Title: Evaluation of progestogen supplementation for luteal phase support in fresh IVF cycles**.**

Running title: Evaluating luteal phase support.

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Structured Abstract:

Objective: To evaluate the effectiveness of progestogen supplementation in improving clinical pregnancy rates in women undergoing fresh IVF cycles and to compare different routes, start times, durations and estrogen co-administration regimen.

Design: Comprehensive systematic review and meta-analysis.

Setting: University.

Patients: Women undergoing fresh IVF cycles who did and did not receive progestogen supplementation.

Intervention(s): Summary odds ratios (ORs) were calculated by binomial logistic regression.

Main Outcome Measure(s): Clinical pregnancy rates.

Results: 82 articles (26,726 women) were included. Clinical pregnancy rates were increased by intramuscular (OR=4.57; $p<0.001$), vaginal (OR=3.34; $p<0.01$), subcutaneous (OR=3.36; $p<0.01$) or oral (OR=2.57; $p<0.05$) progestogen supplementation versus no treatment. Greatest benefit was observed when progestogens were supplemented intramuscularly versus vaginally (OR=1.37; $p<0.001$). The optimal time to commence administration was between oocyte retrieval and embryo transfer (OR=1.31; $p<0.01$), with oocyte retrieval +1 day being most beneficial. Co-administration of estrogen had no benefit (OR=1.33; $p>0.05$) whether progestogens were co-administered vaginally or intramuscularly. Clinical pregnancy rates were equivalent when progestogen supplementation was ceased after ≤3 weeks or continued for up to 12 weeks (OR=1.06; $p>0.05$).

Conclusion: This broad-ranging meta-analysis highlights the need to re-evaluate current clinical practice. The use of progestogens in fresh IVF cycles is substantially beneficial to clinical pregnancy. Critically, the use of intramuscular progestogens should not be dismissed, as it yielded the greatest clinical pregnancy rates. Pregnancy success was impacted by initiation of therapy, with one day after oocyte retrieval being optimal. There is little evidence to support co-administration of estrogen or prolonging progestogen treatment beyond three weeks.

Keywords: (3-5) meta-analysis, progestogen, estrogen, luteal phase support, fresh IVF

Capsule: Luteal phase deficiency commonly occurs after ovarian stimulation in women undergoing assisted reproduction. Progestogen supplementation is a routine and critical component for luteal phase support, however, the optimal regimen remains unresolved.

Introduction

Luteal phase deficiency is a common result of assisted reproductive technologies (ART) and is characterised by inadequate or inappropriate progesterone production. This inevitably compromises the successful establishment and maintenance of pregnancy, and has led to the critical requirement for luteal support protocols. Luteal phase deficiency has been attributed to diminished luteotrophic support from pituitary LH, reduced luteal steroidogenic capacity and/or premature luteolysis (1-3). Hence, the provision of exogenous progestogens to supplement endogenous progesterone production has become a routine component of ART.

In recent years, there has been much debate as to whether the immediate transfer of "fresh" embryos or cryopreservation with subsequent transfer of frozen embryos confers the greatest pregnancy success for patients (4, 5). Indeed, elective freeze-all cycles have been widely advocated and adopted (6), with an anticipated improvement in endometrial receptivity thought to give rise to an improved pregnancy outcome (7). However, recent evidence suggests that whilst a freeze-all strategy is of benefit to women who are highly responsive to ovarian stimulation, it is not beneficial for those women with a low or intermediate response (8). Therefore, there is an ongoing need for the evaluation of luteal phase support in fresh IVF cycles.

The potential supplementation regimens for luteal phase support following ART are numerous; progestogens are available in a number of formulations (of progesterone or synthetic progestins) and can be administered by nasal, rectal, vaginal, oral, subcutaneous or intramuscular routes alone, or via multiple routes in combination. Progestogen administration can commence before oocyte retrieval, on the day of oocyte retrieval or in the days soon afterwards, or on or around the day of embryo transfer. Supplementation can then be maintained for several weeks, until a positive urinary pregnancy test, until fetal heart pulsations have been observed or until week 12 of gestation or later (9). Luteal phase progestogens may also be co-administered with estrogen.

While there is an agreed need for luteal phase support following ART (10), the choice of preparation, route of delivery, time at which to commence treatment and its duration remain a matter of debate (11, 12). The wide variation in clinical approach means that the choice of luteal phase support for couples undergoing ART is far from clear. Evidence from clinical practice suggests a current global preference for luteal phase support via vaginal progestogens in tablet form, administered from the day of oocyte collection and maintained for 8-10 weeks (9).

The current study critically evaluates the efficacy of luteal phase support by analysing the impact of these complex treatment choices on pregnancy rates following fresh embryo

transfer via binomial logistic regression, with the aim of both influencing practice and providing an essential point of reference for patients. In contrast to previous meta-analyses, our use of binomial logistic regression enables the synthesis of results from the numerous studies performed without control groups. This distinctive and robust statistical approach has the benefit of greatly broadening both the scope of questions answered and the number of study groups eligible for each comparison (13). This includes addressing largely overlooked and important questions or those with few existing RCTs (14), such as, determining the optimal day on which to commence progestogen supplementation.

Recent commentary (11) has concluded that the luteal phase in ART is deserving of greater attention, such as provided by this analysis. Furthermore, whilst the clinical approach to luteal phase support may be becoming more consistent (9, 15), the suggested lack of evidence-based decision making (9) may ultimately limit pregnancy success or lead to women undergoing additional treatment for luteal phase support that is of little benefit.

Methods

Search strategy

An extensive systematic literature search was performed using Google Scholar https://scholar.google.com/, PubMed https://www.ncbi.nlm.nih.gov/pubmed and Web of Science http://wok.mimas.ac.uk (last accessed April 2018). Searches were performed in English and included studies (excluding abstracts and conference proceedings) published between 1980 and January 2018. For this purpose, the principal search terms in the title, abstract or keywords were "progesterone supplementation" OR "progestogen supplementation" OR "luteal support" in conjunction with "vaginal" OR "intramuscular" OR "oral" OR "subcutaneous" OR "rectal". A separate search was conducted with the following terms "assisted reproductive technology or ART", "in vitro fertilization or IVF", "intra-cytoplasmic sperm injection or ICSI", or "fresh embryo transfer". The references within these articles including any meta-analysis were scrutinised for any additional articles. These search results were subsequently combined to yield a total of 517 articles (excluding duplicates) (Supplementary Fig 1).

Selection of articles: methodology and criteria

Following the PRISMA guidelines (16), the title, abstract and keywords were screened to confirm that the article was within subject remit and this excluded 327 articles (e.g. animal models). Then, the full text of each manuscript was obtained and reviewed. For articles to be included, the following inclusion criteria were assigned: 1) included sub-fertile women (undefined or defined aetiology) undergoing ART with fresh embryo

transfer; 2) involved ovarian stimulation; 3) included an evaluation of pregnancy outcomes between two groups of women, either progestogen versus untreated control group, or comparing at least 2 different regimens of luteal support involving progestogen; 4) included pregnancy outcome represented as clinical pregnancy (e.g. presence of a gestational sac, with or without a fetal heartbeat on ultrasonography) as defined in the original manuscript.

Articles were excluded for the following reasons: 1) assignment of progestogen treatment occurred after a positive HCG pregnancy test, 2) the only luteal support was HCG treatment; 3) studies involved frozen or donor oocyte cycles. A total of 108 articles were excluded, resulting in 82 articles submitted to the meta-analysis (Supplementary Fig 1).

Assessing the risk of bias

Two authors (RSR & KJW) independently assessed the risk of bias in each included article across several domains according to previous criteria (10); random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other potential sources of bias (e.g. apparent variations in patient management or embryo quality between arms). Studies were classified as being at low, high or unclear risk of bias and the risk of bias analysis was used to generate a risk of bias summary figure (Supplementary Fig 2) and graph (Supplementary Fig 3).

In order to mimimize any risk of bias across studies and in recognition of the difficulty of identifying publication bias and selective reporting, a comprehensive and broad-ranging systematic literature search for eligible studies was conducted. The articles were thoroughly interrogated for any duplication of data.

Data collection process

Data extraction was performed independently by two reviewers (AM & RSR) and discrepancies were resolved by discussion with a third reviewer (KJW). The following data were extracted from each article: route of progestogen administration, dose of progestogen and duration of progestogen treatment, the time when progestogen supplementation commenced and the presence or absence of estrogen co-treatment. Clinical pregnancy was considered the primary pregnancy outcome, with additional data on live birth tabulated when given. Additional extracted information included country/region of origin, publication date, number of patients, mean age of participants, type of ART procedure (e.g. IVF or ICSI or combination), and other treatment information including controlled ovarian stimulation protocol and ovulation trigger.

Classification of study groups

Route of administration: The relative benefit of the different routes of progestogen administration was compared. The most commonly employed routes were intramuscular (IM) injection and vaginal pessary. However, other reported routes included oral, rectal and subcutaneous (SC) injection. There were several articles where no luteal support (N=8 study groups) was administered and this control group was used as the reference in the initial analyses; in the later analyses, the vaginal route of administration was used as the comparator. The dose of progestogen was not analysed since it was intrinsically linked to route and further sub-divisions created groups with too few women.

Time to commence supplementation: A key component of the meta-analysis was to investigate the effect of the time at which progestogen supplementation commenced. The start time of each treatment was classified into one of five groups; (1) before oocyte retrieval; (2) on the day of oocyte retrieval (at oocyte retrieval or evening of); (3) between oocyte retrieval and embryo transfer; (4) on the day of embryo transfer (ET); (5) after embryo transfer. In this analysis, the comparator was the control group which received no luteal support.

In a subsequent analysis, study groups were further categorised as progestogen supplementation that commenced (1) at oocyte retrieval (the day and evening of), or on (2) the first, (3) second and (4) third day after oocyte retrieval. Most articles detailed this information directly, however in several articles this was determined utilising other information such as the oocyte retrieval to embryo transfer time. If there was insufficient information to state the exact day, then this study group was excluded from this analysis.

Estrogen treatment: For this analysis, only articles which included a direct estrogen treatment comparison were included. All study groups which received estrogen as part of the luteal phase support were coded as treated, while the others were incorporated as the control comparator. The route of estrogen treatment (oral, transdermal patch or vaginal) and its timing (around oocyte retrieval or around embryo transfer) was not considered.

Duration of progestogen supplementation: The study groups were classified based on whether progestogen supplementation treatment was 3 weeks or less, or greater than 3 weeks. The cut-off at 3 weeks was selected as the approximate time of HCG pregnancy diagnosis and there was a natural stratification in the studies at 3 weeks of treatment.

Sample size calculation

The sample size for a binomial test (two-sided) was calculated with the overall mean clinical pregnancy rate of 37% being used as the reference. Thus, at a significance level of 0.05 with a 90% power of detection, the number of women in each study group required to detect: 1) an increase of 5 percentage points from the reference rate was 2000, 2) an

increase of 10 percentage points was 510 and 3) an increase of 15 percentage points was 229. Alternatively, if all women (n=26,726) were included then a 2 percentage point change could be detected.

Statistical analysis

The statistical approach utilised was binominal logistic regression. The "number of subjects" was the total number of women who were treated within that study, the "number of successes" was the number of women with a confirmed clinical pregnancy or live birth and "the model fitted" was the factor (e.g. route or start time) that was being compared. The dispersion parameter was set to estimate the residual mean squares of fitted model. The analysis was performed using GenStat 19th Edition (Hemel Hempstead, UK). The data are presented as odds ratio (OR) with 95% confidence intervals (CI) alongside the number of study groups (N) and women (n). The use of binomial logistic regression meant that no estimate of heterogeneity between articles (i.e. estimation of I^2) was feasible.

Comparison between different routes of administration: In the initial analysis, the different routes of administration (IM, oral, rectal, SC and vaginal) were compared to the control (no luteal support) group. A further 11 study groups where progestogen was simultaneously administered by multiple routes (i.e. IM plus vaginal) were excluded from this analysis. None of the control study groups reported live births, thus the effects of route of administration on live births were not analysed.

Comparison between the different times at which progestogen supplementation commenced: The effects of the different start times (before oocyte retrieval, at oocyte retrieval, between oocyte retrieval and ET, at ET and after ET) on clinical pregnancy rate were compared to the control group. For the effects on live birth rates, the start times were compared to commencing supplementation at oocyte retrieval, as no live birth rates were reported in the control group.

Comparison between different start times when progestogen was given via either intramuscular or vaginal routes: The two most commonly employed routes (IM and vaginal) were further analysed to determine if the time at which supplementation commenced affected pregnancy rates. The comparator group was the "at oocyte retrieval" group as this included the most study groups and women. Next, the intramuscular and vaginal routes of progestogen administration were directly compared with vaginal administration as the comparator group. The data was stratified into the following time points: before oocyte retrieval, at oocyte retrieval, between oocyte retrieval and ET, at ET and all times combined (overall).

An additional comparison was performed between the intramuscular and vaginal routes of administration with the data stratified by publication date as follows: 1990-1999, 2000-

2009 and 2010-2017. There were no studies reporting the use of vaginal progestogen supplementation prior to 1990.

Determination of the optimal day after oocyte retrieval to start progestogen supplementation: The database was further interrogated to compare different specific start times of progestogen supplementation, with the day of oocyte retrieval acting as the reference. The times categorised were the first, second and third day after oocyte retrieval.

Effect of co-administration of estrogen with progestogen supplementation: The data was analysed in two separate ways (1) with the data categorised by vaginal, IM or all routes of administration and (2) with data categorised into "at oocyte retrieval" and "between oocyte retrieval and embryo transfer". In all cases, the comparator was the no estrogen treatment group.

Effect of duration of progestogen supplementation: For this, progestogen supplementation for 3 weeks and less (the comparator) was compared with more than 3 weeks. Initially, all routes of administration were included, but this was then stratified according to either intramuscular or vaginal route of administration.

Results

Characteristics of identified studies

A total of 82 articles (Supplementary Table 1) including 26,726 women met the selection criteria, which were published between 1983 and 2018. This created 185 different study groups/treatments. Both prospective and retrospective studies were incorporated into this analysis. The prospective studies included "randomised control trials" however a large proportion of these studies did not have a control-untreated group. More often they were randomised trials in which two or more different treatments were compared. In respect to live births, there were fewer study groups (N=65) with a lower number of women (n=12,006). Consequently, live birth rates were considered as a secondary outcome measure.

The studies were conducted across the World with the greatest proportion of the studies originating from continental Europe (32%), North America (23%), and the Middle East (27%). A relatively low percentage of the studies were performed in Asia (13%), the UK (2%), South America (1%) and Africa (2%).

The youngest reported individual in the dataset was 18 years old, while the oldest was 47. In the majority of articles, the age groups were matched across the different treatments and the overall mean age was 32.8 years old. The fertilisation rates and number of embryos transferred (mean: 3.5) were generally stated but not in all studies. The ovarian stimulation protocol was described in most study groups, with 132 using long GnRH agonist

protocols, 10 using a short GnRH agonist flare protocol and 16 with short GnRH antagonist protocol. However, the induction protocol was not clearly stated in the other study groups (N=27). A variety of ovarian induction hormones were used within these protocols including FSH (N=74), HMG (N=19) and both recombinant FSH and HMG (N=42), while 50 study groups did not mention which type of gonadotrophin was used. The most common treatment used to trigger final oocyte maturation was HCG (N=173), and in the remaining 12 study groups the ovulation trigger was not detailed.

The aetiology of the specific infertility was mentioned in only 5 study groups, where women were at risk of ovarian hyper-stimulation syndrome (OHSS). Thus, there was insufficient information to dissect the benefits of progestogen supplementation according to different underlying pathologies to warrant further investigation.

Risk of bias

Most articles (including those reporting control study groups) were identified as having an unclear or high risk of bias in one or more domain (Supplementary Fig 2 & 3), often resulting from a lack of detail reported in the original methods (e.g. if or how randomisation was generated). Blinding was considered difficult to achieve given the markedly different routes of administration (vaginal vs intramuscular) but was thought unlikely to have introduced significant bias, given the objective nature of pregnancy outcomes and is not expected to have influenced the outcomes.

The potential risk of bias in those articles which included control study groups appeared broadly similar to that observed across all articles (Supplementary Fig 3). Amongst the articles reporting control study groups, one (17) was judged to have a high risk of selection bias relating to one of the treatment groups.

Does progestogen supplementation improve clinical pregnancy rates in women undergoing fresh IVF cycles?

There was a significant benefit to clinical pregnancy rates of either intramuscular (OR=4.57 [CI: 2.19-9.53]; $p<0.001$), vaginal (OR=3.34 [CI: 1.61-6.91]; $p<0.01$), subcutaneous (OR=3.36 [CI: 1.44-7.83]; $p<0.01$) or oral (OR=2.57 [CI: 1.19-5.58]; $p<0.05$) progestogen supplementation (Fig 1A) versus no treatment. Numerically, this was equivalent to increasing mean pregnancy rates from 14.7% for untreated women to 30.7% following oral, 36.4% following vaginal, 36.6% following subcutaneous, and 44.0% following intramuscular progestogen supplementation. While rectal (OR=2.32 [CI: 0.62-8.68]; $p>0.05$) routes of administration offered no benefit, although this route was poorly represented.

When is the optimal time to start progestogen supplementation?

The relative benefit to clinical pregnancy rates of commencing progestogen supplementation at different times was compared with no supplementation (Fig 1B). There was a clear benefit of progestogen supplementation at oocyte retrieval, at embryo transfer or between these events as well as after embryo transfer. The greatest benefit was clearly observed when progestogen administration commenced between oocyte retrieval and embryo transfer (OR=4.76 [CI: 2.35-9.67]; $p<0.001$). Furthermore, when at oocyte retrieval and between oocyte retrieval and embryo transfer were directly compared then there was a clear benefit of starting progesterone administration between oocyte retrieval and embryo transfer (OR=1.31 [CI: 1.10-1.58], $p<0.01$). In contrast, there was no benefit to clinical pregnancy rates versus untreated women when progestogen treatment commenced before oocyte retrieval (OR=2.10 [CI: 0.95-4.66]; $p>0.05$).

Does the optimal time to commence progestogen supplementation vary by route of administration?

In order to address this, the control untreated group was excluded and the different start times were compared to starting progestogen supplementation at oocyte retrieval. There were insufficient study groups and women to include the after ET group. Additionally, intramuscular and vaginal routes of administration were analysed separately.

When progestogen was administered intramuscularly, starting progestogen supplementation before oocyte retrieval (OR=0.32 [CI: 0.16-0.63]; $p<0.01$) was less favourable to clinical pregnancy rates than administration commencing at oocyte retrieval (Fig 2A). There was no statistically significant benefit to clinical pregnancy rates of commencing progestogen supplementation between oocyte retrieval and embryo transfer (OR=1.30 [CI: 0.97-1.75]; $p=0.08$) or at embryo transfer (OR=0.75 [CI: 0.44-1.28]; $p>0.05$).

When progestogen was administered vaginally, the greatest benefit to clinical pregnancy rates was observed when administration began between oocyte retrieval and embryo transfer (OR=1.38 [CI: 1.10-1.74]; $p<0.01$). While not significant ($p>0.05$), the odds ratio for clinical pregnancy rate was numerically lower when starting supplementation before oocyte retrieval (OR=0.77 [CI: 0.46-1.28]) or at embryo transfer (OR=0.85 [CI: 0.68-1.07] when compared with at oocyte retrieval (Fig 2B).

Thus, it appeared that commencing progestogen supplementation before oocyte retrieval vaginally was less detrimental to clinical pregnancy rates than following intramuscular treatment. This indicated that when progestogen was administered intramuscularly or vaginally, the supplementation start times differentially influenced clinical pregnancy outcomes.

In respect to live birth rates, across all routes combined, there was a clear benefit of commencing supplementation between oocyte retrieval and embryo transfer (OR=1.33 [CI: 1.04-1.69], $p<0.05$) when compared with at oocyte retrieval (Fig 2C). In contrast, live birth rates were decreased when progestogen supplementation commenced before oocyte retrieval (OR=0.52 [CI: 0.30-0.92], $p<0.05$). However, live birth rates were no different when progestogen was supplemented at embryo transfer (OR=0.84 [CI: 0.65-1.08]; $p>0.05$).

Which route of progestogen administration (IM or vaginal) is more beneficial in terms of clinical pregnancy and live birth rates?

When all time-points were combined for each route, intramuscular progestogen administration offered the greatest overall benefit to clinical pregnancy rates (OR=1.37 [CI: 1.15-1.63], $p<0.001$) versus vaginal administration. Furthermore, intramuscular progestogen supplementation was more beneficial to clinical pregnancy rates than the vaginal route at oocyte retrieval (OR=1.42 [CI: 1.14-1.76]; $p<0.01$). Similar patterns were observed between oocyte retrieval and embryo transfer (OR=1.33 [CI: 0.96-1.85]) and at embryo transfer (OR=1.24 [CI: 0.68-2.27]) but these failed to reach significance ($p>0.05$; Fig 3A). Conversely, when progestogen supplementation commenced before oocyte retrieval (data not shown), vaginal progestogen administration showed numerically greater clinical pregnancy rates (OR = 0.59 [CI: 0.427-1.32] but this was not significant ($p>0.05$), largely due to a small number of study groups (N=4) in each treatment for this timeframe.

Over time, the proportion of women enrolled in studies administering progestogens intramuscularly (versus vaginal) has decreased (Supplementary Table 2). However, in both the 2000-2009 and 2010-2017 timeframes intramuscular progestogen administration offered a greater benefit to clinical pregnancy rates versus vaginal treatment ($p<0.05$). The data was also analysed to confirm whether intramuscular progestogen supplementation was also of benefit to live birth rates. Fewer studies reported live birth rates (in total 10391 women), with intramuscular (N=27, n=2910) and vaginal (N=31, n=7481) progestogen supplementation having equivalent live birth rates (OR=1.17 [CI: 0.89-1.53]; $p>0.05$).

What is the optimal day after oocyte retrieval to start progestogen supplementation?

The previous analysis demonstrated that progestogen supplementation was most beneficial when it commenced between oocyte retrieval and embryo transfer. Thus, further analysis was performed to determine the exact optimal day within this window. This included 149 study groups and supplementation by intramuscular, vaginal, oral and subcutaneous routes. The day of oocyte retrieval was used as the comparator (Fig 3B).

Commencing progestogen supplementation on the day after oocyte retrieval was most beneficial in terms of clinical pregnancy rates (OR=1.25 [CI: 1.02-1.54]; p<0.05). Starting supplementation on the second day after oocyte retrieval had equivalent clinical pregnancy rates to at oocyte retrieval (OR=1.10 [CI: 0.88-1.36]; p>0.05). In contrast, further delaying supplementation until the third day (OR=0.66 [CI: 0.50-0.87; p<0.01) reduced clinical pregnancy rates versus starting at oocyte retrieval (Fig 3B). No significant differences in live birth rates were detected between the different start days (p>0.05; data not shown).

Does the addition of estrogen treatment to progestogen supplementation improve clinical pregnancy rates?

The co-administration of estrogen was of no overall benefit to clinical pregnancy rates (OR=1.33 [CI: 0.90-1.97; p>0.05]). Furthermore, this lack of benefit was observed when progestogens were co-administered by either the vaginal (OR=1.40 [CI: 0.84-2.34; p>0.05) or intramuscular route (OR=1.04 [CI: 0.50-2.14; p>0.05) (Fig 4A). Clinical pregnancy rates were similar following the addition of estrogen to progestogen supplementation that commenced at oocyte retrieval (OR=1.29 [CI: 0.72-2.34]; p>0.05) and between oocyte retrieval and embryo transfer (OR=1.59 [CI: 0.85-2.95]; p>0.05) (Fig 4B). Similarly, live birth rates were not improved by the addition of estrogen supplementation (p>0.05; data not shown).

Does the duration of progestogen supplementation effect clinical pregnancy and live birth rates?

Overall, there was no difference in clinical pregnancy rate when progestogen supplementation was continued for up to 12 weeks (OR=1.06 [CI: 0.87-1.29]; N=115, n=17,215; p>0.05) compared with ceasing after 3 weeks (N=41, n=5357). Similarly, if the duration of progestogen supplementation was categorised into smaller 2 or 3 weekly intervals, then there was no particular timeframe that was of greater benefit than ceasing supplementation after three weeks (data not shown). When the data was subdivided based on route of progestogen supplementation (intramuscular or vaginal) then extending progestogen supplementation was not beneficial to clinical pregnancy rates when it was administered intramuscularly (OR=1.23 [CI: 0.85-1.78]; N=17, n=1486 [≤3 weeks] vs N=29, n=3771 [3-12 weeks]; p>0.05) or vaginally (OR= 0.94 [CI: 0.75-1.19]; N=15, n=3338 [≤3 weeks] vs N=72, n=10654 [3-12 weeks]; p>0.05).

The dataset was more limited when considering live births. Overall, continuing progestogen supplementation for >3 weeks similarly had no benefit to live birth rates (OR=1.11 [CI: 0.88-1.46]; N=17, n=3411 [≤3 weeks] vs N=46, n=8121 [3-12 weeks]; p>0.05). When the data was subdivided based on whether progestogen supplementation

was via intramuscular or vaginal routes then extending progestogen supplementation had no benefit when administered vaginally (OR=1.31 [CI: 0.99-1.74]; N=6, n=2431 [≤3 weeks] vs N=24, n=4878 [3-12 weeks]; p=0.06) or when administered intramuscularly (OR= 0.72 [CI: 0.43-1.20]; N=10, n=960 [≤3 weeks] vs N=16, n=1648 [3-12 weeks]; p>0.05).

Discussion

Progestogen supplementation was of benefit to clinical pregnancy rates when administered intramuscularly, subcutaneously, orally or vaginally. The best response was observed when administration commenced at or following oocyte retrieval. The benefit was less however, if progestogen supplementation was delayed for 2 or more days after oocyte retrieval, likely reflecting the benefit of exogenous progestogen prior to embryo transfer. The most commonly reported routes of progestogen supplementation were intramuscular and vaginal. Both routes improved clinical pregnancy rate versus no treatment, with most benefit observed following intramuscular administration.

Progestogen supplementation was found to be of some benefit to clinical pregnancy rates, ongoing pregnancy and live birth versus placebo or no-treatment in a Cochrane review of 875 women across 8 randomised controlled trials (10). Also in that review intramuscular progestogen was of more benefit than vaginal/rectal (OR=1.24, [CI: 1.03-1.50]) in respect to live birth rates. However, a difference between these two routes was not detected when clinical pregnancy rates were considered (13 RCTs, 2932 women). The present study utilised a distinctive and robust statistical approach, enabling a broad-ranging scope which incorporates retrospective studies. Importantly, it revealed that intramuscular progestogen was of greater benefit to clinical pregnancy rates than vaginal progestogen (153 study groups, 22852 women). This was particularly evident when administration commenced at oocyte retrieval.

Intramuscular administration offered most benefit to clinical pregnancy rate in the current study, however, it represented only 26% of treatments, with the majority (62%) of supported cycles using vaginal administration. In a survey of luteal phase support in 408 treatment centres from 82 countries, vaginal progestogens were administered alone in 77% of supplemented cycles (9). Furthermore, the clinical use of intramuscular progestogens for the support of assisted reproduction has declined in recent years from 13% to around 5%, although it has traditionally been the most popular form of luteal support in the United States (9, 15).

Vaginal progestogen preparations may be preferred by patients to intramuscular preparations (18, 19). Vaginal treatments are reportedly well tolerated, due to their ease

and relative convenience, whilst patients find the injections painful and report high rates of irritation at the intramuscular injection site (18). In addition, rare but significant side effects have been reported following intramuscular luteal support (20-23). Vaginal progestogens are not free from disadvantages however; they may require multiple daily applications, can lead to vaginal irritation or discharge in some women (24) and the preparations may leak which is unpleasant and leads to variable exposure.

The routes of progestogen administration exhibit different pharmacological profiles. Oral progesterone has very poor bioavailability, does not produce a sustained plasma progesterone concentration (25), fails to elicit an adequate endometrial secretory response (26) and produces sedative metabolites (27). In addition, a negative impact on implantation was observed following oral micronized progesterone versus intramuscular or vaginal progesterone (28, 29). Administration of the orally effective dydrogesterone led to clinical pregnancy rates similar to those following intravaginal micronized progesterone support (30, 31). Recent evidence also suggests that oral dydrogesterone is well tolerated and is not associated with significant fetal or maternal safety risk (32). Despite this, oral progesterone has very low current clinical use (9).

Intramuscular administration of progesterone results in higher more sustained serum levels than vaginal administration, however vaginal regimens undergo rapid absorption to achieve higher endometrial tissue concentrations (33). This preferential uptake of progesterone has been described as the "first uterine pass effect", with direct local transport of progesterone from vagina to uterus thought to explain the enhanced uterine concentrations (34). It has been suggested however, that these raised local progesterone concentrations may not provide optimal support for ongoing pregnancy (35). Indeed, in the interim analysis of a recent large-scale randomised control trial evaluating progesterone replacement in frozen transfer cycles, vaginal progesterone administration resulted in significantly reduced ongoing pregnancy rates versus intramuscular supplementation (35), thought to result from early pregnancy loss. Intramuscular progesterone has been suggested to better support early pregnancy via greater uterine quiescence (36) and different progestogen formulations may also result in varied luteotrophic metabolites (37).

In a sub-analysis, it was observed that categorising progestogen dosage into low vs high within intramuscular or vaginal routes revealed that clinical pregnancy rates were not affected by dosage in either route (data not shown). This is in agreement with the Cochrane review (10) which demonstrated no effect of dose on live birth rates when progestogen was administered vaginally.

The time at which progestogen treatment began had an impact on its degree of benefit. This aspect of luteal phase support has received less attention and was not reported on by van der Linden et al (10). The present study has clearly demonstrated that commencing luteal support following oocyte retrieval but before embryo transfer provided most benefit to clinical pregnancy rates, irrespective of route. However, the timing of administration appeared to be more critical for intramuscular progestogen, where the difference in response before oocyte retrieval (OR=0.32) was markedly lower than in the window between oocyte retrieval and embryo transfer (OR=1.30). Similarly, live birth rates were improved when progestogen supplementation commenced between oocyte retrieval and embryo transfer compared with commencing at oocyte retrieval. Previous studies (14) have similarly suggested an ideal window for the initiation of luteal support, between the evening of oocyte retrieval and day 3, based on a small number of randomised controlled trials. Others have also suggested that the initiation of intravaginal progestogen is critical (19), with unfavourable results associated with the early initiation of intravaginal gel. Furthermore, it has been suggested that the greater bioavailability of vaginal progestogen to the endometrium may result in precocious development of the endometrial receptivity window (19).

In the current study the benefit observed within the window from oocyte retrieval and embryo transfer, largely resulted from luteal support that began on the first day after oocyte retrieval (OR=1.25 vs at oocyte retrieval). This delay in the initiation of luteal support does not reflect current clinical practice, where in a survey of IVF units, 80.1% of luteal phase support began on the day of oocyte retrieval, whilst in 15.4% of cycles progestogen began on the day of embryo transfer (9).

The co-administration of estrogen plus progestogen was of no overall benefit to clinical pregnancy rates (OR=1.33; $p>0.05$). When routes of progestogen supplementation were considered separately, clinical pregnancy rates did not benefit from the addition of estrogen to progestogen co-administered by either the vaginal or intramuscular routes. Equally, there was no benefit to clinical pregnancy rates of estrogen treatment when different times of progestogen supplementation were considered.

Progesterone supplementation is considered obligatory following the luteal deficiency observed in ART. However, whether supplemental estrogen is also required to ameliorate the effects of declining luteal estradiol remains a matter of debate. Experimental results in human and non-human primates have suggested that normal endometrial function requires only low levels of estradiol (38). Despite this, elevated serum and endometrial estradiol levels following vaginal administration have been suggested to enhance endometrial thickness and implantation (39), whilst others report that it may be detrimental to endometrial receptivity (40). Other studies have failed to find a link between

declining estradiol in early or mid-luteal phase of ART cycles and clinical pregnancy or miscarriage (41).

No differences in endometrial histology were observed in women following GnRH downregulation and progesterone replacement, with or without varying doses of estrogen (42). In contrast, estrogen receptor antagonist treatment delayed endometrial maturation, suggesting a requirement for luteal phase estradiol (43). In addition, endometrial gene expression is altered by controlled ovarian stimulation (44, 45) and proteins were differentially expressed by human endometrial cells in response to high estradiol (46).

There are conflicting clinical reports regarding the value of adding estrogen to luteal support regimes. Progestogens plus estrogen have been associated with higher clinical pregnancy rates than progestogens alone (47), although this has varied by route of administration (48). In contrast other studies have shown no beneficial effect of adding estrogen (10, 49, 50); indeed adverse effects, such as increased miscarriage, have also been reported (51). Equally, it is possible that circulating E2 levels following ovarian stimulation might influence whether further boosting estrogen levels is of benefit (52, 53), however, analysis of this was not possible due to the lack of reporting of E2 levels.

An important consideration in meta-analysis is the consistency between articles, such as the quantity I^2 (54). In the current study, estimation of I^2 was not feasible as within each statistical comparison there were relatively few articles in which both treatments were performed. Thus, reporting I^2 or Cochran's Q statistic would be invalid and not representative of the data presented.

Equally, it is clear from other similar meta-analyses, using different statistical approaches, that there is often moderate to considerable heterogeneity between studies for a particular comparison. For example, in the Cochrane review by van der Linden et al (10) I^2 was estimated to be 71% when comparing intramuscular versus vaginal routes and 56% when comparing the effect of estrogen supplementation. Potential causes of this heterogeneity include variations in ovarian stimulation and / or treatment protocols employed and the characteristics of the patient populations within the different articles.

A limitation of the present study was that the number of articles reporting live birth data was markedly lower than those reporting clinical pregnancy rates. Consequently, the potential for valid sub-analyses in relation to live birth is more restricted. Another important consideration is that the different estrogen regimes (start time and route) were not analysed separately, due to the low number of study groups within each comparison. It is feasible that the different routes and start time could influence pregnancy outcome and this warrants further investigation.

In a worldwide survey of IVF centres, the majority of luteal phase support was continued up to 8-10 weeks of gestation (44%) or beyond (28%), despite suggestions that it can be safely discontinued following a positive HCG test (55, 56) or fetal heart pulsations (57). The rationale for prolonged progesterone is unclear, given that the luteo-placental shift causes placental progesterone to dominate from the 8th week of pregnancy (58). The presence of significant luteotrophic HCG levels by week 5 of pregnancy also lead others to support suspending progesterone early (59). Indeed, in the current study there was no benefit to clinical pregnancy rates of continuing treatment beyond 3 weeks OR=1.06 [CI: 0.87-1.29]; p>0.05). Similarly, live birth rates were not improved by prolonged progesterone (intramuscular; >3 weeks v ≤3 weeks; OR= 1.11).

Conclusion

Our results have clearly established that progestogen supplementation via the IM route offers the most benefit to clinical pregnancy. These results demonstrate that the optimal time to commence supplementation is the day after oocyte retrieval, and that clinical pregnancy rates were not improved by continued supplementation for greater than 3 weeks or by the additional treatment with estrogens. This lack of improvement occurred whether the progestogen was administered by the vaginal or intramuscular route.

These outcomes are in contrast to current trends in global clinical practice and collectively suggest that the clinical approach to luteal phase support may not be delivering optimal benefits. Therefore this study enables the evidence-based re-evaluation of clinical protocols for progestogen supplementation, the provision of improved informed choice for patients and ultimately greater pregnancy success for women undergoing fresh IVF cycles.

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Figure captions

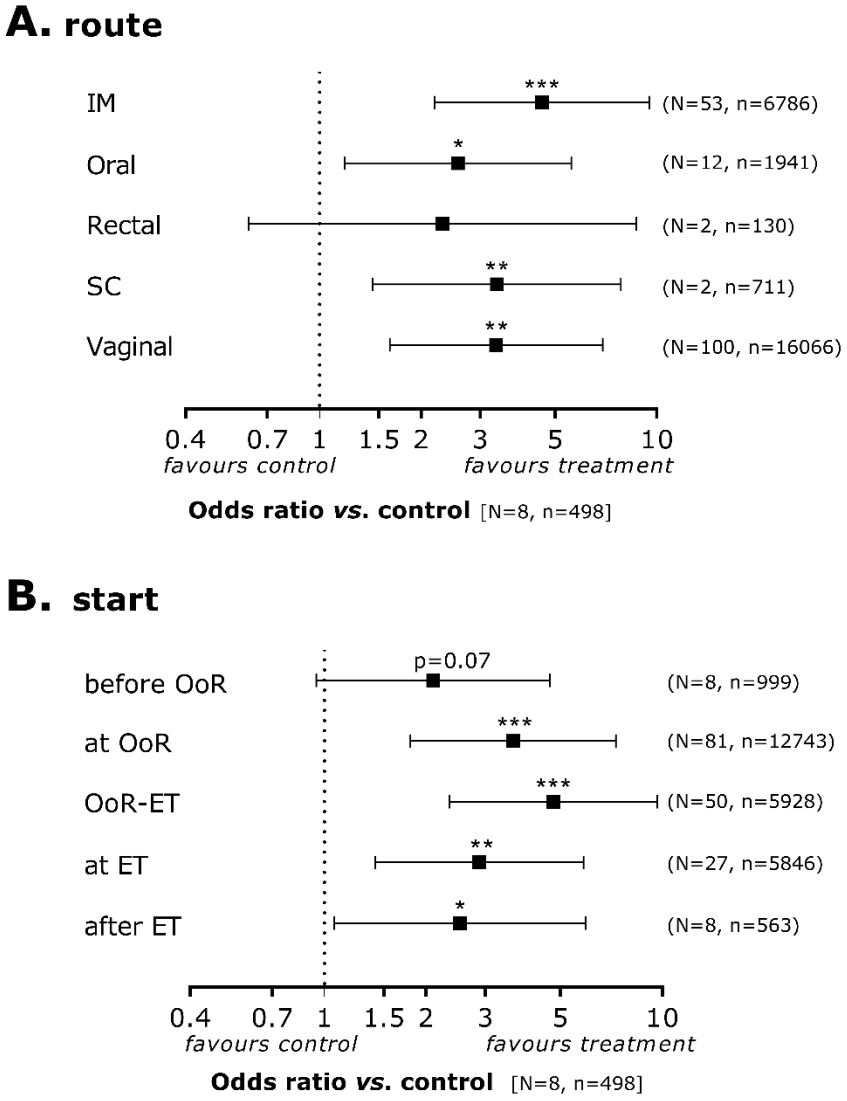
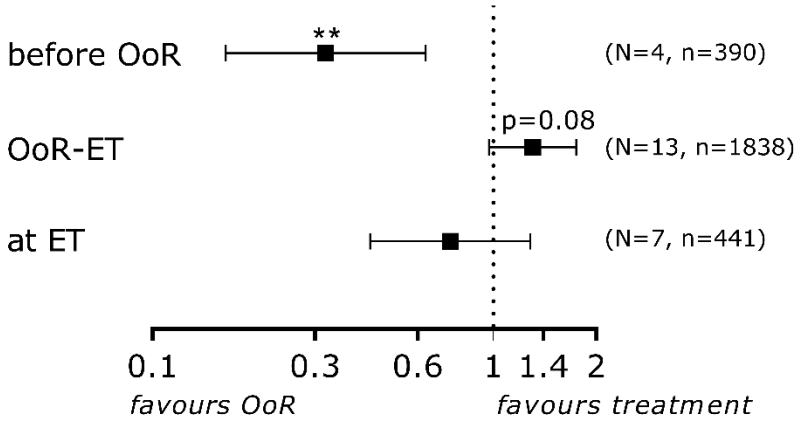


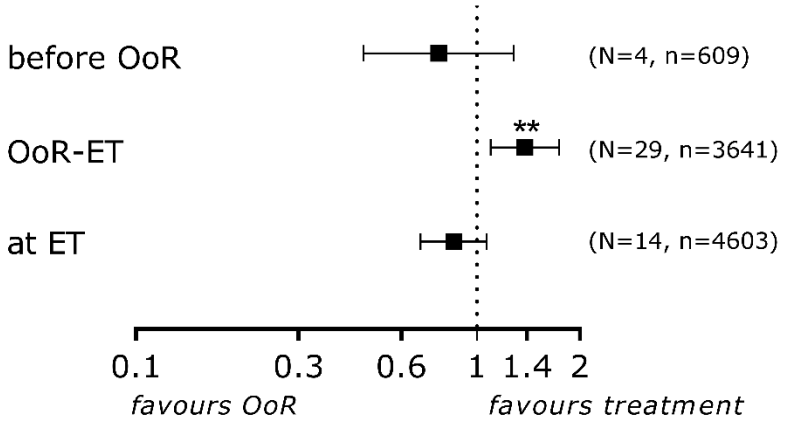
Figure 1: The odds ratio (± 95% confidence intervals) for the relative benefit to clinical pregnancy of the (A) different routes and (B) start time of progestogen supplementation versus no progestogen treatment in women undergoing fresh IVF cycles. In (A), progestogens were administered by intramuscular (IM), oral, rectal, subcutaneous (SC) or vaginal routes. In (B), the different start times for progestogen supplementation were; before the day of oocyte retrieval (before OoR); at oocyte retrieval (at OoR); between oocyte retrieval and the day of embryo transfer (OoR-ET); on the day of embryo transfer (at ET); after the day of embryo transfer (after ET). The dotted line represents the comparative odds ratio for the control (untreated) group. Significant differences between treatment and control are indicated as follows: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. N = number of study groups, n = total number of women, for each treatment or comparator.

A. intramuscular (clinical pregnancy)



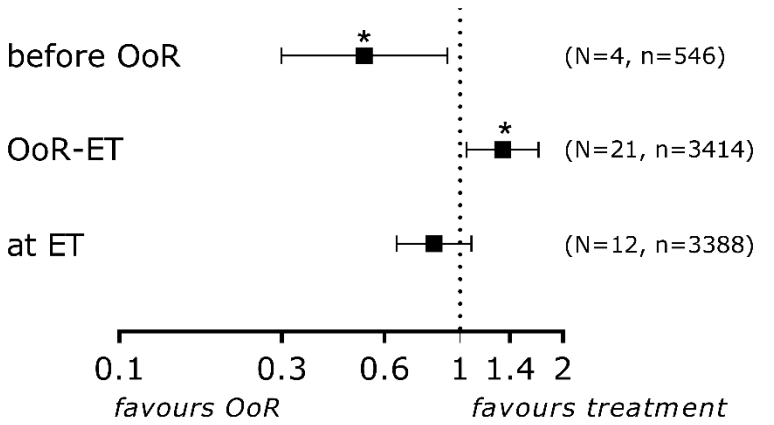
Odds ratio *vs*. at OoR [N=26, n=3968]

B. vaginal (clinical pregnancy)



Odds ratio *vs*. at OoR [N=47, n=6824]

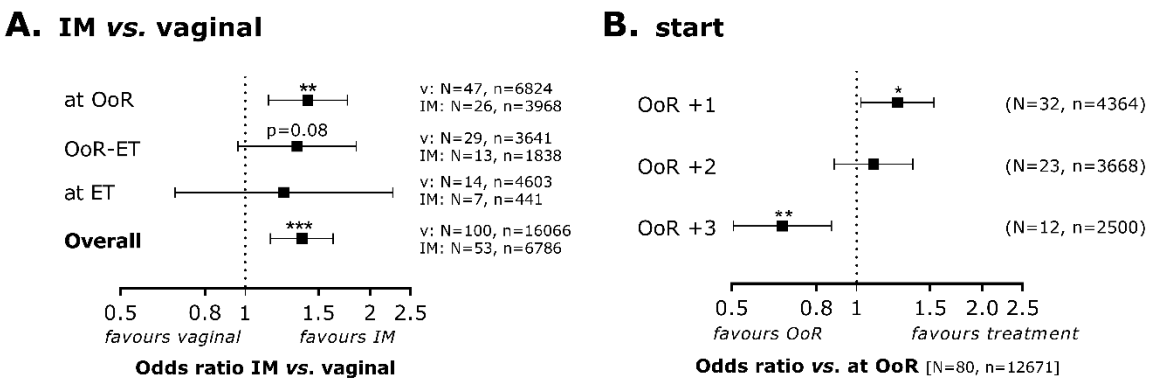
C. all routes (live birth)



Odds ratio *vs*. at OoR [N=26, n=4375]

783

Figure 2: The odds ratio (± 95% confidence intervals) for the relative benefit to clinical pregnancy of the different times to commence A) intramuscular and B) vaginal progestogen supplementation, while C) shows the relative benefit to live births of the different times to commence progestogen supplementation with all routes combined, in women undergoing fresh IVF cycles. The start times were; before the day of oocyte retrieval (before OoR); at oocyte retrieval (at OoR); between oocyte retrieval and the day of embryo transfer (OoR-ET); on the day of embryo transfer (at ET). The dotted line represents the comparative odds ratio for commencing progestogen at oocyte retrieval (OoR). Significant differences between treatment and comparator (at OoR) are indicated as follows: *, $p<0.05$; **, $p<0.01$. N = number of study groups, n = total number of women, for each treatment or comparator.



796

797 **Figure 3:** The odds ratio (± 95% confidence intervals) for the relative benefit to clinical
798 pregnancy of (A) intramuscular versus vaginal progestogen supplementation and (B)
799 commencing on specific days after oocyte retrieval (OoR) in women undergoing fresh IVF
800 cycles. In (A), the analysis was split into the different times that treatment began as
801 follows: at oocyte retrieval (at OoR); between oocyte retrieval and the day of embryo
802 transfer (OoR-ET); on the day of embryo transfer (at ET); at all start times combined
803 (Overall). The dotted line represents the comparative odds ratio for vaginal administration
804 of progestogen. In (B), all routes of administration were included and the different times
805 were as follows: Oocyte retrieval plus 1 day (OoR +1), plus 2 days (OoR +2) and plus 3
806 days (OoR +3). The dotted line represents the comparative odds ratio for administration
807 of progestogen at oocyte retrieval (OoR). Significant differences between treatment and
808 comparator are indicated as follows: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. N = number
809 of study groups, n = total number of women, for each treatment or comparator. IM,
810 intramuscular; v, vaginal.

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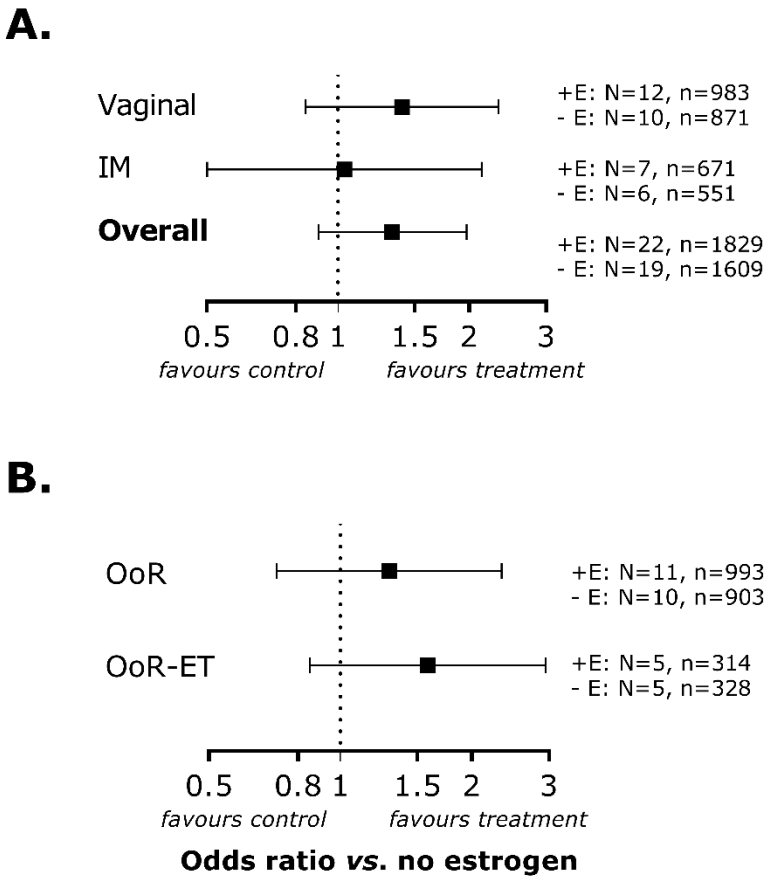
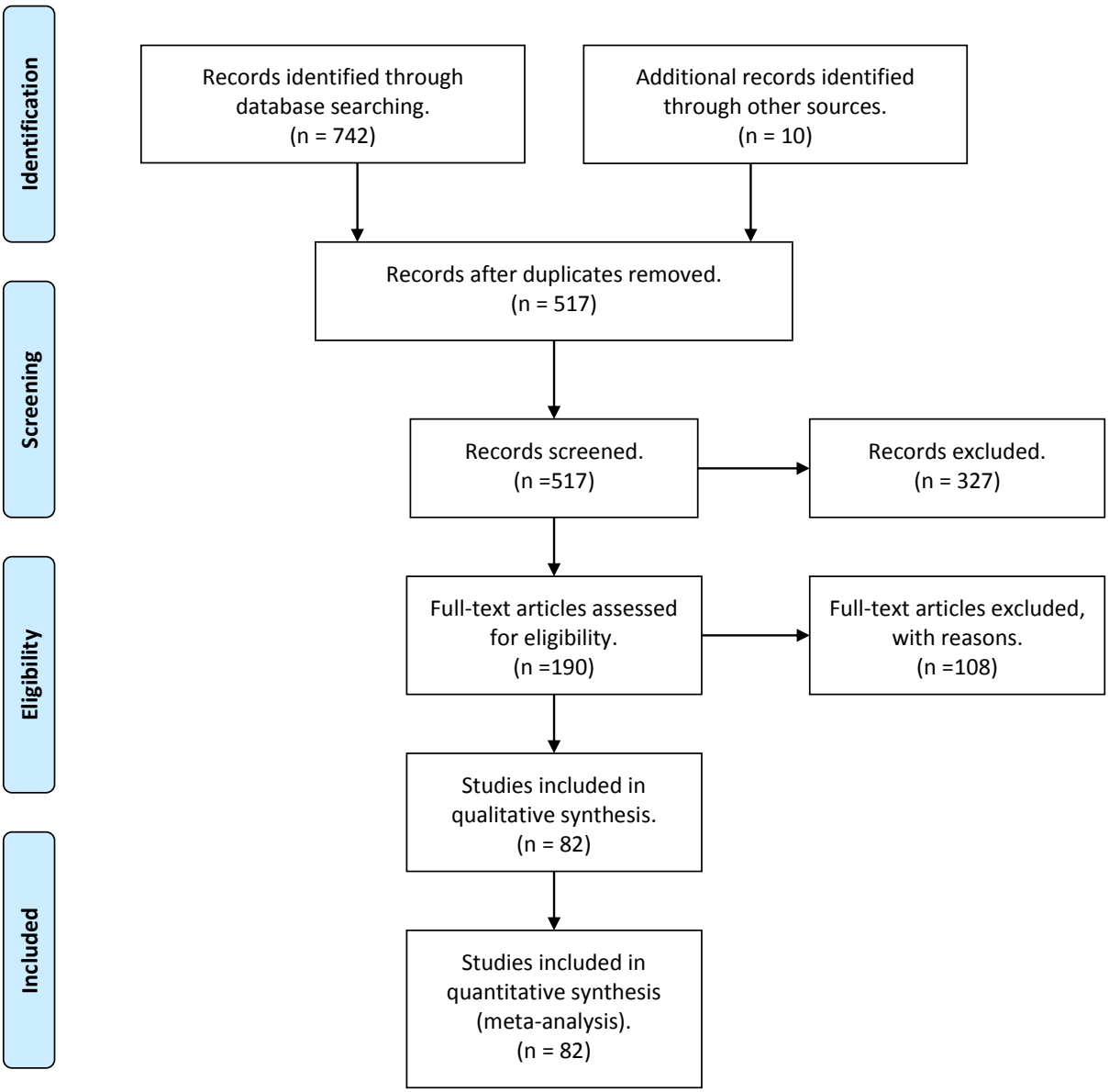


812

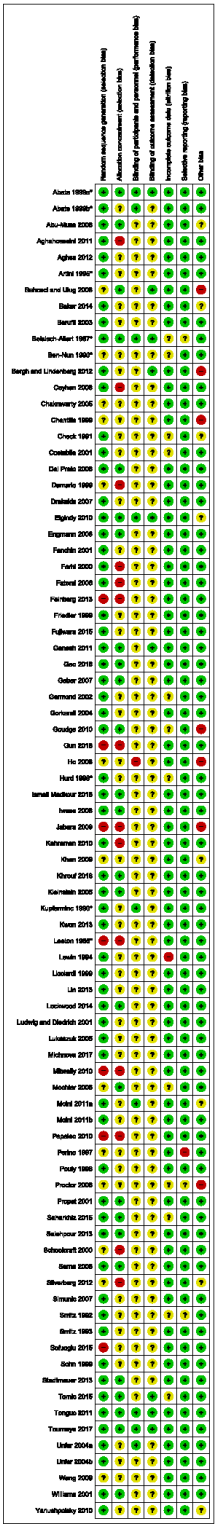
813 **Figure 4:** The odds ratio (± 95% confidence intervals) for the relative benefit to clinical
814 pregnancy of combining estrogen and progestogen supplementation in women undergoing
815 fresh IVF cycles. In A), progestogen was administered by vaginal or intramuscular (IM)
816 routes and all progestogen routes combined (Overall). In B), progestogen administration
817 commenced at oocyte retrieval (OoR) or between oocyte retrieval and embryo transfer
818 (OoR–ET). The dotted line represents the comparative odds ratio for progestogen-only
819 treatment (no estrogen). There were no significant differences between treatment and
820 comparators. N = number of study groups, n = total number of women, for each treatment
821 or comparator. +E, plus estrogen; -E, no estrogen.

822

Figure captions – Supplementary



Supplementary Figure 1: PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) 2009 flow diagram.



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830 **Supplementary Figure 2**: Risk of bias summary: review authors' judgements about
831 each risk of bias item for each included study. Articles including control study groups are
832 indicated by *.

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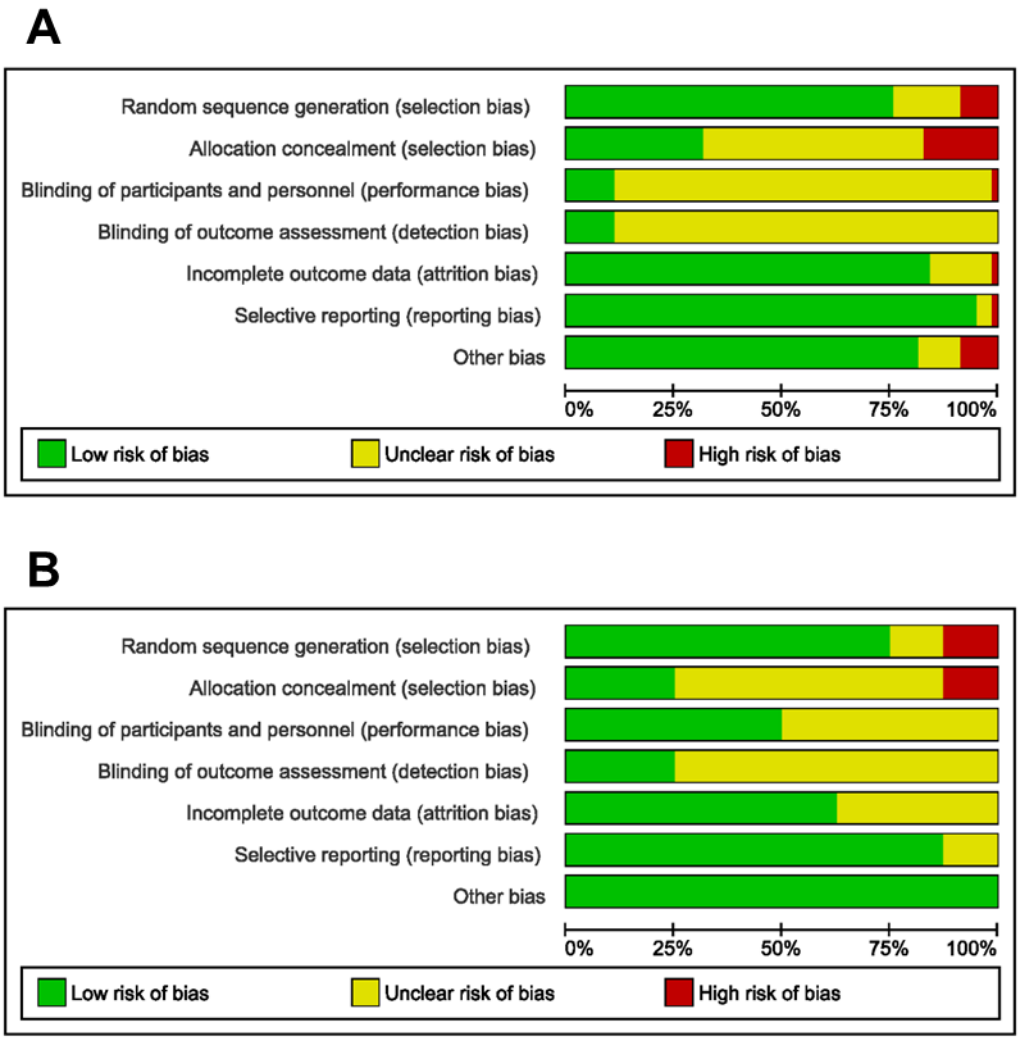

834

835 **Supplementary Figure 3**: Risk of bias graphs: review authors' judgements about each
836 risk of bias item presented as percentages across a) all included studies, and b) those
837 articles which included control study groups.

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839

Supplementary Table 1: Summary and bibliography of the studies investigating the effects of progestogen supplementation in women undergoing ART, which met the inclusion criteria and from which data was extracted.

No.	Reference	Year	Region	No. of women	No. of study groups
1	Abate, et al. (1)	1999a	EUROPE	86	2*
2	Abate, et al. (2)	1999b	EUROPE	156	3*
3	Abu-Musa, et al. (3)	2008	MIDDLE EAST	125	2
4	Aghahosseini, et al. (4)	2011	MIDDLE EAST	108	2
5	Aghsa, et al. (5)	2012	MIDDLE EAST	147	2
6	Artini, et al. (6)	1995	EUROPE	132	3*
7	Bahceci and Ulug (7)	2008	MIDDLE EAST	2013	4
8	Baker, et al. (8)	2014	USA	782	2
9	Baruffi, et al. (9)	2003	S.AMERICA	103	2
10	Belaisch-Allart, et al. (10)	1987	EUROPE	258	2*
11	Ben-Nun, et al. (11)	1990	MIDDLE EAST	111	3*
12	Bergh and Lindenberg (12)	2012	EUROPE	1983	2
13	Ceyhan, et al. (13)	2008	MIDDLE EAST	44	2
14	Chakravarty, et al. (14)	2005	ASIA PACIFIC	430	2
15	Chantilis, et al. (15)	1999	USA	206	2
16	Check, et al. (16)	1991	USA	127	2
17	Costabile, et al. (17)	2001	EUROPE	300	2
18	Dal Prato, et al. (18)	2008	EUROPE	412	3
19	Damario, et al. (19)	1999	USA	271	2
20	Drakakis, et al. (20)	2007	EUROPE	77	2
21	Elgindy, et al. (21)	2010	MIDDLE EAST	270	3
22	Engmann, et al. (22)	2008	UK	166	2
23	Fanchin, et al. (23)	2001	EUROPE	84	2
24	Farhi, et al. (24)	2000	MIDDLE EAST	285	4
25	Fatemi, et al. (25)	2006	EUROPE	182	2
26	Feinberg, et al. (26)	2013	USA	681	2
27	Friedler, et al. (27)	1999	MIDDLE EAST	64	2
28	Fujiwara (28)	2015	ASIA PACIFIC	90	2
29	Ganesh, et al. (29)	2011	ASIA PACIFIC	1363	3
30	Gao, et al. (30)	2018	ASIA PACIFIC	197	2
31	Geber, et al. (31)	2007	MIDDLE EAST	244	2
32	Germond, et al. (32)	2002	EUROPE	114	2
33	Gorkemli, et al. (33)	2004	MIDDLE EAST	288	2
34	Goudge, et al. (34)	2010	USA	97	2
35	Gun, et al. (35)	2016	MIDDLE EAST	177	2
36	Ho, et al. (36)	2008	ASIA PACIFIC	144	2
37	Hurd, et al. (37)	1996	USA	79	2*
38	Ismail Madkour, et al. (38)	2016	USA	220	2
39	Iwase, et al. (39)	2008	ASIA PACIFIC	40	2
40	Jabara, et al. (40)	2009	USA	292	2
41	Kahraman, et al. (41)	2010	MIDDLE EAST	426	2
42	Khan, et al. (42)	2009	USA	240	4
43	Khrouf, et al. (43)	2016	AFRICA	186	3
44	Kleinstein (44)	2005	EUROPE	430	2
45	Kupferminc, et al. (45)	1990	MIDDLE EAST	105	2*

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No.	Reference	Year	Region	No. of women	No. of study groups
46	Kwon, et al. (46)	2013	ASIA PACIFIC	108	2
47	Leeton, et al. (47)	1985	ASIA PACIFIC	186	3*
48	Lewin, et al. (48)	1994	MIDDLE EAST	100	2
49	Licciardi, et al. (49)	1999	USA	43	2
50	Lin, et al. (50)	2013	ASIA PACIFIC	402	4
51	Lockwood, et al. (51)	2014	UK	640	2
52	Ludwig and Diedrich (52)	2001	EUROPE	126	2
53	Lukaszuk, et al. (53)	2005	EUROPE	224	3
54	Michnova, et al. (54)	2017	EUROPE	100	2
55	Mitwally, et al. (55)	2010	USA	544	2
56	Mochtar, et al. (56)	2006	EUROPE	298	3
57	Moini, et al. (57)	2011a	MIDDLE EAST	98	2
58	Moini, et al. (58)	2011b	MIDDLE EAST	153	3
59	Papaleo, et al. (59)	2010	EUROPE	172	2
60	Perino, et al. (60)	1997	EUROPE	300	2
61	Pouly, et al. (61)	1996	EUROPE	283	2
62	Proctor, et al. (62)	2006	USA	358	2
63	Propst, et al. (63)	2001	USA	201	2
64	Saharkhiz, et al. (64)	2015	MIDDLE EAST	210	2
65	Salehpour, et al. (65)	2013	MIDDLE EAST	80	2
66	Schoolcraft, et al. (66)	2000	USA	89	2
67	Serna, et al. (67)	2008	EUROPE	160	2
68	Silverberg, et al. (68)	2012	USA	474	2
69	Simunic, et al. (69)	2007	EUROPE	266	2
70	Smitz, et al. (70)	1992	EUROPE	262	2
71	Smitz, et al. (71)	1993	EUROPE	378	2
72	Sofuoglu, et al. (72)	2015	MIDDLE EAST	463	2
73	Sohn, et al. (73)	1999	USA	282	2
74	Stadtmauer, et al. (74)	2013	USA	1297	2
75	Tomic, et al. (75)	2015	EUROPE	831	2
76	Tonguc, et al. (76)	2011	MIDDLE EAST	285	3
77	Tournaye, et al. (77)	2017	EUROPE	967	2
78	Unfer, et al. (78)	2004a	EUROPE	734	2
79	Unfer, et al. (79)	2004b	EUROPE	284	2
80	Wang, et al. (80)	2009	ASIA PACIFIC	460	2
81	Williams, et al. (81)	2001	USA	126	2
82	Yanushpolsky, et al. (82)	2010	USA	407	2

907 **Supplementary Table 2:** The relative benefit to clinical pregnancy between
908 intramuscular and vaginal progestogen supplementation in women undergoing fresh IVF
909 cycles, stratified by decade.

Decade	**Number of study groups (N), number of women (n); pregnancy rate**	**Odds ratio[1] (*95% CI*)**	**P-value**
1990-1999	N=21, n=2379; 32.3% (vaginal) N=23, n=2277; 36.8% (IM)	**1.27** *(0.94-1.71)*	ns
2000-2009	N=58, n=11086; 39.4% (vaginal) N=18, n=3289; 44.3% (IM)	**1.38** (*1.07-1.79*)	p<0.05
2010-2017	N=21, n=2601; 33.2% (vaginal) N=10, n=1114; 51.2% (IM)	**2.33** (*1.62-3.35*)	p<0.001

910 [1]reference route of administration is vaginal. IM: intramuscular
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