 controls confirmed similar matching parameters between the groups for maternal age (36.5 ± 4.3 years versus 36.7 ± 4.2 years), body mass index (25.4 ± 4.9 versus 25.0 ± 4.6 kg/m^2), cycle number

Table 1 Fresh cycles: demographics and cycle details.[a]

	Cases	*Controls*
Patient characteristics		
Adjuvants	324	324
Adjuvants without luteal oestradiol	125/324 (38.6%)	NA
Adjuvants with luteal oestradiol	199/324 (61.4%)	NA
Age (years)	36.8 (±4.0)	36.9 (±4.0)
BMI (kg/m^2)	24.7 (±4.9)	24.7 (±4.5)
Smoker	10/324 (3.1%)	3/324 (0.9%)
Cause		
Endometriosis	16/324 (4.9%)	7/324 (2.2%)
Tubal	13/324 (4.0%)	28/324 (8.6%)[a]
Multiple	65/324 (20.1%)	71/324 (21.9%)
Ovarian	58/324 (17.9%)	41/324 (12.7%)
Male factor only	75/324 (23.1%)	77/324 (23.8%)
Unexplained	97/324 (29.9%)	100/324 30.9%)
Clinical characteristics		
Stimulation cycle number	3.3 (±2.1)	3.1 (±2.2)
Treatment		
IVF	59/324 (18.2%)	62/324 (19.1%)
ICSI	265/324 (81.8%)	262/324 (80.9%)
Stimulation protocol		
Agonist cycle	295/324 (91.0%)	299/325 (92.3%)
Antagonist cycle	29/324 (9.0%)	25/324 (7.7%)
Start dose FSH (IU)	324 (±127)	313 (±122)
Total FSH dose (IU)	3149 (±1330)	3085 (±1303)
OHSS	3 (0.9%)	2 (0.6%)
Embryo transfer characteristics		
Single cleavage transfer	58/324 (17.9%)	59/324 (18.2%)
Double cleavage transfer	125/324 (38.6%)	126/324 (38.9%)
Single blastocyst transfer	55/324 (17.0%)	54/324 (16.7%)
Double blastocyst transfer	86/324 (26.5%)	85/324 (26.2%)

ICSI, intracytoplasmic sperm injection; NA, not applicable; OHSS, ovarian hyperstimulation syndrome.
[a]Values are represented by means (±standard deviation) or frequency (%).
[b]$P = 0.02$.

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Table 2 Fresh cycles: ovarian response and pregnancy outcome.[a]

	Cases	*Controls*
Number of oocytes collected		
Total	10.4 (±6.3)	10.9 (±6.5)
One to three oocytes retrieved	29/324 (9.0%)	30/324 (9.3%)
Four to eight oocytes retrieved	98/324 (30.2%)	81/324 (25.0%)
Nine to 15 oocytes retrieved	132/324 (40.7%)	143/324 (44.1%)
>15 oocytes retrieved	65/324 (20.1%)	70/324 (21.6%)
Number of oocytes inseminated	8.8 (±5.6)	9.3 (±5.7)
Number of oocytes fertilised	6.0 (±4.2)	6.5 (±4.4)
Fertilization rate	1931/2846 (67.8%)	2107/3010 (70.0%)
Number of oocytes discarded	3.1 (±3.5)	3.2 (±3.4)
Number of embryos transferred	1.65 (±4.8)	1.65 (±4.8)
Number of embryos frozen	1.2 (±2.0)	1.7 (±2.0)[b]
Implantation rate	161/535 (30.1%)	158/535 (29.5%)
Spontaneous abortion rate	19/126 (15.1%)	17/125 (13.6%)
Pregnancy outcome		
Clinical pregnancy	126/324 (38.9%)	125/324 (38.6%)[c]
Viable pregnancy	120/324 (37.0%)	117/324 (36.1%)
Live birth		
Total	106 /324(32.7/%)	106/324 (32.7%)
Stimulation cycle < 3	49/149 (32.9%)	52/169 (30.8%)
Stimulation cycle ≥ 3	57/175 (32.6%)	54/155 (34.8%)
Adjuvants without luteal oestradiol	45/125 (36.0%)	49/125 (39.2%)[d]
Adjuvants with luteal oestradiol	61/199 (30.7%)	57/199 (28.6%)[d]
Multiple birth	30/324 (9.3%)	30/324 (9.3%)

[a]Values are represented by means (±standard deviation) or frequency (%).
[b]$P = 0.01$.
[c]This includes one ectopic pregnancy.
[d]Controls were matched to cases with adjuvant protocols specified but not exposed to adjuvants.

(2.8 ± 2.0 versus 2.5 ± 2.0), stimulation protocol and insemination method used, day of embryo transfer, the number of embryos transferred (Table 3) and number of self reported smokers (1.2% versus 0.0%).

No significant differences were observed between the two groups in the implantation or spontaneous abortion rate. No differences were observed in the clinical pregnancy (OR 0.68, 95% CI 0.39 to 1.18) or viable pregnancy rate (OR 0.66, 95% CI 0.37 to 1.21) between the treatments and controls. The LBR was significantly lower in the treatment group (OR 0.49, 95% CI 0.25t o 0.95) (Table 4).

A sub-analysis of live birth for stimulation cycle number (<3 versus ≥3) showed no benefit of adjuvant treatment. The LBR, however, was lower in the treatment group (7.8%) compared with controls (18.6%) for cycles without the luteal phase estradiol patches and their matched controls (OR 0.37, 95% CI 0.17 to 0.80) (Table 4).

Non-matched cases

A significant proportion of cases (15.7%) could not be appropriately matched. The potential for selection bias was assessed by comparing the cycle outcomes for matched and non-matched cases.

For cases undergoing a fresh transfer, the maternal age, BMI and cycle number were not significantly different. Compared with matched cases, however, non-matched cases had significantly lower clinical (38.9% versus 24.2%; $P = 0.03$) (OR 0.50, 95% CI 0.27 to 0.92) and viable pregnancy rates (37.0% versus 24.2%; $P = 0.05$) (OR 0.54, 95% CI 0.30 to 1.00). The live birth rates were 11.5% lower in the non-matched group but this did not reach statistical significance (32.7% versus 21.2) (OR 0.55, 95% CI 0.30 to 1.1).

For cases undergoing a frozen transfer, the maternal age, BMI and cycle number were not significantly different. Compared with matched cases, non-matched cases had similar clinical (16.8% versus 16.7%) (OR 1.00, 95% CI 0.31 to 3.1) and viable pregnancy rates (13.7% versus 8.3%) (OR 0.57, 95% CI 0.13 to 2.61) and live birth rates (9.3% versus 8.3%) (OR 0.88, 95% CI 0.19 to 4.14).

Discussion

The use of several adjuvantia, such as aspirin, glucocorticosteroids, antibiotics and luteal phase oestrogen support have been studied previously in IVF; however, to the best of our knowledge, these have not previously been studied in combination. Although there may be theoretical considerations why such a combination may result in better pregnancy outcomes, our study found no evidence to support the use of this combined adjuvant strategy in patients undergoing IVF treatment. Overall, no differences were observed in

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Table 3 Frozen embryo transfer cycles: demographics and cycle details.[a]

	Cases	*Controls*
Patient characteristics		
Adjuvants	161	161
Adjuvants without luteal oestradiol	129 (80.1%)	NA
Adjuvants with luteal oestradiol	32 (19.9%)	NA
Age (years)	36.5 (±4.3)	36.7 (±4.2)
BMI (kg/m^2)	25.4 (±4.9)	25.0 (±4.6)
Smoker	2/161 (1.2%)	0/161 (0.0%)
Cause		
Endometriosis	2/161 (1.2%)	6/161 (3.7%)
Tubal	6/161 (3.7%)	10/161 (6.2%)
Multiple	48/161 (29.8%)	49/161 (30.4%)
Ovarian	33/161 (20.5%)	26/161 (16.1%)
Male factor only	40/161 (24.8%)	26/161 (16.1%)
Unexplained	32/161 (19.9%)	44/161 (27.3%)
Clinical characteristics		
IVF cycle number	2.8 (±2.0)	2.5 (±2.0)
Treatment		
FET–IVF	41/161 (25.5%)	40/161 (24.8%)
FET–ICSI	120/161 (74.5%)	121/161 (75.2%)
Stimulation protocol		
Natural cycle	51/161 (31.7%)	51/161 (31.7%)
HRT cycle	90/161 (55.9%)	90/161 (55.9%)
Agonist-HRT cycle	20/161 (12.4%)	20/161 (12.4%)
Embryo transfer characteristics		
Single cleavage transfer	53/161 (32.9%)	56/161 (34.8%)
Double cleavage transfer	56/161 (34.8%)	55/161 (34.2%)
Single blastocyst transfer	29/161 (18.0%)	27/161 (16.8%)
Double blastocyst transfer	23/161 (14.3%)	23/161 (14.3%)

BMI, body mas index; FET, frozen embryo transfer; HRT, hormone replacement therapy; ICSI, intracyotplasmic sperm injection; NA: Not applicable.
No statistically significant differences were found.
[a]Values are represented by means (±standard deviation) or frequency (%).

Table 4 Frozen embryo transfer cycles: pregnancy outcome.[a]

	Cases	*Controls*
Number of embryos transferred	1.5 (±0.5)	1.5 (±0.5)
Implantation rate	31/240 (12.9%)	40/239 (16.7%)
Spontanous abortion rate	12/27 (44.4%)	9/37 (24.3%)
Pregnancy outcome		
Clinical pregnancy	27/161 (16.8%)	37/161 (23.0%)
Viable pregnancy	22/161 (13.7%)	31/161 (19.3%)
Live birth		
Total	15/161 (9.3%)	28/161 (17.4%)
Stimulation cycle < 3	13/90 (14.4%)	21/101 (20.8%)
Stimulation cycle ≥ 3	2/71 (2.8%)	7/60 (11.7%)
Adjuvants without luteal oestradiol	10/129 (7.8%)	24/129 (18.6%)[c,d]
Adjuvants with luteal oestradiol	5/32 (15.6%)	4/32 (12.5%)[c]
Multiple birth	6/161 (3.7%)	1/161 (0.6%)

[a]Values are represented by means (±standard deviation) or frequency (%).
[b]P =0.03.
[c]Controls were matched to cases with the adjuvant protocols specified but not exposed to adjuvants.
[d]P = 0.01.

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ovarian response, fertilization rates, implantation rate, viable pregnancy rate, spontaneous abortion rate or live birth rate in women undergoing a fresh IVF cycle. Women in the combined adjuvant group had a smaller total number of good quality embryos (the sum of embryos transferred and embryos frozen), which may have a neatigely affect the cumulative live birth rate.

Of particular concern was the finding that women undergoing a FET cycle with combined adjuvant therapy had a lower live birth rate.

Our conclusion that there is no clinical benefit and possible harm in using a combination of aspirin, antibiotics and prednisolone, either with or without luteal oestradiol, is broadly in keeping with other studies that have investigated these adjuvantia individually.

Aspirin has long been proposed as an adjuvant in poor prognosis patients, including those with implantation failure, because it inhibits platelet aggregation, promotes vasodilatation and increases uterine blood perfusion (Rubinstein et al., 1999). Several studies have suggested that impaired uterine blood flow may decrease uterine receptivity (Chien et al., 2002; Ferreira et al., 2007; Habara et al., 2002; Nakatsuka et al., 2003). In addition, the presence of anti-phospholipid antibodies and thrombophilias has been claimed to reduce the likelihood of implantation, although evidence to support this is conflicting (Penzias, 2012). Five meta-analyses have been published on the subject thus far, with most showing no beneficial effect of aspirin. Three of these meta-analyses (Gelbaya et al., 2007; Khairy et al., 2007; Poustie et al., 2007) reported no significant effect of aspirin. The fourth conventional meta-analysis (Ruopp et al., 2008) suggested a statistically significant effect of aspirin with an OR of 1.2 (95% CI 1.1 to 1.4). An individual patient data meta-analysis of six studies was carried out by Groeneveld et al. (2011) showing an odds ratio of 0.86 (95% CI 0.69 to 1.1).

Recurrent implantation failure has also been linked to abnormal fetal–maternal tolerance. In this context, glucocorticoids with their potent anti-inflammatory and immunosuppressive properties have been proposed as another class of potentially useful adjuvants. Several studies have investigated the effects of prednisolone in assisted reproduction techniques. A meta-analysis by Boomsma et al. (2012) found no evidence that glucocorticoids improved clinical pregnancy rates (13 RCTs; OR 1.16, 95% CI 0.94 to 1.44) in assisted reproduction technique cycles. Nevertheless, a subgroup analysis of 650 women which excluded cryopreservation cycles revealed a significantly higher pregnancy rate (OR 1.50, 95% CI 1.05 to 2.13). Furthermore, the authors asserted that the available evidence does not permit any conclusions to be drawn regarding the benefit of glucocorticoids in women with unexplained infertility, endometriosis or recurrent implantation failure. Furthermore, several studies have shown promising results in women with auto-antibodies (Ando et al., 1996; Geva et al., 2000; Kim et al., 1997), although Shohayeb and Demerdash (2005) found no significant differences in pregnancy rates in a randomized controlled trial using glucocorticoids in women with anti-thyroid antibodies. Two randomized controlled trials included in the meta-analysis considered pregnancy outcomes after co-treatment after prednisolone and low dose aspirin (Duvan et al., 2006; Ubaldi et al., 2002). Both failed to show a significant increase in either implantation or pregnancy rates in unselected women. The combination of low dose aspirin and prednisolone has also been associated with a significantly increased risk of hypertension, diabetes mellitus and premature birth (Laskin et al., 1997).

The role of prophylactic antibiotics in IVF has also been investigated following the study by Wittemer et al. (2004) who screened 951 couples before IVF and found that positive vaginal and semen cultures were associated with reduced clinical pregnancy rates and increased spontaneous abortion rates. In another study, microbial cultures of the catheter tip after transfer showed that the heaviest contamination was associated with the lowest clinical pregnancy rate (Brook et al., 2006). Despite these findings, two randomized contolled trials have so far failed to find a benefit for antibiotic co-treatment in IVF cycles (Brook et al., 2006; Peikrishvili et al., 2004).

Abnormalities in luteal phase sex steroid serum concentrations and asynchronous endometrial histology have been detected in cycles stimulated with exogenous gonadotropins (Evans et al., 2012). It has, therefore, been proposed that the addition of oestrogens in the luteal phase may correct this asynchronicity. Two systematic reviews and meta-analyses by Jee et al. (2010) and by Gelbaya et al. (2008) failed to show any improvement in pregnancy outcomes with luteal phase oestrogen supplementation. Nevertheless, both reviews again conclude that the available data are limited, and heterogeneous and definite conclusions will have to await further large-scale studies. We undertook a sub-analysis stratified by the administration of luteal oestradiol in the form of transdermal patches. No differences were observed in the fresh cycles, whereas there appeared to be a lower LBR when cases did not receive luteal oestradiol in a FET cycle. This effect of luteal oestradiol was not observed in fresh cycles and it only reversed the negative effect of combined adjuvant therapy on FET cycles with no net gain. Although it may be tempting to speculate further on this finding, the number of available matched pairs in the stratified FET analysis is too small to draw meaningful conclusions.

Overall, the individual use of these adjuvantia does not seem to be supported by the available evidence. In line with this, the main finding of our study was that the live birth rate is indeed unchanged with combined adjuvant therapy in fresh cycles. Live birth rates, however, were significantly lower in patients using the combined adjuvant therapy in a FET cycle, indicating that such treatment has the potential to harm. Nevertheless, it has previously been proposed that these adjuvantia may deliver greater benefit in defined subgroups, such as women with repeat IVF failures (Geva et al., 2000). An a-priori planned sub-analysis comparing women who had undergone less than three compared with those who have undergone three or more cycles showed no difference in fresh or frozen cycles. This argues against the notion that adjuvant strategies are more effective in women with repeat IVF failures.

While not a randomized trial, this observational study has a number of significant strengths, including its large size, the careful and extensive matching of suitable controls using nine important confounders to minimize selection bias and the fact that multiple outcomes were assessed, including live birth rate. Nevertheless, the study has a number of limitations. Although the risk of recall and observer bias was limited, selection bias cannot be completely controlled with this study design even with the extensive matching that took place. In addition, a small number of cases could not be included because the BMI could not be retrieved from the clinical notes,

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and a larger number of cases were not included because no appropriate matches were found. It is unlikely, however, that the non-inclusion of these unmatched cases would have improved the outcomes. In the FET group, the non-matched cases had lower clinical and viable pregnancy rates than the matched cases. If anything, the exclusion of these unmatched cases in our analysis has provided an overly optimistic estimate of the treatment effect in the adjuvant group. In the FET group, no obvious differences were found in the patient characteristics or the outcomes, and it is unlikely that the reported outcomes would have significantly improved by the inclusion of the unmatched cases. The effect of the smaller number of excess frozen embryos in the combined adjuvant group has yet to be explored, and future investigation into the cumulative pregnancy rate may yet provide further insight in to the clinical efficacy of the combined treatment regimen.

Cancelled cycles were also not considered for inclusion, as we were concerned with the effect of the luteal phase adjuvants on endometrial receptivity. It was, therefore, appropriate to limit the criteria to cycles that involved an embryo transfer. The cycle cancellation rate, however, was similar between the groups for both fresh and FET cycles.

In conclusion, the treatment of patients with repeated implantation failure in IVF clearly remains a challenge, and there are many drivers that may tempt clinicians to offer unproven or poorly documented treatment options (Barnhart, 2014). The present study adds further to published research that adjuvantia, either used alone or in combination, are unlikely to improve live birth rates. Of even greater concern is that the adjuvant combinations used in this study may be detrimental in IVF cycles using frozen embryos, although prospective randomized controlled trials are required to confirm these observations.

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