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Systematic review

Effectiveness of seminal plasma in in vitro fertilisation treatment: a systematic review and meta-analysis

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Background With *in vitro* fertilization (IVF) techniques, only 20–25% of the transferred embryos lead to a pregnancy.

Objective To evaluate the beneficial effects of seminal plasma (SP) or semen applied at the time of oocyte aspiration or embryo transfer.

Search strategy Electronic databases were searched from their inception up to August 2017.

Selection criteria We included all randomized controlled trials (RCTs) evaluating the effects of SP or semen in IVF treatment. Trials were considered if women were exposed to any kind of SP or semen (either SP/semen injection or sexual intercourse) around the time of oocyte pickup and embryo transfer.

Data collection and analysis The primary outcome was clinical pregnancy rate (CPR).

Main results Eight RCTs on women undergoing IVF (2128 in total) were included in the meta-analysis. Women randomized in the intervention group had a significantly higher CPR compared with controls (30.0 versus 25.1%; RR 1.20; 95% CI, 1.04–1.39). No significant differences were found in the secondary outcomes, including livebirth rate, biochemical pregnancy, miscarriage, multiple pregnancies, and birth weight. The subgroup analyses (four RCTs, 780 participants), including only those RCTs in which prepared undiluted SP was injected just after oocyte pickup, conformed with the overall analysis for the primary outcome (46.3 versus 37.2%; RR 1.23; 95% CI, 1.05–1.45).

Conclusion Because intravaginal or intracervical SP application around the time of oocyte pickup is associated with higher CPR, local application SP may be considered as a potential treatment to improve implantation.

Keywords Fertility, ICSI, implantation, *in vitro* fertilization, oocyte, seminal plasma.

Tweetable abstract SP at the time of oocyte pickup is associated with higher CPR.

Linked article This article is commented on by DH Barad, p. 226 in this issue. To view this article visit https://doi.org/10.1111/1471-0528.15102.

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Introduction

With *in vitro* fertilization (IVF) techniques, only 20–25% of the transferred embryos lead to a pregnancy.[1] Besides embryo quality, endometrial receptivity plays an important role in the establishment of a pregnancy.[1,2] Around implantation, a feto–maternal dialogue and a unique state of maternal immune tolerance is needed to avoid an immune attack on the implanting and developing semi-allograft conceptus.[2] This requires a well-balanced activation and modulation of pro-inflammatory factors to induce inflammatory pathways in the endometrium during implantation. Endometrial function is highly sensitive to a number of factors including supraphysiological concentrations of estrogen in conventional gonadotropin-stimulated IVF. Accordingly, several studies have revealed functional alterations of the endometrium in IVF therapies, including endometrial immune cell signaling.[1–3]

Seminal plasma (SP), fluid without sperm, has been shown to stimulate the expression of pro-inflammatory cytokines *in vivo* in animal studies and in humans *in vitro*.[1–5] SP and semen have therefore been suggested to support implantation through their beneficial effects on endometrial function and the maternal immune system.[5] Several authors have suggested that SP application might improve implantation in IVF therapies both because the functionally advantageous sexual intercourse is typically avoided around oocyte pickup, and hyperstimulation in IVF therapies seems to negatively affect endometrial function.[5] As SP application possibly compensates for these negative effects, several clinical studies have been performed. In these studies on gonadotropin-stimulated IVF therapies, SP or semen was applied to the vagina or cervix by intercourse or vaginal or cervical injection around the time of follicle aspiration or embryo transfer to improve the outcome. We conducted a systematic review of randomized controlled trials (RCTs) using SP and semen and performed a meta-analysis to summarize and evaluate the effect of this kind of intervention on the IVF outcome.

Methods

Search strategy

This review was performed according to a protocol designed a priori and recommended for systematic reviews. Electronic databases (MEDLINE, Scopus, ClinicalTrials.gov, EMBASE, ScienceDirect, the Cochrane Library at the CENTRAL Register of Controlled Trials, Scielo) were searched from their inception up to August 2017. Search terms used were the following: 'seminal plasma', '*in vitro* fertilization', 'pregnancy rate', 'labor', 'trial', 'randomized', 'review', 'study', 'live birth rate', 'IVF', 'endometrium', 'meta-analysis', 'metaanalysis', 'implantation', 'ICSI', 'coitus', 'intercourse', 'randomised', 'effectiveness', 'guidelines', and 'clinical trial'. No restrictions for language or geographic location were applied. In addition, the reference lists of all identified articles were examined to seek out studies not captured by the electronic searches. The searches and the eligibility of the studies were independently assessed by two authors (GS, AC). Differences were discussed with a third reviewer (ADS).

Study selection

We included all RCTs evaluating the effects of SP on outcome during IVF treatment. Trials were considered if women were exposed to any kind of SP or semen (either SP/semen injection or sexual intercourse) at the time of oocyte pickup and embryo transfer. Analyses included all RCTs comparing the outcome of IVF treatment in women exposed to SP or semen (i.e. intervention group) or not exposed (either placebo or no treatment or abstinence) (i.e. control group).

Quasi RCTs (i.e. trials in which allocation was done on the basis of a pseudo-random sequence, e.g. odd/even hospital number or date of birth, alternation) were excluded.

Risk of bias assessment

The risk of bias in each included study was assessed using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions*. Seven domains related to risk of bias were assessed in each included trial because there is evidence that they are associated with biased estimates of treatment effect: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessment; (5) incomplete outcome data; (6) selective reporting; and (7) other bias. Review authors' judgments were categorized as 'low risk', 'high risk', or 'unclear risk' of bias.

For this review, the GRADE approach was used to assess the quality of the body of evidence relating to the primary and secondary outcomes. The GRADEpro Guideline Development Tool was used to import data from Review Manager 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) to create 'Summary of findings' tables. A summary of the intervention effect and a measure of quality for each of the above outcomes was produced using the GRADE approach. The evidence can be downgraded by one level from 'high quality' for serious (or by two levels for very serious) limitations, depending on assessments for risk of bias, indirectness of evidence, serious inconsistency, imprecision of effect estimates, or potential publication bias.

Outcomes

All analyses were done using an intention-to-treat approach, evaluating women according to the treatment group to which they were randomly allocated in the original trials. The primary outcome was clinical pregnancy rate (CPR) as defined by the original trial. Biochemical pregnancies were not included in the primary outcome.

Secondary outcomes were livebirth rate, biochemical pregnancy rate, incidence of miscarriage and of multiple pregnancy (including twin and higher order pregnancies), and mean birth weight in grams.

Live birth was defined as any delivery of a live infant after 22 weeks of gestation. Biochemical pregnancy was defined as positivity to hCG. Miscarriage was defined as pregnancy loss before 22 weeks, using CPR as denominator.

A subgroup analysis of the primary outcome included only those RCTs in which prepared undiluted SP was injected into the vagina and/or cervix at the time oocyte pickup was planned. We also performed subgroup analyses on sperm-containing and sperm-void inseminations.

Data analysis

Data analysis was completed by two authors independently (GS, ADS) using Review Manager v. 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). The completed analyses were then compared, and any differences resolved by discussion with a third reviewer (AC).

Data from each eligible study were extracted without modification onto custom-made data collection forms. A two-by-two table was assessed for relative risk (RR); continuous outcomes means ± SD were extracted and imported into Review Manager.

Meta-analysis was performed using the random effects model of DerSimonian and Laird to produce summary treatment effects in terms of either a RR or a mean difference (MD) with a 95% confidence interval (CI). Heterogeneity was measured using *I*-squared (Higgins I^2).

Potential publication biases were assessed statistically using Begg's and Egger's tests.

The meta-analysis was reported following the Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) statement.[6] Before data extraction, the review was registered with the PROSPERO international prospective register of systematic reviews (Prospero registration number: 42016054354).

Results

Study selection and study characteristics

The flow of study identification is shown in Figure 1. Eight RCTs on women undergoing IVF (2128 in total) were identified as relevant and included in the meta-analysis.[7–14] No quasi-randomized trials were identified. Publication bias, assessed using Begg's and Egger's tests, was not significant ($P = 0.75$ and 0.84, respectively).

All the included studies had 'low risk' of bias in 'random sequence generation' and 'performance bias'. Allocation concealment was not adequate in all the trials (Figure 2).

The interventions varied: four studies analysed the effect of prepared undiluted SP just after oocyte pickup; one study analysed thawed diluted SP; two studies analysed the effect of sexual intercourse around the time of oocyte aspiration and/or embryo transfer; and one used untreated diluted semen (Table S1).

In detail, the interventions included 0.5 ml of undiluted SP in most of the included studies, while Aflatoonina et al.[11] used sexual intercourse as intervention at least once 12 hours after embryo transfer. Tremellen et al.[12] was a multicentre RCT including women who underwent IVF in two centers. In centre 1 (Australia), intervention included sexual intercourse at least once in a four-day period, from two days before to two days after thawed embryo transfer. In centre 2 (Spain), intervention included sexual intercourse 12 hours before and 12 hours after fresh embryo transfer. All data from both centres were used for this meta-analysis. As control, four trials used 0.5 ml of placebo (sodium chloride solution), two used no insemination, and two used abstinence from sexual intercourse. All RCTs used progesterone for both groups (Table S1).

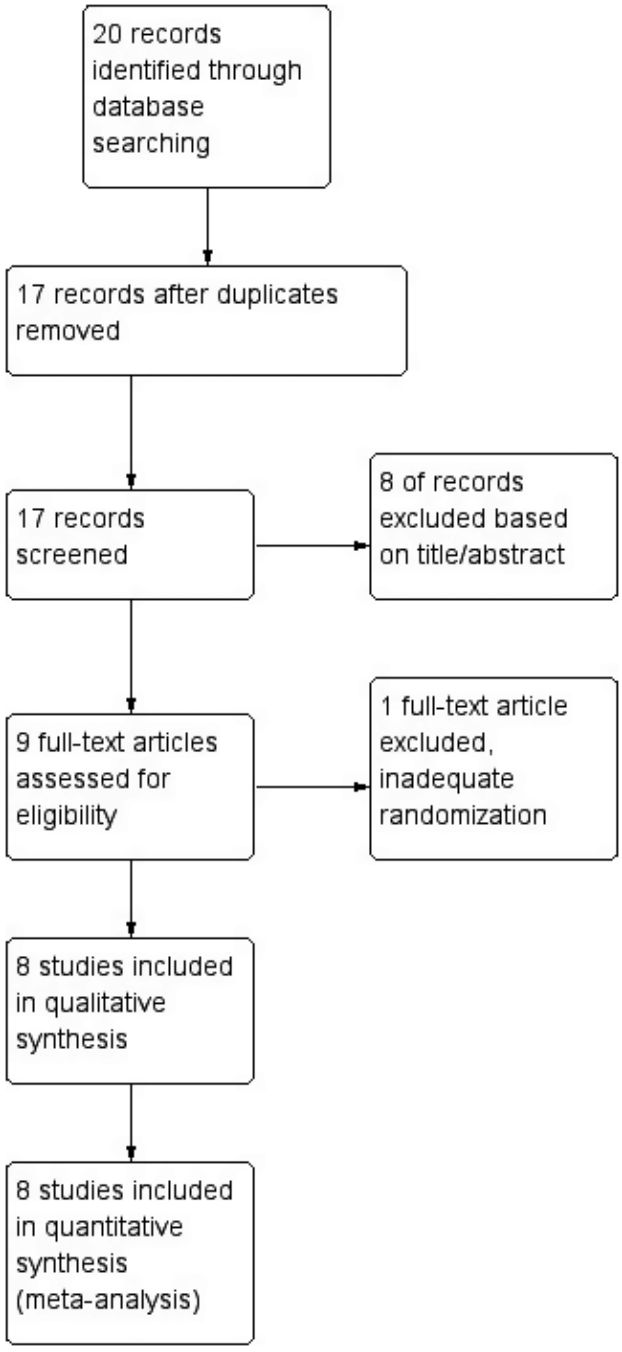

Figure 1. Flow diagram of studies identified in the systematic review. [Prisma template (Preferred Reporting Item for Systematic Reviews and Meta-analyses)].

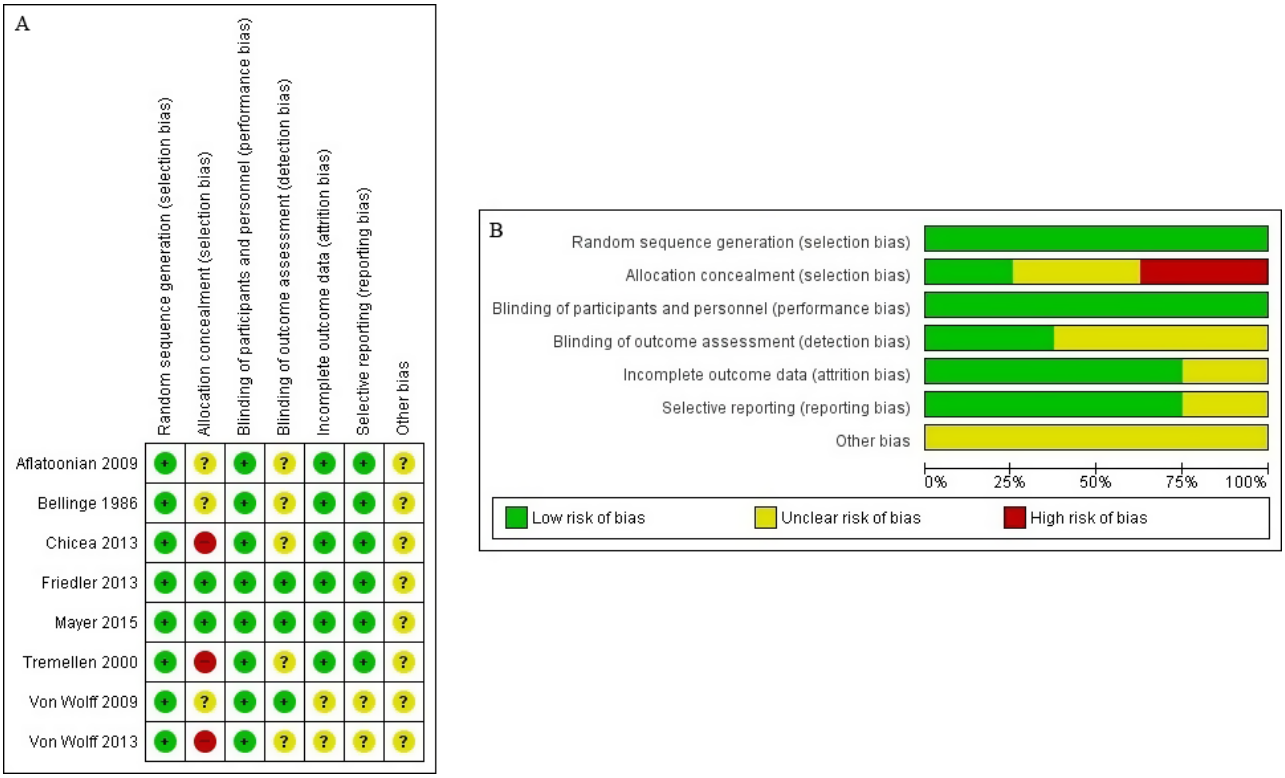

Figure 2. Assessment of risk of bias. (A) Summary of risk of bias for each trial. Plus sign, low risk of bias; minus sign, high risk of bias; question mark, unclear risk of bias. (B) Risks of bias presented as percentages across all included studies.

All trials included couples with an aetiology of infertility (e.g. male factor, tubal factor, mixed factors, unexplained infertility) (Table S2).

Synthesis of results

Table S3 shows the primary and secondary outcomes of the meta-analysis. Women randomized in the intervention group had a significantly higher CPR compared with controls (30.0% versus 25.1%; RR 1.20; 95% CI, 1.04–1.39; Figure 3). No significant differences were found in the secondary outcomes.

The subgroup analyses (four RCTs, 780 participants) including only those RCTs in which prepared undiluted SP was injected just after oocyte pickup were in agreement with the overall analysis for the primary outcome (RR 1.23; 95% CI, 1.05–1.45; Figure 4).

The subgroup analyses of sperm-containing and sperm-void inseminations both accord with the overall analysis for the primary outcome (RR 1.20; 95%, CI 1.09–1.72; and RR 1.26; 95% CI, 1.08–1.66, respectively)

The quality of evidence was downgraded because of serious 'imprecision' in the secondary outcomes. Outcomes were imprecise because studies included relatively few patients and few events and therefore had wide CIs around the estimates of the effect and because the optimal information size was not reached. The quality of the evidence was also downgraded another level because of serious 'indirectness' that was due to differences in the interventions for both primary and secondary outcomes.

Discussion

Main findings

This meta-analysis from eight RCTs on women undergoing IVF (2128 in total) showed that SP or semen application near the time of oocyte pick up (OPU) was associated with higher CPR. Most data come from RCTs using 0.5 ml of undiluted SP injected into the vaginal vault or cervical canal after OPU.

Strengths and limitations

Our study has several strengths: the eight trials included had a low risk of allocation bias based on a Cochrane Collaboration tool assessment; intent-to-treat analysis was used; and statistical analysis showed that publication bias was not apparent. These are key elements that are needed to evaluate the reliability of a meta-analysis. To our knowledge, no prior meta-analysis on this issue is as large, up-to-date, or comprehensive.

Limitations of our study are mostly inherent in the limitations of the included studies. Only four studies

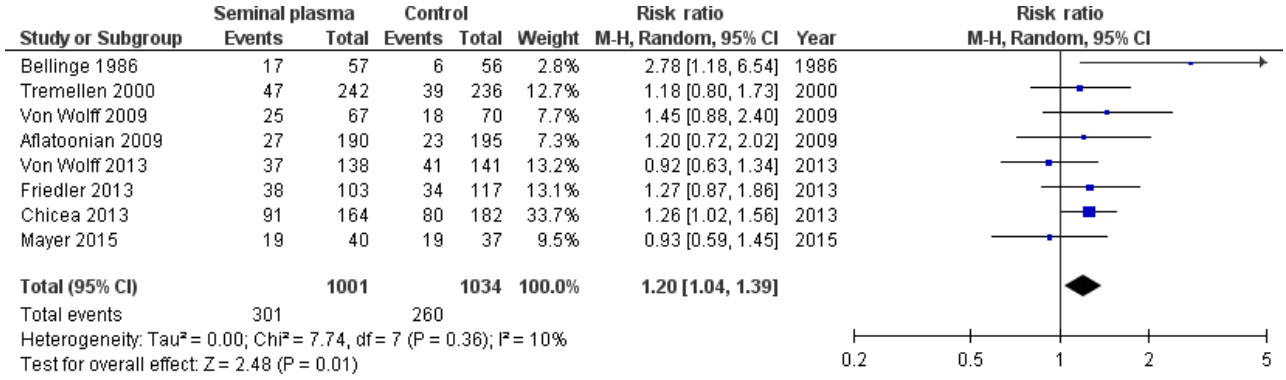

Figure 3. Forest plot of clinical pregnancy rates in the overall analysis. CI, confidence interval.

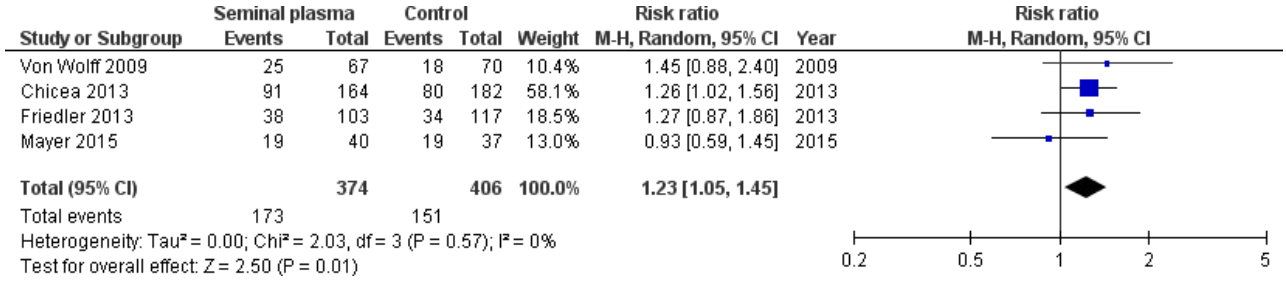

Figure 4. Forest plot of clinical pregnancy rates in trials using only prepared undiluted seminal plasma injected immediately after oocyte pickup. CI, confidence interval.

used a placebo as control and were double blind. We acknowledge that some outcomes were underpowered, but those were uncommon outcomes (e.g. miscarriage, multiple pregnancy) with an estimated overall rate of <10%. The major shortcomings of this meta-analysis lie in the differences in the intervention protocols used and the different definitions of CPR. The observed effect may be based on endometrial factors and not simply exposure to SP. The definition of clinical pregnancy was also different between the trials. Finally, there was a lack of core outcome sets which affects infertility research owing to a lack of standardization of study outcomes. The timing of insemination as well as unknown or unmeasured factors not reported in publications could have modified the observed associations. While we did not include as-per-protocol biochemical pregnancies in the primary outcome (i.e. CPR), one trial did not specify if biochemical pregnancies were included in the total numbers reported for CPR.

Interpretation

This review included different interventions, including different SP application at the time of oocyte pick up. So far, this analysis only allows us to judge the effect of any kind of SP or semen exposure. To analyse if SP may be used as a therapy in conventional gonadotropin-stimulated IVF, subgroup analyses according to type of intervention have been assessed.[15,16]

As assessed by GRADE, the quality level of summary estimates was moderate for the primary outcome and low for the secondary outcomes, indicating that the true effect may or is likely to be substantially different from the estimated effect.

Our study was in agreement with a prior review.[16] Crawford et al., in a meta-analysis of seven RCTs, found significantly improved outcomes when women were exposed to SP around the time of ovum pickup or embryo transfer. Our review included more RCTs and more randomized women, however. We also obtained additional unpublished data and performed subgroup analyses.

Conclusion

SP may be able to stimulate the expression of pro-inflammatory cytokines *in vitro* like interleukin (interleukin-1β) and leukaemia inhibiting factor. A few studies have shown that endometrial immune response to SP antigens could activate inflammatory pathways that may have a positive effect on the implantation rate.[2–4] Our review, based on 8 RCTs, shows a statistically significant increase in CPR in women who were exposed to SP during their IVF cycle. These findings could add value to the role of SP in women undergoing IVF.

In summary, based on these level-1 data, there is a significant association of a higher CPR not only with all kinds of SP and semen applications around the time of oocyte pickup and embryo transfer, but also specifically with intravaginal and intracervical injection of prepared undiluted SP exactly at the time of oocyte pickup. These findings support the hypothesis that SP has a positive effect on endometrial function and the maternal immune system, thereby supporting implantation. Furthermore it suggests SP application as a potential therapeutic tool to improve implantation in IVF therapy. However, as secondary outcomes, including live birth and miscarriage, were not statistically different, further studies need to be undertaken to better understand whether and under what circumstances the use of SP injection near the time of OPU translates into better clinical outcomes. Future trials should report on all pertinent pregnancy outcomes and include cost-effectiveness analyses. Most importantly, future studies should include a clear protocol (e.g. progesterone, intravaginal, or intracervical injection), so they may be easily evaluated and replicated.

Disclosure of interests

None declared. Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship

All authors conceived, designed, and performed the experiments, contributed materials and tools, and wrote the paper. GS and AC analysed the data. All authors approved the final version of the manuscript.

Details of ethics approval

Not applicable.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of the included trials.

Table S2. Characteristics of the included women.

Table S3. Primary and secondary outcomes. ■

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