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The use of androgens or androgen-modulating agents in poor responders undergoing *in vitro* fertilization: a systematic review and meta-analysis

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BACKGROUND: The aim of this meta-analysis was to evaluate the role of androgens or androgen-modulating agents on the probability of pregnancy achievement in poor responders undergoing IVF.

METHODS: Medline, EMBASE, CENTRAL, Scopus and Web of Science databases were searched for the identification of randomized controlled trials evaluating the administration of testosterone, dehydroepiandrosterone (DHEA), aromatase inhibitors, recombinant luteinizing hormone (rLH) and recombinant human chorionic gonadotrophin (rhCG) before or during ovarian stimulation of poor responders.

RESULTS: In two trials involving 163 patients, pretreatment with transdermal testosterone was associated with an increase in clinical pregnancy [risk difference (RD): +15%, 95% confidence interval (CI): +3 to +26%] and live birth rates (RD: +11%, 95% CI: +0.3 to +22%) in poor responders undergoing ovarian stimulation for IVF. No significant differences in clinical pregnancy and live birth rates were observed between patients who received DHEA and those who did not. Similarly, (i) the use of aromatase inhibitors, (ii) addition of rLH and (iii) addition of rhCG in poor responders stimulated with rFSH for IVF were not associated with increased clinical pregnancy rates. In the only eligible

study that provided data, live birth rate was increased in patients who received rLH when compared with those who did not (RD: +19%, 95% CI:+1 to +36%).

CONCLUSIONS: Based on the limited available evidence, transdermal testosterone pretreatment seems to increase clinical pregnancy and live birth rates in poor responders undergoing ovarian stimulation for IVF. There is insufficient data to support a beneficial role of rLH, hCG, DHEA or letrozole administration in the probability of pregnancy in poor responders undergoing ovarian stimulation for IVF.

Key words: poor response / androgens / aromatase inhibitors / recombinant luteinizing hormone / recombinant human chorionic gonadotrophin

Introduction

Despite the advancement in assisted reproduction technologies, poor ovarian response is still considered to be one of the most challenging tasks in reproductive medicine (Tarlatzis *et al.*, 2003; Kyrou *et al.*, 2009). Although a universal definition of poor ovarian response has not been utilized in the relevant studies that are currently available in the literature (Surrey and Schoolcraft, 2000; Kyrou *et al.*, 2009), poor ovarian response is considered to be an inadequate response to ovarian stimulation, defined usually by a low number of oocytes retrieved or a low number of developing follicles and low estradiol concentration in a previous or in the running, respectively, IVF cycle (Tarlatzis *et al.*, 2003).

Poor responders represent a significant proportion among women undergoing ovarian stimulation for IVF, ranging from 9 to 24% (Ben-Rafael *et al.*, 1991; Jenkins *et al.*, 1991; Surrey and Schoolcraft, 2000). Given the severely diminished probability of pregnancy after IVF in these patients, various interventions have been proposed, without, however, the identification of an indisputably efficacious treatment (Kyrou *et al.*, 2009). Among these interventions, data suggest that growth hormone addition (Kolibianakis *et al.*, 2009), as well as embryo transfer (ET) performance on Day 2 instead of Day 3, might be beneficial, whereas no benefit has been confirmed regarding other proposed treatments (Kyrou *et al.*, 2009). Nevertheless, in most of these cases data are quite limited and thus, a potential beneficial effect cannot be excluded (Kyrou *et al.*, 2009; Venetis *et al.*, 2010).

It has been suggested that the accumulation of androgens in the micro milieu of the primate ovary, plays a critical role in early follicular development and granulosa cell proliferation (Weil *et al.*, 1998). Androgen excess has been shown to stimulate early stages of follicular growth (Vendola *et al.*, 1998, 1999; Weil *et al.*, 1998) and increase the number of preantral and antral follicles (Hillier *et al.*, 1997; Weil *et al.*, 1998, 1999). In addition, increased intraovarian concentration of androgens seems to augment follicle stimulating hormone (FSH) receptor expression in granulosa cells (Weil *et al.*, 1998, 1999) and thus, potentially lead to enhanced responsiveness of ovaries to FSH (Hillier and De Zwart, 1981; Harlow *et al.*, 1986; Vendola *et al.*, 1998; Weil *et al.*, 1998). Besides these experimental data, further clinical observations on women with polycystic ovary syndrome or testosterone-treated female transsexuals, suggest that exposure to exogenous androgens may lead to increased number of developing follicles, regardless of gonadotrophin stimulation (Futterweit and Deligdisch, 1986; Spinder *et al.*, 1989; Hugues and Durnerin, 2005). Furthermore, it has been reported that inadequate levels of endogenous androgens are associated with decreased ovarian sensitivity to FSH and low pregnancy rates after IVF (Frattarelli and Peterson, 2004).

On the basis of these data, it has been hypothesized that increasing androgen concentration in the ovarian micro milieu in poorly responding patients might lead to an increase in the number and the maturity of oocytes after ovarian stimulation for IVF. Hence, the use of various androgens and/or androgen-modulating agents has been proposed. These interventions include: (i) pretreatment with transdermal testosterone (Balasch *et al.*, 2006), (ii) pretreatment with dehydroepiandrosterone (DHEA) (Casson *et al.*, 2000), (iii) addition of aromatase inhibitors (Mitwally and Casper, 2001, 2002), (iv) addition of recombinant luteinizing hormone (rLH) (Ferraretti *et al.*, 2004) and (v) addition of human chorionic gonadotrophin (hCG) during ovarian stimulation (Ferrari *et al.*, 2002; Berkkanoglu *et al.*, 2007).

Transdermal testosterone or DHEA pretreatment

Pretreatment with transdermal testosterone or DHEA has been suggested as a safe and effective way of increasing the intraovarian androgen concentration (Casson *et al.*, 2000; Balasch *et al.*, 2006). Recently published, randomized controlled trials (RCTs) have evaluated transdermal testosterone (Massin *et al.*, 2006; Kim *et al.*, 2011) or DHEA pretreatment (Wiser *et al.*, 2010) in poor responders undergoing ovarian stimulation for IVF, with inconclusive results.

Addition of aromatase inhibitors

Similarly, it has been advocated that the use of aromatase inhibitors could be also beneficial. This is based on the fact that aromatase activity is the key component for the production of estrogens from androgens in granulosa cells. Thus, inhibition of aromatase activity could increase the intraovarian concentration of androgens by blocking their aromatization to estrogens (Mitwally and Casper, 2002). The RCTs that have recently been conducted, evaluating aromatase inhibitors addition during ovarian stimulation are also inconclusive (Kashyap *et al.*, 2005; Ozmen *et al.*, 2009).

Pretreatment or addition of rLH during ovarian stimulation with gonadotrophins

According to the 'two cell–two gonadotrophin' theory, follicular steroidogenesis would not take place without the presence of luteinizing hormone (LH), since LH stimulates the production of androgens from theca cells (Hillier *et al.*, 1994). Thus, luteinizing hormone activity is fundamental in maintaining adequate concentrations of intraovarian androgens and promoting adequate steroidogenesis and follicular growth. At the same time, it has been suggested that using GnRH analogues for pituitary suppression during ovarian stimulation may eventually lead to extremely low levels of endogenous LH and compromise

the probability of pregnancy (Westergaard *et al.*, 2000; Humaidan *et al.*, 2002). Therefore, it has been supported that pretreatment with rLH (Durnerin *et al.*, 2008) or addition of rLH (Humaidan *et al.*, 2004) during ovarian stimulation with recombinant gonadotrophins could optimize ovarian stimulation and thus, might be beneficial for poor responders (Mochtar *et al.*, 2007). Although many investigators have attempted to evaluate the effectiveness of rLH addition in this category of patients in the context RCTs (Ferraretti *et al.*, 2004; Berkkanoglu *et al.*, 2007; Barrenetxea *et al.*, 2008), it still remains a considerable controversy.

Addition of hCG

It has already been demonstrated that LH activity provided by hCG addition in the late stages of ovarian stimulation is able to promote and complete the growth of large follicles (Filicori *et al.*, 2002, 2005). Hence, similarly to rLH, pretreatment with or addition of hCG during ovarian stimulation might be a promising alternative approach for poor responders undergoing IVF.

In view of the conflicting or inconclusive data regarding the efficacy of the proposed interventions, the aim of this systematic review and meta-analysis is to evaluate the administration of androgens and androgen-modulating agents on the probability of pregnancy achievement in poor responders undergoing ovarian stimulation with gonadotrophin releasing hormone (GnRH) analogues and gonadotrophins for IVF.

Methods

Search strategy

A computerized literature search in MEDLINE, EMBASE, CENTRAL, ISI Web of Science and SCOPUS covering the period until April 2011 was performed independently by two reviewers (C.A.V. and J.K.B) aiming to identify RCTs that evaluated the following research question: does administration of testosterone or DHEA or aromatase inhibitors or recombinant LH or hCG increase the probability of pregnancy in poor responders undergoing ovarian stimulation with GnRH analogues and gonadotrophins for IVF? For this purpose, a search strategy with keywords aiming to identify three different terms in each case was used. These terms included 'Intervention', 'Population' and 'Setting'. Various synonyms describing each term were entered as free-text terms in the electronic databases in an attempt to maximize the sensitivity of the search strategy (Table I). Additionally, the citation lists of all relevant publications and review articles were hand-searched. Meeting proceedings of the European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine were also hand-searched for the identification of relevant studies. No language limitations were applied.

Selection of studies

Criteria for inclusion/exclusion of studies were established prior to the literature search. Studies had to fulfill the following criteria for eligibility: (i) administration of androgens or androgen modulators in the intervention group; (ii) inclusion of women being subjected to IVF using gonadotrophins and GnRH analogues for ovarian stimulation; (iii) inclusion of women characterized as poor responders and (iv) a parallel comparative design using random allocation of patients in the groups to be compared should have been employed. Studies that included asymmetric interventions (co-interventions), besides the one evaluated in each study (e.g. addition of rLH), were excluded. All parallel RCTs evaluating the relevant interventions were included in the current systematic review and meta-analysis, irrespective of the definition of poor ovarian response, the dose and protocol of intervention proposed, the type of gonadotrophin injected, or the type and protocol of GnRH analogue used. Selection of the studies was performed independently by two of the reviewers (C.A.V. and J.K.B). Any disagreement was resolved by discussion.

Data extraction

Data extraction was performed independently by two of the reviewers (C.A.V. and J.K.B). The following data were recorded from each of the eligible studies: demographic (citation data, country, study period, number of patients included), methodological (method of randomization,

Table I Search strategy used for the identification of eligible studies (these terms were search as 'Free-text' terms).

Interventions	Terms				
	Intervention	AND	Population	AND	Setting
Testosterone	(testosteron*)		[(poor) OR (low) OR (slow) OR (inadequate) OR (suboptimal) OR (decreas*) OR (diminish*)] AND (respon*)		(*in-vitro* fertilization) OR (*in vitro* fertilization) OR (IVF) OR (ICSI) OR (intra-cytoplasmic sperm injection) OR (ICSI)
DHEA	(Dehydroepiandrosteron*) OR (DHEA)				
Aromatase inhibitors	(Aromatase inhib*) OR (letrozol*) OR (anastrazol*)				
Recombinant LH	(recombinant luteinizing hormone) OR (recombinant LH) OR (rLH) OR (r-LH) OR (recLH) OR (rec LH) OR (rec-LH) OR (luveris) OR (lutropin alfa)				
Recombinant hCG	(human chorionic gonadotrop*) OR (hCG) OR (recombinant chorionic gonadotrop*) OR (recombinant hCG) OR (rhCG) OR (r-hCG) OR (rec-hCG) OR (rec hCG) OR (Ovitrel*) OR (Ovidrel*)				

*, ?: Wildcards have been used where available to increase the sensitivity of the search. The asterisk (*) stands for none or any number of characters. The question mark (?) stands for any single character.

allocation concealment), procedural (whether financial support was declared, type of GnRH analogue and protocol used for LH surge inhibition, dose and protocol of the intervention proposed, type and starting dose of gonadotrophin administered for ovarian stimulation, type and dose of medication used for triggering final oocyte maturation, criteria used for triggering final oocyte maturation, type of fertilization, day of ET, type of luteal support, adverse events associated with the type of intervention). Any disagreement between the two reviewers responsible for data extraction was resolved by discussion.

Outcomes

The main outcome measure chosen for the current meta-analysis was achievement of pregnancy per patient randomized, expressed as clinical pregnancy (evidence of intrauterine sac with fetal heart activity at 6–8 weeks of gestation) or as live birth. Secondary outcome measures included duration of gonadotrophin stimulation, total dose of gonadotrophins required for ovarian stimulation, number of cumulus–oocyte complexes (COCs) retrieved, number of embryos transferred, number of metaphase II (MII) oocytes, number of patients with retrieved oocytes, number of patients having an ET, fertilization rate, estradiol levels on the day of hCG, endometrial thickness on the day of hCG, number of follicles $\geq$17 mm on the day of hCG, global cancellation rate and cancellation rate due to poor response. In case of missing information, the study authors were contacted in order to retrieve relevant data where available.

Quantitative data synthesis

The dichotomous data results for each of the eligible for meta-analysis studies were expressed as risk difference (RD) with 95% confidence intervals (CIs). These results were combined for meta-analysis using the Mantel/Haenszel model (Mantel and Haenszel, 1959), when using the fixed effects method, and the DerSimonian and Laird (1986), when using the random effects method. When the outcome of interest was of a continuous nature, the differences were pooled across the studies, which provided information on this outcome, resulting in a weighted mean difference (WMD) with 95% CI. The inverse variance method (Hedges and Olkin, 1985) and the DerSimonian and Laird method (DerSimonian and Laird, 1986) were used when the fixed or random effects method were applied, respectively. All results were combined for meta-analysis with Revman Software (Version 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Study-to-study variation was assessed by using the χ^2 statistic (the hypothesis tested was that the studies are all drawn from the same population, i.e. from a population with the same effect size). A fixed effects model was used where no statistically significant heterogeneity was present, whereas in the presence of statistically significant heterogeneity, a random effects model was applied. Statistical significance was set at a *P*-level of 0.05. The presence of publication bias was tested by using the Harbord–Egger's test (Harbord *et al.*, 2006).

Subgroup analyses according to the protocol of the proposed intervention (onset of administration, dose, etc.) were a priori planned to be performed, where necessary.

Results

Systematic review

The literature search yielded 2126 publications in total. The titles of these manuscripts were screened, resulting in 106 studies considered potentially eligible to be included in the review. Of the total of 106 relevant manuscripts identified, 76 studies were excluded after the examination of the abstracts and 30 studies were further evaluated by retrieving the full text. Where necessary, the authors were contacted in an attempt to clarify methodological aspects of the study. Finally, after the exclusion of 17 studies (Supplementary data, Table SI), 13 studies were included in the present systematic review and meta-analysis [the study by Berkkanoglu *et al.* (2007) contributed two comparisons (rLH versus control and rhCG versus control) in the final data set and for this reason is counted as two studies] (Ferraretti *et al.*, 2004; Demirol *et al.*, 2005; Kashyap *et al.*, 2005; Fernández Ramírez *et al.*, 2006; Massin *et al.*, 2006; Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008; Ozmen *et al.*, 2009; Wiser *et al.*, 2010; Kim *et al.*, 2011). A detailed flow chart of this process is presented in Fig. 1. Characteristics of the studies included in the systematic review are presented in Tables II–IV.

The eligible studies were published between 2004 and 2011 and the number of patients included in the study ranged from 33 to 136 patients (median 84). All studies were randomized controlled parallel studies. In the study by Wiser *et al.*, patients who did not achieve a pregnancy in their first cycle, were subsequently submitted to a second cycle with increased duration of DHEA pretreatment (Wiser *et al.*, 2010). From this study, only the first cycle was included in the present analysis, so that the assumption of independent observations would not be violated. As stated earlier, the study of Berkkanoglu *et al.* (2007) was a three-armed RCT comparing the addition of rLH or rhCG with a control group. In this case, the comparison between the rLH and the control group was extracted for the calculation of the pooled effect in rLH addition and the data from the comparison of the rhCG group with the control group were used for the rhCG addition meta-analysis.

Randomization method and allocation concealment were reported in 7 (Ferraretti *et al.*, 2004; Kashyap *et al.*, 2005; Massin *et al.*, 2006; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008; Wiser *et al.*, 2010; Kim *et al.*, 2011) and 5 (Fernández Ramírez *et al.*, 2006; Barrenetxea *et al.*, 2008; Ozmen *et al.*, 2009; Wiser *et al.*, 2010; Kim *et al.*, 2011) out of the 12 individual studies, respectively. Definition of poor ovarian response, as well as the primary outcome varied among studies (Table II). Power analysis was performed in 4 (Kashyap *et al.*, 2005; Massin *et al.*, 2006; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008) studies and financial support was also declared in 4 (Fernández Ramírez *et al.*, 2006; Massin *et al.*, 2006; Ruvolo *et al.*, 2007; Wiser *et al.*, 2010) out of the 12 individual studies (Table II). To inhibit premature LH surge, GnRH agonists were used in six studies (Ferraretti *et al.*, 2004; Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008; Wiser *et al.*, 2010), whereas in five studies a GnRH antagonist protocol was applied (Demirol *et al.*, 2005; Kashyap *et al.*, 2005; Fernández Ramírez *et al.*, 2006; Ozmen *et al.*, 2009; Kim *et al.*, 2011). In the study by Massin *et al.* (2006) both GnRH agonists, as well as GnRH antagonists were used (Table III), although the proportions of the different GnRH analogue protocols were not statistically different between the two groups compared.

Apart from the evaluated intervention in each study (i.e. transdermal testosterone, DHEA, aromatase inhibitors, rLH or rhCG), ovarian stimulation was performed with the use of recombinant FSH in most of the studies, except for the one study that tested

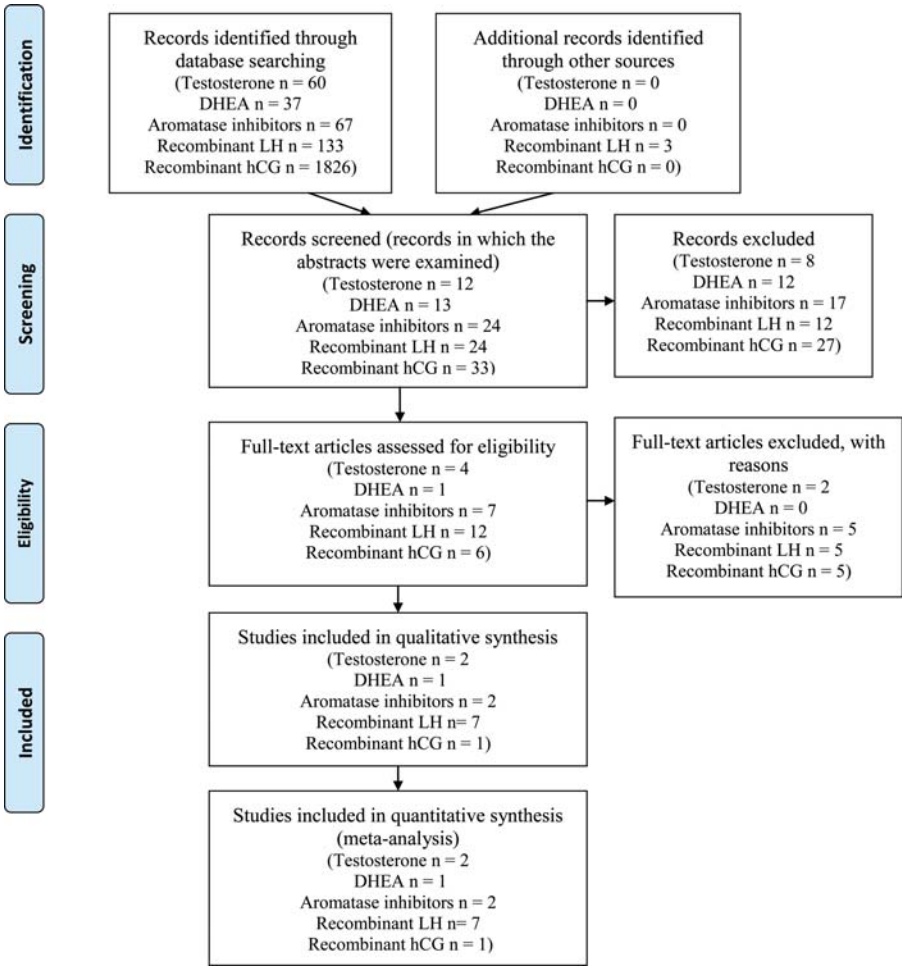

Figure 1 PRISMA flow diagram detailing selection of studies for inclusion.

the addition of DHEA in which a combination of recombinant FSH and recombinant LH was used for ovarian stimulation (Wiser *et al.*, 2010). Gonadotrophin adjustments were reported in eight studies (Ferraretti *et al.*, 2004; Demirol *et al.*, 2005; Fernández Ramírez *et al.*, 2006; Massin *et al.*, 2006; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008; Ozmen *et al.*, 2009; Kim *et al.*, 2011). HCG was used to trigger final oocyte maturation in all studies, except for three in which this information was not reported (Kashyap *et al.*, 2005; Polidoropoulos *et al.*, 2007; Ozmen *et al.*, 2009), while the criteria for hCG administration varied across studies (Table III). Oocyte retrieval was performed 35–36 h after hCG administration in 10 studies (Ferraretti *et al.*, 2004; Demirol *et al.*, 2005; Fernández Ramírez *et al.*, 2006; Massin *et al.*, 2006; Berkkanoglu *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008; Ozmen *et al.*, 2009; Wiser *et al.*, 2010; Kim *et al.*, 2011), whereas in the two remaining studies (Kashyap *et al.*, 2005; Polidoropoulos *et al.*, 2007), the timing of oocyte retrieval was not reported. Fertilization methods included IVF ($n = 2$) (Ferraretti *et al.*, 2004; Wiser *et al.*, 2010), ICSI ($n = 6$) (Demirol *et al.*, 2005; Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008; Ozmen *et al.*, 2009), IVF/ICSI ($n = 3$) (Fernández Ramírez *et al.*, 2006; Massin *et al.*, 2006; Kim *et al.*, 2011), whereas in one study the information was not available (Kashyap *et al.*, 2005). ETs were performed on Day 2 or 3 after oocyte retrieval, while luteal support varied among studies (Table III). Three studies did not provide details about the type of luteal support used, as only the abstract was available and, although pursued, no contact was established with the authors (Demirol *et al.*, 2005; Kashyap *et al.*, 2005; Polidoropoulos *et al.*, 2007).

Interventions

Pretreatment with transdermal testosterone

Regarding the type of intervention performed, pretreatment with transdermal testosterone gel was applied in two studies (Massin *et al.*, 2006; Kim *et al.*, 2011), in a dose of 10 and 12.5 mg/day, respectively, starting from 15 to 21 days in the period preceding ovarian stimulation (Table IV).

Pretreatment with DHEA

DHEA was administered in one study, in a dose of 75 mg/day, 6 weeks before ovarian stimulation (Wiser *et al.*, 2010; Table IV).

Table II Methodological characteristics of eligible RCTs

Study, country of origin, journal or meeting	Study period	Number of patients	Definition of poor responders	Type of study	Randomization method	Allocation concealment	Blinding	Power analysis	Primary outcome assessed	Financial support	Authors contacted
Testosterone											
Massin *et al.* (2006), France, Hum Reprod	October 2001–December 2003	53 (T:27, C:26)	A poor response to ovarian stimulation in a previous IVF/ICSI attempt defined as plasma estradiol E2 < 1200 pg/ml at day of hCG and ≤5; COCs retrieved and day 3 FSH > 12 IU/l, E2 > 70 pg/ml and inhibin B < 45 pg/ml	Randomized placebo controlled trial	Blocked randomization with fixed blocks of four	Not reported	Double-blind	Yes	COCs retrieved	Yes, testosterone gel and placebo gel were provided by commercial company	Yes
Kim *et al.* (2011), South Korea, Fertil Steril	March 2005–January 2009	110 (T:55, C:55)	A poor response to ovarian stimulation in a previous IVF/ICSI attempt defined as ≤3; COCs retrieved despite the use of a high gonadotrophin dose (>2500 IU)	RCT	Computer-generated list	Sealed envelopes	No	Not reported	Mature oocytes retrieved	Not reported	Contact attempted but not established
DHEA											
Wiser *et al.* (2010), Israel, Hum Reprod	January 2008–July 2009	33 (T: 17, C: 16)	A poor response to ovarian stimulation in a previous IVF attempt defined as <5; COCs retrieved, poor quality embryos, or cycle cancellation due to poor response to ovarian stimulation whenever the gonadotrophin starting dose ≥ 300 IU/day	RCT	Computer-generated random numbers	Sealed envelopes, a sealed envelope was chosen by each patient	Open labeled	No	COCs retrieved	DHEA was provided at no cost by SuperPharm, Ltd	Contact attempted but not established

Aromatase inhibitors											
Kashyap *et al.* (2005), North America, J Soc Gynecol Investig	Not reported	55 (T: 29, C: 26)	Not reported	RCT	Computer generated, stratified randomization in blocks of 4–6	Yes, method not reported	Not reported	Yes	Pregnancy per cycle started	Not reported	Contact attempted but not established
Ozmen *et al.* (2009), Turkey, RBM Online	Not reported	70 (T: 35, C:35)	Previous ICSI cycle cancellation due to E2 < 130 pg/ml on day 6 or E2 < 450 pg/ml on hCG day and/or <4 oocytes retrieved	RCT	Not reported	Sealed envelopes	Not reported	Not reported	Not reported	Not reported	Contact attempted but not established
rLH											
Ferraretti *et al.* (2004), Italy, Fertil Steril	January 2002–April 2003	104 (T:54, C:50)	Patients with normal initial follicular recruitment presenting a plateau on follicular growth (no increase in the E2 level and in the follicular size) between Day 7 and Day 10 of stimulation	RCT	Alternate allocation	Not reported	Not reported	Not reported	Pregnancy rate per ET, implantation rate and live birth rate per started cycles	Not reported	Yes
Demirol *et al.* (2005), Turkey Hum Reprod (abstract)	Not reported	106 (T:53, C:53)	At least two failed cycles with (i) ≤3 oocytes retrieved or (ii) ≤3 follicles of 16 mm in diameter on hCG day or (iii) E2 < 500 pg/ml	RCT	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Contact attempted but not established
Fernández Ramírez *et al.* (2006), Spain, Rev Iberoamer Fertil	January–June 2006	34 (T:16, C:18)	≤3 follicles or E2 ≤ 600 pg/ml on the day of hCG or cycle cancellation, or ≤2 MII oocytes or basal FSH ≥ 8.5 mIU/ml or a previous cancelled cycle due to poor ovarian response	RCT	Not reported	Sealed envelopes	Not reported	Not reported	Not reported	Partly funded by Ensayo Clinico de Laboratorios Serono	Contact attempted but not established
Berkkanoglu *et al.* (2007), Turkey, Fertil Steril	Not reported	97 (T:46, C:51)	Patients with <12 AFC and having >3 growing follicles on the seventh day of stimulation	RCT (3 arms)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Contact attempted but not established

Continued

Table II *Continued*

Study, country of origin, journal or meeting	Study period	Number of patients	Definition of poor responders	Type of study	Randomization method	Allocation concealment	Blinding	Power analysis	Primary outcome assessed	Financial support	Authors contacted
Ruvolo *et al.* (2007), Italy, Fertil Steril	September 2004–February 2005	42 (T:24, C:18)	Patients with E2 < 180 pg/ml and ≥6 follicles with diameters 7–10 mm and no follicle with a diameter > 12 mm on Day 8 of stimulation	RCT	Computer-generated random number tables with blocks of three	Not reported	Double-blind	Yes	The apoptosis rate in cumulus cells	Supported in part by the Italian Ministero Instruzione Universita Ricerca (M.I.U.R)	Yes
Polidoropoulos *et al.* (2007), Greece, Hum Reprod (abstract)	Not reported	136 (T:68, C:68)	A previous attempt on a GnRH-a long protocol with <5 oocytes retrieved, age ≤ 37 years and basal FSH < 10 mIU/ml	RCT	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Contact attempted but not established
Barrenetxea *et al.* (2008), Spain, Fertil Steril	January–June 2005	84 (T:42, C:42)	Age ≥ 40 years and 3-day FSH ≥ 10 mIU/ml undergoing first IVF cycle and 2 ovaries	RCT	Computer-generated block randomization	Sealed envelopes	Double-blind	Yes	Ongoing pregnancy rate per cycle started and implantation rate per embryo transferred (the study was powered to detect a difference in clinical pregnancy rate)	No financial support was provided for the study	Yes
r-hCG											
Berkkanoglu *et al.* (2007), Turkey, Fertil Steril	Not reported	99 (T:48, C:51)	Patients with <12 AFC and having >3 growing follicles on the seventh day of stimulation	RCT (3 arms)	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Contact attempted but not established

T, treatment group; C, control group; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; E2, estradiol; hCG, human chorionic gonadotrophin; COC, cumulus–oocyte complex; FSH, follicle stimulating hormone; ET, embryo transfer; MII oocytes, metaphase II oocytes; AFC, antral follicle count; GnRH, gonadotrophin releasing hormone.

Table III Cycle characteristics of eligible RCTs

Study	GnRH-analogue	Type of analogue protocol	Gonadotrophin type/starting dose	Gonadotrophin adjustments	Signal for triggering final oocyte maturation	Criteria for hCG administration	OPU	Fertilization	ET	Type of luteal support
Testosterone										
Massin *et al.* (2006)	Triptorelin or Cetrorelix	Mainly long GnRH agonist but also short GnRH agonist and antagonist (the proportions of GnRH analogue protocols were not statistically different between the two arms)	rFSH/300–450 IU	Yes, no further information provided	10 000 IU u-hCG	At least three follicles of 17 mm in diameter	36 h after hCG	IVF/ICSI	Day 2/3	Micronized progesterone 200 mg/b.i.d. vaginally and 2500 IU hCG at 3, 6, 9 days after hCG for triggering final oocyte maturation
Kim *et al.* (2011)	Cetrorelix 0.25 mg/day	Flexible protocol (when the lead follicle reached 13–14 mm in diameter)	r-hFSH/300 IU	Yes, every 3–4 days according to ovarian response	250 μg r-hCG	At least one follicle of at least 18 mm in diameter	36 h after hCG	IVF/ICSI	Day 3	90 mg vaginal progesterone gel/day
DHEA										
Wiser *et al.* (2010)	Triptorelin acetate 0.1 mg/day	Long luteal	rFSH/ 450 IU + rLH/ 150 IU	Not reported	500 μg r-hCG	When the leading follicle(s) achieved a diameter of 18 mm	36 h after hCG	IVF	Day 2/3	Vaginal progesterone and single injection of 250 μg r-hCG 3 days after OPU
Aromatase inhibitors										
Kashyap *et al.* (2005)	Antagonist	Not reported	Not reported/≥450 IU	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Ozmen *et al.* (2009)	GnRH antagonist 0.25 μg/day	Flexible protocol (when the lead follicle was >13–14 mm in diameter and/or E2 >350 pg/ml)	rFSH/450 IU	Yes, on Day 5 or 7	10 000 IU hCG	Not reported	35–36 h after hCG	ICSI	Day 3	Micronized progesterone 200 mg/t.i.d vaginally

Continued

Table III *Continued*

Study	GnRH-analogue	Type of analogue protocol	Gonadotrophin type/starting dose	Gonadotrophin adjustments	Signal for triggering final oocyte maturation	Criteria for hCG administration	OPU	Fertilization	ET	Type of luteal support
rLH										
Ferraretti *et al.* (2004)	GnRH agonist	Long protocol	rFSH/150 IU in women <30 yrs, 225 IU in women 30–37 years, 300 IU in women ≥ 38 years	Yes, according to follicular growth	7500 IU hCG	At least two follicles of 18 mm in diameter	36 h after hCG	IVF	Day 3	50 mg progesterone oil/day
Demirol *et al.* (2005)	Cetrorelix 0.25 mg/day	Fixed day 6	rFSH/450 IU	Yes, step-down protocol according to individual response	hCG (dose not reported)	At least two follicles of ≥16 mm in diameter	36 h after hCG	ICSI	Day 3	Not reported
Fernández Ramírez *et al.* (2006)	Cetrorelix 0.25 mg/day	Flexible (as soon as the dominant follicle ≥ 14 mm	rFSH/300–450 IU	Yes, according to E2 and US	250 μg hCG	At least one follicle of >18 mm and 2 follicles of 16 mm in diameter and appropriate E2 concentrations	36 h after hCG	IVF/ICSI	Day 2	Micronized progesterone 400 mg/day vaginally
Berkkanoglu *et al.* (2007)	Leuprolide acetate 40 μg b.i.d.	Short agonist following 3 weeks of OCP use	rFSH/600 IU	None	10 000 IU hCG	At least two follicles of >17 mm in diameter	35 h after hCG	ICSI	Day 2	90 mg intravaginal progesterone gel b.i.d.
Ruvolo *et al.* (2007)	Buserelin 0.2 ml/day	Long mid- luteal	rFSH/225 IU	Yes from Day 6 according to E2 and US	10 000 IU hCG	At least three follicles of ≥18 mm in diameter	36 h after hCG	ICSI	Day 2	Prontogest 100 mg i.m. 3 times a week starting from Day 1 after pick-up for 2 weeks
Polidoropoulos *et al.* (2007)	GnRH agonist	Short protocol	rFSH/450 IU	Not reported	Not reported	Not reported	Not reported	ICSI	Day 2/3	Not reported
Barrenetxea *et al.* (2008)	Leuprolide acetate 0.5 mg	Flare-up protocol starting on Day 2	rFSH/375 IU	Yes	250 μg r-hCG	At least two follicles of ≥18 mm in diameter	36 h after hCG	ICSI	Day 3	Micronized progesterone 600 mg/day vaginally

r-hCG										
Berkkanoglu *et al.* (2007)	Leuprolide acetate 40 μg b.i.d.	Short agonist following 3 weeks of OCP use	rFSH/600 IU	None	10 000 IU hCG	At least two follicles of >17 mm in diameter	35 h after hCG	ICSI	Day 2	90 mg intravaginal progesterone gel b.i.d.

GnRH, gonadotrophin releasing hormone; hCG, human chorionic gonadotrophin; OPU, oocyte pick up; ET, embryo transfer; rFSH, recombinant follicle stimulating hormone; u-hCG, urinary human chorionic gonadotrophin; h, hours; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; b.i.d, twice daily; r-hFSH, recombinant human follicle stimulating hormone; r-hCG, recombinant human chorionic gonadotrophin; rLH, recombinant luteinizing hormone; E2, estradiol; t.i.d, three times a day; US, ultrasound; OCP, oral contraceptive pills.

Addition of aromatase inhibitors

In two studies, the aromatase inhibitor, letrozole, was proposed (Kashyap *et al.*, 2005; Ozmen *et al.*, 2009). One study was an abstract, reporting only minimal information regarding the intervention protocol (Kashyap *et al.*, 2005). Communication with the authors to retrieve missing data was attempted, yet no reply was received. In the remaining RCT (Ozmen *et al.*, 2009), 2.5 mg of letrozole was administered twice a day (2 × 2.5 mg) from Day 3 of ovarian stimulation for five consecutive days (Table IV).

Addition of recombinant LH

Addition of recombinant LH was evaluated in seven studies, in which the dose was 75 IU/day ($n = 2$) (Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007), 75–150 IU/day ($n = 3$) (Ferraretti *et al.*, 2004; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008) and 150 IU/day ($n = 2$) (Demirol *et al.*, 2005; Fernández Ramírez *et al.*, 2006). The timing of initiation of rLH addition varied among studies, whereas in all eligible studies rLH was administered until the hCG criteria were met (Table IV).

Addition of recombinant hCG

One study (Berkkanoglu *et al.*, 2007) evaluated the addition of 75 IU/day of recombinant hCG to rFSH from the 7th day until completion of ovarian stimulation (Table IV).

Meta-analysis

Transdermal testosterone pretreatment

Clinical pregnancy rate and live birth rate

Clinical pregnancy rate was significantly increased by 15% in patients that were pretreated with transdermal testosterone when compared with those who were not (RD: +15%, 95% CI: +3 to +26%; fixed effects model; heterogeneity: $P = 0.60$; Fig. 2a). Similarly, live birth rate was also increased by 11% in patients who were pretreated with transdermal testosterone (RD: +11%, 95% CI: +0.3 to +22%; fixed effects model; heterogeneity: $P = 0.20$; Fig. 2b). Two small studies (Massin *et al.*, 2006; Kim *et al.*, 2011), evaluating the use of transdermal testosterone pretreatment in 163 patients in total, offered data for these outcomes. The Harbord–Egger's test for the assessment of publication bias could not be performed due to the limited number of studies ($n = 2$).

Total dose of gonadotrophins required for ovarian stimulation

The total dose of gonadotrophins required for ovarian stimulation was significantly decreased in patients who were pretreated with transdermal testosterone when compared with those who were not (WMD: −446.2 IUs, 95% CI: −600.9 to −291.5; fixed effects model; heterogeneity: $P = 0.91$).

Duration of ovarian stimulation

Significantly fewer days were required to complete ovarian stimulation in the transdermal testosterone group when compared with the control group (WMD: −0.80 days, 95% CI: −1.26 to −0.33; fixed effects model; heterogeneity: $P = 0.34$).

Table IV Protocol characteristics of the intervention proposed in the eligible studies.

Study	Type of intervention proposed				
	Type/route	Dose	Timing of initiation	Duration of administration	Adverse effects
Testosterone					
Massin et al. (2006)	Testosterone gel 1%/transdermal	10 mg/day (applied on the external side of the thigh)	15–20 days in the period preceding ovarian stimulation	15–20 days in the period preceding ovarian stimulation	None
Kim et al. (2011)	Testosterone gel 1%/transdermal	12.5 mg/day (applied over both upper arms in the morning)	Sixth day of Estrogen-Progesterone pretreatment[a]	21 days (ovarian stimulation commenced the day after the last testosterone gel application)	None
DHEA					
Wiser et al. (2010)	DHEA/orally	75 mg/day	Six weeks before ovarian stimulation	6 weeks	None
Aromatase inhibitors					
Kashyap et al. (2005)	Letrozole	Not reported	Not reported	Not reported	Not reported
Ozmen et al. (2009)	Letrozole/orally	2 × 2.5 mg/day	Day 3	5 days	Not reported
rLH					
Ferraretti et al. (2004)	rLH	75–150 IU/day	Between day 7 and 10 depending on when the ovarian response showed the plateau	Until hCG criteria were met	Not reported
Demirol et al. (2005)	rLH	150 IU/day	Day 1	Until hCG criteria were met	Not reported
Fernández Ramírez et al. (2006)	rLH	75 IU/b.i.d (150 IU/day)	On the day of GnRH antagonist initiation	Until hCG criteria were met	Not reported
Berkkanoglu et al. (2007)	rLH	75 IU/day	7th day of ovarian stimulation	Until hCG criteria were met	Not reported
Ruvolo et al. (2007)	rLH	75–150 IU/day	8th day of ovarian stimulation	Until hCG criteria were met	Not reported
Polidoropoulos et al. (2007)	rLH	75 IU/day	Not reported	Until hCG criteria were met	Not reported
Barrenetxea et al. (2008)	rLH	150 IU (day 7–10) and 75 IU (day 11–14)	7th day of ovarian stimulation	Until hCG criteria were met	Not reported
r-hCG					
Berkkanoglu et al. (2007)	hCG	75 IU/day	7th day of ovarian stimulation	Until hCG criteria were met	Not reported

DHEA, dehydroepiandrosterone; rLH, recombinant luteinizing hormone; hCG, human chorionic gonadotrophin; b.i.d, twice daily; GnRH, gonadotrophin releasing hormone.
[a]All of the patients received estrogen- progesterone pretreatment using E2 valerate 1 mg/day and norethridone 5 mg/day for 21 days in the cycle preceding COS.

COCs retrieved

The number of COCs retrieved was significantly increased in the patients who were pretreated with transdermal testosterone when compared with the patients who were not (WMD: +1.5 COCs, 95% CI: +0.9 to +2.1; fixed effects model; heterogeneity: $P = 0.22$).

Data on remaining secondary outcomes regarding transdermal testosterone pretreatment are presented in Table V.

DHEA administration before ovarian stimulation

One small study (Wiser *et al.*, 2010), evaluating the addition of DHEA in 33 patients in total, offered data for the following outcomes.

Clinical pregnancy rate and live birth rate

Clinical pregnancy rates (RD: +11%, 95% CI: −15 to +37%; fixed effects model; heterogeneity: not applicable), and live birth rates (RD: +11%, 95% CI: −10 to +33%; fixed effects model; heterogeneity: not applicable) were not significantly different between patients treated with DHEA before ovarian stimulation and the control group. The Harbord–Egger's test for the assessment of publication bias could not be performed because only one study was available.

COCs retrieved

The number of COCs retrieved was not significantly different in the patients who received DHEA when compared with the patients

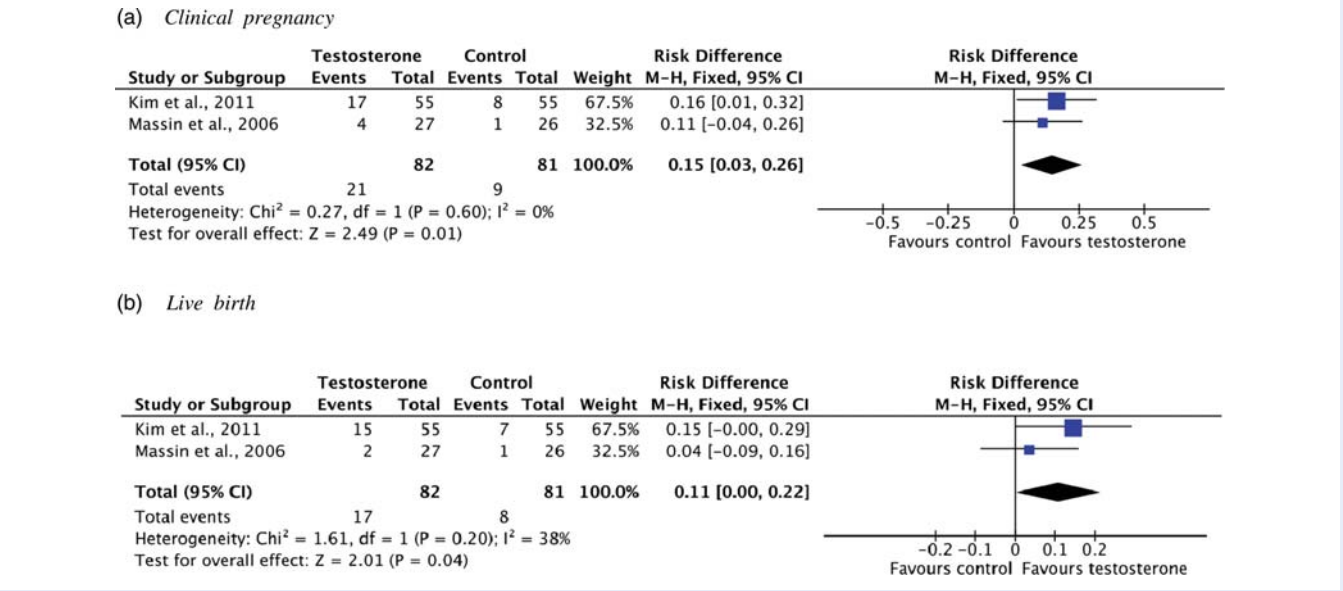

Figure 2 Risk difference for clinical pregnancy (**a**) and live birth (**b**) in patients treated with testosterone or not.

who did not (WMD: −1.0, 95% CI: −2.23 to +0.23; fixed effects model; heterogeneity: not applicable).

Data on remaining secondary outcomes regarding DHEA pretreatment in poor responders undergoing IVF are presented in Table V.

Aromatase inhibitors

Clinical pregnancy rate and live birth rate

Clinical pregnancy rates were not significantly different between patients who received aromatase inhibitor and those who did not (RD: +8%, 95% CI: −4.0 to +19.0%; fixed effects model; heterogeneity: $P = 0.84$; Fig. 3). Two studies (Kashyap *et al.*, 2005; Ozmen *et al.*, 2009) offered data for this outcome. Data on live birth rates were not available in any of the eligible studies. The Harbord–Egger's test for the assessment of publication bias could not be performed due to the limited number of studies ($n = 2$).

Total dose of gonadotrophins required for ovarian stimulation

The addition of aromatase inhibitors during ovarian stimulation of poor responders undergoing IVF was associated with a significant decrease in gonadotrophin consumption (WMD: −870 IUs, 95% CI: −1110.19 to −629.81; fixed effects model; heterogeneity: not applicable). Only one study ($n = 70$) offered data for this outcome (Ozmen *et al.*, 2009).

COCs retrieved

The number of COCs retrieved was not significantly different between patients who received aromatase inhibitors during ovarian stimulation and those who did not (WMD: +0.10 COCs, 95% CI: −0.60 to +0.80; fixed effects model; heterogeneity: not applicable). Only one study (Ozmen *et al.*, 2009) offered data for this outcome.

Data on remaining secondary outcomes regarding the addition of aromatase inhibitors are presented in Table V.

Recombinant LH addition

Clinical pregnancy rate and live birth rate

A non-significant increase of 6% in clinical pregnancy rates was detected in patients who received rLH when compared with those who did not (95% CI: −0.3 to +13%; fixed effects model; heterogeneity: $P = 0.59$; Fig. 4). Seven studies (Ferraretti *et al.*, 2004; Demirol *et al.*, 2005; Fernández Ramírez *et al.*, 2006; Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008), evaluating the addition of recombinant LH in 603 patients in total, offered data for this outcome. Publication bias was not detected (Harbord–Egger's test: $P = 0.69$).

Moreover, a subgroup analysis according to dose of rLH administered was performed and no significant difference in the pooled clinical pregnancy RDs was detected between the three subgroups ($P = 0.39$). When the two studies (Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007) in which a dose of 75 IU of rLH was used were combined, clinical pregnancy rates were similar (RD: +2%, 95% CI: −9 to +13%; fixed effects model; heterogeneity: $P = 0.85$) between the rLH and the control groups. In the three studies that used a variable dose of the dose of 75–150 IU (Ferraretti *et al.*, 2004; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008) a slightly significant increase of +12% (95% CI: +1 to +24%; fixed effects model; heterogeneity: $P = 0.37$) in favor of rLH addition was detected, while in the other two studies, where a dose of 150 IU of rLH was used (Demirol *et al.*, 2005; Fernández Ramírez *et al.*, 2006), clinical pregnancy rate was not significantly different between patients who received rLH and those who did not (RD: +3%, 95% CI: −9 to +15%; fixed effects model; heterogeneity: $P = 0.49$).

Regarding clinical pregnancy per timing of onset, no significant difference was present with rLH addition either during mid-to-late follicular phase in six studies (Ferraretti *et al.*, 2004; Fernández Ramírez *et al.*, 2006; Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008) (RD: +7%, 95% CI: −1 to

Table V Secondary outcomes.

Outcome	Number of studies	Sample size	Method applied	Effect size, (95% CI)
Testosterone				
E2 level on the day of hCG[a]	1	49	fixed effects model, heterogeneity: n/a	WMD: 41.00, (−296.54, 378.54)
Number of embryos transferred[b]	2	137	fixed effects model, heterogeneity: $P = 0.25$	WMD: 0.08, (−0.20, 0.35)
Number of MII oocytes[c]	2	145	fixed effects model, heterogeneity: $P = 0.32$	WMD: 1.32, (0.79, 1.84)
Number of available embryos[c]	1	36	fixed effects model, heterogeneity: n/a	WMD: 0.66, (−0.69, 2.01)
Endometrial thickness on the day of hCG[a]	1	109	fixed effects model, heterogeneity: n/a	WMD: −0.10, (−0.59, 0.39)
Number of patients reaching oocyte retrieval[d]	2	163	random effects model, heterogeneity: $P = 0.006$	RD: −0.07, (−0.41, 0.27)
Number of patients having an ET[d]	2	163	random effects model, heterogeneity: $P = 0.002$	RD: −0.10, (−0.51, 0.31)
Fertilization rate[c]	1	36	fixed effects model, heterogeneity: n/a	WMD: −0.10, (−18.06, 17.86)
Number of follicles ≥ 17 mm on the day of hCG[a]	2	158	random effects model, heterogeneity: $P < 0.0001$	WMD: 0.38, (−1.90, 2.65)
Implantation rate[b]	1	30	fixed effects model, heterogeneity: n/a	WMD: 4.40, (−5.08, 13.88)
DHEA				
E2 level on the day of hCG[a,d]	1	33	fixed effects model, heterogeneity: n/a	WMD: −392.00, (−616.94, −167.06)
Number of embryos transferred[b,d]	1	33	fixed effects model, heterogeneity: n/a	WMD: −0.30, (−0.89, 0.29)
Endometrial thickness on the day of hCG[a,d]	1	33	fixed effects model, heterogeneity: n/a	WMD: −1.10, (−3.01, 0.81)
Aromatase inhibitors				
E2 level on the day of hCG[a]	1	70	fixed effects model, heterogeneity: n/a	WMD: −145.00, (−223.33, −66.67)
Number of embryos transferred[b]	1	56	fixed effects model, heterogeneity: n/a	WMD: 0.30, (−0.36, 0.96)
Number of MII oocytes[c]	1	70	fixed effects model, heterogeneity: n/a	WMD: 0.30, (−0.67, 1.27)
Endometrial thickness on the day of hCG[a]	1	70	fixed effects model, heterogeneity: n/a	WMD: −0.40, (−1.77, 0.97)
Number of patients reaching oocyte retrieval[d]	2	118	fixed effects model, heterogeneity: $P = 0.75$	WMD: 0.01, (−0.11, 0.12)
Number of patients having an ET[d]	2	118	fixed effects model, heterogeneity: $P = 0.48$	RD: 0.12, (−0.03, 0.28)
Number of follicles ≥ 17 mm on the day of hCG[a]	1	70	fixed effects model, heterogeneity: n/a	WMD: 0.50, (−0.23, 1.23)
Global cancellation rate[d]	1	70	fixed effects model, heterogeneity: n/a	RD: −0.17, (−0.35, 0.01)
Cancellation rate due to poor response[d]	1	70	fixed effects model, heterogeneity: n/a	RD: −0.20, (−0.38, −0.02)
Recombinant LH				
E2 level on the day of hCG[a]	5	325	random effects model, heterogeneity: $P < 0.00001$	WMD: 81.10, (−368.34, 530.55)
Number of embryos transferred[b]	3	189	fixed effects model, heterogeneity: $P < 0.00001$	WMD: −0.04, (−0.10, 0.03)
Number of MII oocytes[c]	3	185	fixed effects model, heterogeneity: $P = 0.88$	WMD: −0.49, (−0.70, −0.28)
Endometrial thickness on the day of hCG[a]	1	34	fixed effects model, heterogeneity: n/a	WMD: 0.70, (−0.68, 2.08)
Number of patients reaching oocyte retrieval[d]	4	319	fixed effects model, heterogeneity: $P = 0.76$	RD: 0.01, (−0.06, 0.07)

Continued

Table V *Continued*

Outcome	Number of studies	Sample size	Method applied	Effect size, (95% CI)
Number of patients having an ET[d]	3	235	fixed effects model, heterogeneity: $P = 0.19$	RD: −0.04, (−0.15, 0.07)
Global cancellation rate[d]	4	319	fixed effects model, heterogeneity: $P = 0.30$	RD: 0.03, (−0.06, 0.11)
Recombinant hCG				
E2 level on the day of hCG[a]	1	87	fixed effects model, heterogeneity: n/a	WMD: 465.60, (−548.28, 1479.48)
Number of embryos transferred[b]	1	70	fixed effects model, heterogeneity: n/a	WMD: −0.40, (−0.96, 0,16)
Number of MII oocytes[c]	1	87	fixed effects model, heterogeneity: n/a	WMD: −1.80, (−3.38, −0.22)
Number of patients reaching oocyte retrieval[d]	1	99	fixed effects model, heterogeneity: n/a	RD: 0.03, (−0.09, 0.16)
Number of patients having an ET[d]	1	99	fixed effects model, heterogeneity: n/a	RD: −0.04, (−0.22, 0.14)
Global cancellation rate[d]	1	99	fixed effects model, heterogeneity: n/a	RD: 0.04, (−0.14, 0.22)
Cancellation rate due to poor response[d]	1	99	fixed effects model, heterogeneity: n/a	RD: −0.03, (−0.16, 0.09)

CI, confidence interval; E2, estradiol; hCG, human chorionic gonadotrophin; n/a, not applicable; WMD, weighted mean difference; RD, risk difference; MII oocytes, metaphase II oocytes; DHEA, dehydroepiandrosterone; LH, luteinizing hormone.
[a]Per patient reaching hCG administration.
[b]Per patient reaching ET.
[c]Per patient having at least one oocyte retrieved.
[d]Per patient randomized.

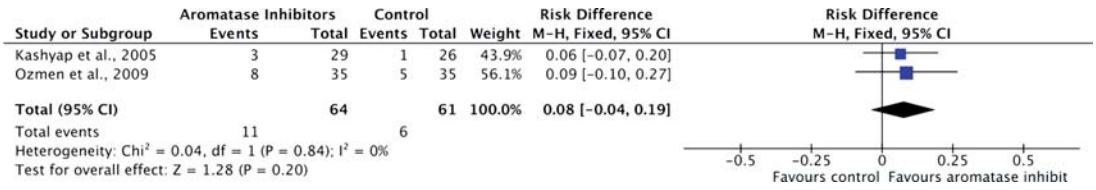

Figure 3 Risk difference for clinical pregnancy in patients treated with aromatase inhibitors or not.

+14%; fixed effects model; heterogeneity: $P = 0.50$) or during early follicular phase in one study (Demirol *et al.*, 2005) (RD: +6%, 95% CI: −9 to +20%; fixed effects model; heterogeneity: not applicable). Testing for differences between these two subgroups was also not significant ($P = 0.97$).

Data on live birth rate were available only in one (Ferraretti *et al.*, 2004) out of seven eligible studies. In that study, live birth rate was significantly increased in poor responders who received rLH when compared with poor responders who did not (RD: +19%, 95% CI: +1 to +36%; fixed effects model; heterogeneity: not applicable).

Total dose of FSH required for ovarian stimulation

No significant difference in the total dose of FSH required for ovarian stimulation was present between patients stimulated with rFSH and rLH when compared with patients who were stimulated with rFSH alone (WMD: −272.85 IUs, 95% CI: −600.52 to +54.83; random effects model; heterogeneity: $P = 0.0004$). Six studies (Ferraretti *et al.*, 2004; Fernández Ramírez *et al.*, 2006; Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008), consisting of 461 patients undergoing ovarian stimulation, provided information about this outcome.

Duration of ovarian stimulation

Duration of stimulation was not significantly different between patients treated with rLH and those who were not (WMD: −0.31 days, 95% CI: −0.66 to +0.04; random effects model; heterogeneity: $P = 0.02$). Six studies (Ferraretti *et al.*, 2004; Fernández Ramírez *et al.*, 2006; Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008) offered data for this outcome.

COCs retrieved

The number of COCs retrieved was not significantly different between patients who did or did not received rLH (WMD: −0.04, 95% CI: −0.61 to + 0.54; random effects model; heterogeneity: $P = 0.002$). Five eligible studies (Ferraretti *et al.*, 2004; Fernández Ramírez *et al.*, 2006; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008) consisting of 376 patients offered data for this outcome.

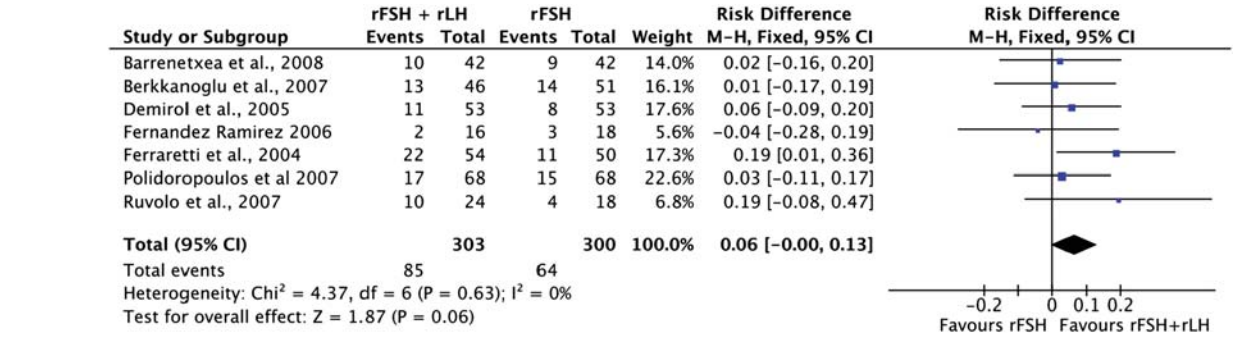

Figure 4 Risk difference for clinical pregnancy in patients treated with rFSH + rLH or rFSH alone.

Data on remaining secondary outcomes regarding the addition of rLH in poor responders are presented in Table V.

Recombinant hCG addition

One study (Berkkanoglu *et al.*, 2007), evaluating the addition of recombinant hCG in 99 patients in total, offered data for the following outcomes.

Clinical pregnancy rate and live birth rate

Clinical pregnancy rate was not significantly different between patients who received rhCG besides rFSH when compared with those stimulated with rFSH alone (RD: −5%, 95% CI: −20 to +10%; fixed effects model; heterogeneity: not applicable). The Harbord–Egger's test for the assessment of publication bias could not be performed because only one study was available. Data on live birth rate were not provided by the only eligible study.

Total dose of gonadotrophins required for ovarian stimulation

The addition of rhCG was associated with a significant decrease in the total dose of FSH required for ovarian stimulation when compared with the control group (WMD: −552.10 IUs, 95% CI: −1035.16 to −69.04; fixed effects model; heterogeneity: not applicable).

Duration of ovarian stimulation

Duration of stimulation was significantly decreased in patients treated with rhCG when compared with those who were not (WMD: −1.00 days, 95% CI: −1.71 to −0.29; fixed effects model; heterogeneity: not applicable).

Data on remaining secondary outcomes regarding the value of hCG addition in poor responders are presented in Table V.

Discussion

The present systematic review and meta-analysis summarizes the best available evidence regarding the use of androgens or androgen-modulating agents before or during ovarian stimulation with gonadotrophin in poor responders undergoing IVF. Based on the RCTs included in the present meta-analysis, there is limited evidence suggesting that transdermal testosterone pretreatment improves clinical pregnancy and live birth rates. On the other hand, no beneficial effect on pregnancy rates after IVF was detected in poor responders treated with DHEA, aromatase inhibitors or hCG before or during ovarian stimulation.

In the case of rLH addition, data originating only from one study (Ferraretti *et al.*, 2004) support that adding rLH might be beneficial in terms of live birth. Regarding clinical pregnancy rates, however, pooling the data from seven eligible studies did not detect any significant benefit in patients who received rLH when compared with those who did not (Ferraretti *et al.*, 2004; Demirol *et al.*, 2005; Fernández Ramírez *et al.*, 2006; Berkkanoglu *et al.*, 2007; Polidoropoulos *et al.*, 2007; Ruvolo *et al.*, 2007; Barrenetxea *et al.*, 2008). Although statistical significance was not reached, the magnitude of the effect size and the width of the 95% CI in terms of clinical pregnancy rate (RD: +6%, 95% CI: −0.3 to +13%) might indicate a potentially clinically significant finding (Brahman, 1991). However, until the addition of further studies confirms or rejects such a theory, the beneficial role of rLH administration in poor responders undergoing ovarian stimulation for IVF cannot be adequately supported by the available data.

Subgroup analyses were only feasible in the case of rLH addition, due to the limited number of studies in the other sets. When the studies were analyzed according to the exact time of initiation of rLH addition no significant differences were detected both within and between the two subgroups of studies. Similarly, when the studies were analyzed according to the doses of rLH used in these studies, no statistically significant difference was present between the three subgroups (75, 75–150 and 150 IU). Nevertheless, it should be noted that in the 75–150 IU subgroup, the pooled effect estimate from the three studies was statistically significant ($P = 0.03$). Although it cannot be excluded that this finding might reflect an actual underlying effect (i.e. through an optimal dose of rLH), the possibility that it represents a type I error (i.e. a false-positive finding) should also be strongly considered, especially given the multiple statistical comparisons performed (O'Brien and Shampo, 1988).

This systematic review and meta-analysis of RCTs follows a holistic approach on the role of androgens and androgen-modulating agents in poor responders undergoing IVF. Increasing the production of endogenous intraovarian androgens (through the addition of LH activity in the forms of rLH or hCG), decreasing the conversion of androgens to estrogens (through the addition of aromatase inhibitors), administering exogenous androgens (such as transdermal testosterone or DHEA), are all interventions that aim to increase intra-ovarian

androgen concentrations. According to the underlying theory, intra-ovarian androgens promote FSH sensitivity to growing follicles (Hillier and De Zwart, 1981; Vendola *et al.*, 1998), and therefore might increase oocyte yield and oocyte maturity after ovarian stimulation and improve pregnancy rates. By reviewing the available data regarding all relevant interventions, it might be easier for the reader to critically evaluate the plausibility of this theory and draw appropriate conclusions.

In this context, it should be noted, that in all the interventions analyzed in this meta-analysis the direction of the effect in terms of clinical pregnancy rates, even though statistically significant only in the case of transdermal testosterone, was in favor of the addition of androgens or androgen-modulating agents. This might be indicative of the plausibility of the underlying theory.

Interestingly though, based on the proposed theory for enhanced follicular development with the increase of intra-ovarian androgens (Hillier and De Zwart, 1981; Hillier *et al.*, 1994; Weil *et al.*, 1999), it would be expected that the interventions evaluated in the current meta-analysis would (i) increase the number of COCs retrieved, (ii) decrease the total dose of gonadotrophins required and (iii) decrease the duration of ovarian stimulation. Only in the case of transdermal testosterone administrations were all these hypotheses confirmed. Regarding aromatase inhibitors, the total dose of gonadotrophins was indeed significantly decreased, yet the number of COCs was not significantly different. Addition of hCG was associated with reduced consumption of gonadotrophins and decreased duration of stimulation. On the other hand, rLH addition was not associated with differences between the two groups compared in terms of total dose of FSH, duration of ovarian stimulation and COCs retrieved. These inconsistent results highlight the possibility that the evaluated interventions might not all have the same effect or might not act through the same mechanism. Moreover, various parameters such as the type of substance, the timing and the duration of the treatment are likely to be important determinants of the efficacy of these interventions.

Safety is one of the most important parameters of every proposed treatment and therefore the safety of the interventions evaluated in this meta-analysis deserves to be commented. Aromatase inhibitor use for ovarian stimulation has been associated with an increased incidence of congenital malformations (Biljan *et al.*, 2005), although further published data seem to be reassuring (Tulandi *et al.*, 2006). The effect of letrozole on perinatal outcome could not be evaluated in this meta-analysis, since no relevant data were provided by the eligible studies. Similarly, the administration of exogenous androgens could also raise concerns for the health of the patient (Kroboth *et al.*, 1999) and the pregnancy that might follow (Kim *et al.*, 2011). The administration of testosterone through the percutaneous route though, and the use of DHEA in doses of 75 mg appear to be safe, since no adverse effects (Massin *et al.*, 2006; Wiser *et al.*, 2010; Kim *et al.*, 2011) nor any congenital malformations were identified in any of the relevant eligible studies (Kim *et al.*, 2011).

This meta-analysis is also characterized by certain limitations that must be taken into consideration. First of all, the definition of poor ovarian response varied markedly among studies (Table II), which in turn might lead to problems in terms of extrapolating the findings of this meta-analysis. The lack of a universal definition of poor responders has been identified previously (Tarlatzis *et al.*, 2003; Kyrou *et al.*, 2009; Venetis *et al.*, 2010), and unfortunately represents a drawback of the relevant literature. Recently, in an attempt to address this issue, universal criteria for the definition of poor ovarian response have been proposed following a consensus meeting in Bologna (Ferraretti *et al.*, 2011). The use of the Bologna criteria will hopefully lead to more homogeneous studies in the future that can be more easily compared and combined.

The protocols of the proposed interventions and ovarian stimulation protocols also presented marked variation in terms of doses, timing of initiation, duration of stimulation, GnRH analogue protocols used etc. In most cases, a proper investigation of this clinical heterogeneity was not feasible due to the limited number of studies. The addition of more studies in the future will allow for the evaluation of a potential moderating effect of these parameters on the efficacy of the intervention examined.

Recently, another meta-analysis on the effect of androgen supplementation or modulation on ovarian stimulation outcome in poor responders was published (Sunkara *et al.*, 2011). In that meta-analysis the effect of treatment with testosterone, DHEA, and aromatase inhibitors was evaluated. Recombinant LH and rec-hCG were not evaluated. That meta-analysis concluded that there is not currently sufficient evidence to support the use of androgen supplementation or modulation to improve live birth rate in poor responders undergoing IVF (Sunkara *et al.*, 2011). That meta-analysis included both prospective and retrospective studies, while in the current meta-analysis it was a priori decided to include only parallel RCTs in an attempt to solidify the conclusions drawn.

Furthermore, the meta-analysis by Sunkara *et al.* (2011) included two RCTs (Goswami *et al.*, 2004; Fabregues *et al.*, 2009) that were excluded from the current meta-analysis. The reasons for this exclusion were that in these studies co-interventions were performed in the one arm and, thus, it was not the addition of letrozole (Goswami *et al.*, 2004) or transdermal testosterone (Fabregues *et al.*, 2009) in poor responders that was actually evaluated (Supplementary data, Table S1). Moreover, the current meta-analysis includes the RCT by Kim *et al.* (2011), which was recently published and is the largest RCT evaluating transdermal testosterone pretreatment in poor responders undergoing IVF, as well as a RCT by Kashyap *et al.* (2005) in which letrozole addition is examined.

Currently, based on the limited available evidence, transdermal testosterone pretreatment seems to increase clinical pregnancy and live birth rates in poor responders undergoing ovarian stimulation for IVF. There is insufficient data to support a beneficial role of rLH, hCG, DHEA or letrozole administration in the probability of pregnancy in poor responders undergoing ovarian stimulation for IVF. In all cases, further properly designed RCTs evaluating the role of androgens or androgen-modulating agents in poor ovarian response are warranted in order for firm conclusions to be drawn.

Supplementary data

Supplementary data are available at http://humupd.oxfordjournals.org/.

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Authors' roles

J.K.B. contributed to study conception and design, selected the articles and extracted the data, drafted the manuscript. C.A.V. contributed to study conception and design, selected the articles and extracted the data, analyzed and interpreted the data and revised the manuscript. E.M.K., K.A.T., D.G.G. contributed to the analysis and interpretation of the data, critically revised the manuscript for important intellectual content. L.Z. contributed to the interpretation of the data, critically revised the manuscript for important intellectual content. B.C.T. contributed to study conception and design, critically revised the manuscript for important intellectual content. All the authors approved the final version of the manuscript.

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Conflict of interest

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