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REVIEW

The effect of intrauterine HCG injection on IVF outcome: a systematic review and meta-analysis

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Abstract In this systematic review and meta-analysis, the effect of intrauterine HCG infusion before embryo transfer on IVF outcomes (live birth rate, clinical pregnancy rate and spontaneous aboretion rate) was investigated. Searches were conducted on *MEDLINE*, *EMBASE* and The *Cochrane Library*. Randomized studies in women undergoing IVF and intracytoplasmic sperm injection comparing intrauterine HCG administration at embryo transfer compared with no intrauterine HCG were eligible for inclusion. Eight randomized controlled trials were eligible for inclusion in the meta-analysis. A total of 3087 women undergoing IVF and intracytoplasmic sperm injection cycles were enrolled (intrauterine HCG group: $n = 1614$; control group: $n = 1473$). No significant difference was found in the live birth rate (RR 1.13; 95% CI 0.84 to 1.53) and spontaneous abortion rate (RR 1.00, 95% CI 0.74 to 1.34) between women who received intrauterine HCG and those who did not receive HCG. Although this review was extensive and included randomized controlled trials, no significant heterogeneity was found, and the overall included numbers are relatively small. In conclusion the current evidence does not support the use of intrauterine HCG administration before embryo transfer. Well-designed multicentre trials are needed to provide robust evidence.

KEYWORDS: HCG injection, human chorionic gonadotrophin, intrauterine HCG

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Introduction

Embryo implantation remains low despite advances in asssted reprodction techniques (Norwitz et al., 2001). Although most of the causes of implantation failure are embryonic in origin, endometrial contribution cannot be underestimated (Macklon and Brosens, 2014). This has led investigators to propose several interventions to improve endometrial receptivity (Derks et al., 2009; Nastri et al., 2012). Among these interventions is intrauterine infusion of HCG before embryo transfer.

The role played by HCG in natural as well as in assisted conception is important. It is produced by the trophoblastic cells to facilitate implantation and its use has been extended as a substitute for LH surge to trigger ovulation in IVF when pituitary suppression is used. It also maintains progesterone production from the corpus luteum for luteal phase support and suppression of uterine myometrial contractility (Doheny et al., 2003).

Both animal and human studies have shown that HCG is implicated in the process of embryo implantation (Licht et al., 2001; Sherwin et al., 2007). It has been detected as early as 7 days after fertilization in culture media (Hay and Lopata, 1988), and results in the inhibition of insulin-like growth factor-binding protein 1, which could lead to prolongation of the window of endometrial receptivity (Licht et al., 1998). It also stimulates angiogenesis by increasing vascular endothelial growth factor release; modulate implantation by increasing leukemia inhibitory factor and tissue remodelling through stimulating endometrial matrix-metalloproteinases (MMP-9) (Licht et al., 1998; Paiva et al., 2011; Psychoyos, 1973). Additionally, evidence shows that HCG is secreted by the endometrium in the secretory phase and that full-length HCG receptors are expressed mostly in the mid-luteal phase (Zimmermann et al., 2009), suggesting that HCG produced by the endometrium has a paracrine role that can contribute to endometrial pre-decidualization (Licht et al., 2001).

These molecular functions have encouraged clinicians to investigate the effect of intrauterine HCG infusion at the time of embryo transfer on pregnancy rates in IVF programmes. To date, several studies have been conductd with conflicting results (Aaleyasin et al., 2015; Hong et al., 2014; Mansour et al., 2011; Santibáñez et al., 2014; Wirleitner et al., 2015; Zarei et al., 2014). The aim of this systematic review was to establish whether intrauterine infusion of HCG at the time of embryo transfer could improve IVF outcome.

Materials and methods

Literature search methodology

The following databases were searched for randomized controlled trials: *MEDLINE* (1950 to 31 August 2015), *EMBASE* (1980 to 31 August 2015), and *The Cochrane Library*. A combination of Medical Subject Headings (MeSH) and text words were used to generate two subsets of citations, one including studies of "intrauterine HCG" or "human chorionic gonadotrophin" or "HCG injection" and the second "IVF" or "implantation". These subsets were combined using "AND" to generate a subset of citations relevant to our research question. The reference lists of all known primary and review articles were examined to identify cited articles not captured by the electronic searches. No language restrictions were placed on any of our searches. The searches were conducted independently by AO and ME.

Study selection

Studies were selected if they were randomized, and the target population was women undergoing IVF and intracytoplasmic sperm injection (ICSI), who were given intrauterine HCG at the time of embryo transfer and were compared with women who had embryo transfer with no intrauterine HCG administration. The primary outcome measure was the live birth rate (LBR). Secondary outcomes were the clinical pregnancy (CPR) and the spontaneous abortion rates.

A two-stage process was used for study selection. First, two reviewers (AO and ME) scrutinized the titles and abstracts from the electronic searches independently and full manuscripts of all citations that were likely to meet the predefined selection criteria were obtained. Second, final inclusion or exclusion decisions were made on examination of the full manuscripts. In cases of duplicate publication, the most recent or complete versions were selected. Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer (TET).

Assessment of methodological quality and data extraction

Each study included was assessed for method of randomization, allocation concealment, blinding, and completeness of outcome data, intention to treat analysis, outcome reporting and other potential sources of bias. The selected studies were assessed for methodological quality by using the components of study design that are related to internal validity. Assessment of methodological quality was based on the guidelines in the Cochrane Handbook for Systematic Reviews of Interventions v 5.1.0 (Julian PT Higgins and Sally Green). Two reviewers (AO and SD) completed data extraction and quality assessment (Berlin and Rennie, 1999).

Statistical analysis

From each study, two reviewers extracted outcome data. The relative risks with 95% confidence interval for dichotomous measures are calculated for each study and then these relative risks are pooled to get an overall relative risk. $P < 0.05$ is considered statistically significant. The results from individual studies were pooled using either a fixed effect (Mantel and Haenszel, 1959) or random effects model as appropriate (DerSimonian and Laird, 1986). Heterogeneity of the exposure effect was evaluated graphically using forest plots (Lewis and Clarke, 2001) and statistically using the I^2 statistic (Higgins and Thompson, 2002). If the I^2 value was greater than 50%, showing significant heterogeneity, a random effect model was used. A chi-squared test for heterogeneity was also performed and the *P*-values were presented. Exploration of causes of heterogeneity was planned using variations in

features of population, exposure and study quality. We adhered to published guidance for conducting systematic reviews, i.e, *The Cochrane Handbook* throughout. RevMan 5.2.7 software (Cochrane Collaboration, Oxford, UK) was used for statistical analyses.

Results

Literature search

The process of literature identification and selection is summarized in **Figure 1**. Of the 2401 publications identified by the search, 12 were selected during the initial screening. After examination of the full manuscripts, four studies were excluded (**Figure 1**). Eight studies satisfied the selection criteria and were included in this review.

Study characteristics

The eight included studies enrolled 3087 participants. The sample size per study varied across the trials and ranged from 44 to 1186 participants. The characteristics of the included trials are presented in **Table 1**, which indicates the inclusion and exclusion criteria, method of randomization, sample size, treatment protocol, dose, type and timing of intrauterine HCG used before embryo transfer, and all outcomes reported. Methodological randomization and quality is presented in **Table 2**, which indicates the follow up rate for patients. In total, 1614 women were randomized to treatment with intrauterine HCG and 1473 women were randomized not to receive intrauterine HCG at the time of embryo transfer. Out of the eight studies, two were published as oral conference abstracts (Cambiaghi et al., 2013; Janati et al., 2014). Risk of bias in the included trials is represented in **Figures 2 and 3**.

Primary outcome measure

Live birth rate

Three studies (Aaleyasin et al., 2015; Mansour et al., 2011; Wirleitner et al., 2015) reported LBR. Pooling the results of the three studies (n = 2164) showed no significant difference between the group who received intrauterine HCG and the control group (RR 1.13; 95% CI 0.84 to 1.53) (**Figure 4**). Significant heterogeneity was found between the studies (I^2 = 86%; P = 0.0008); therefore, a random effects model was used for pooling of results.

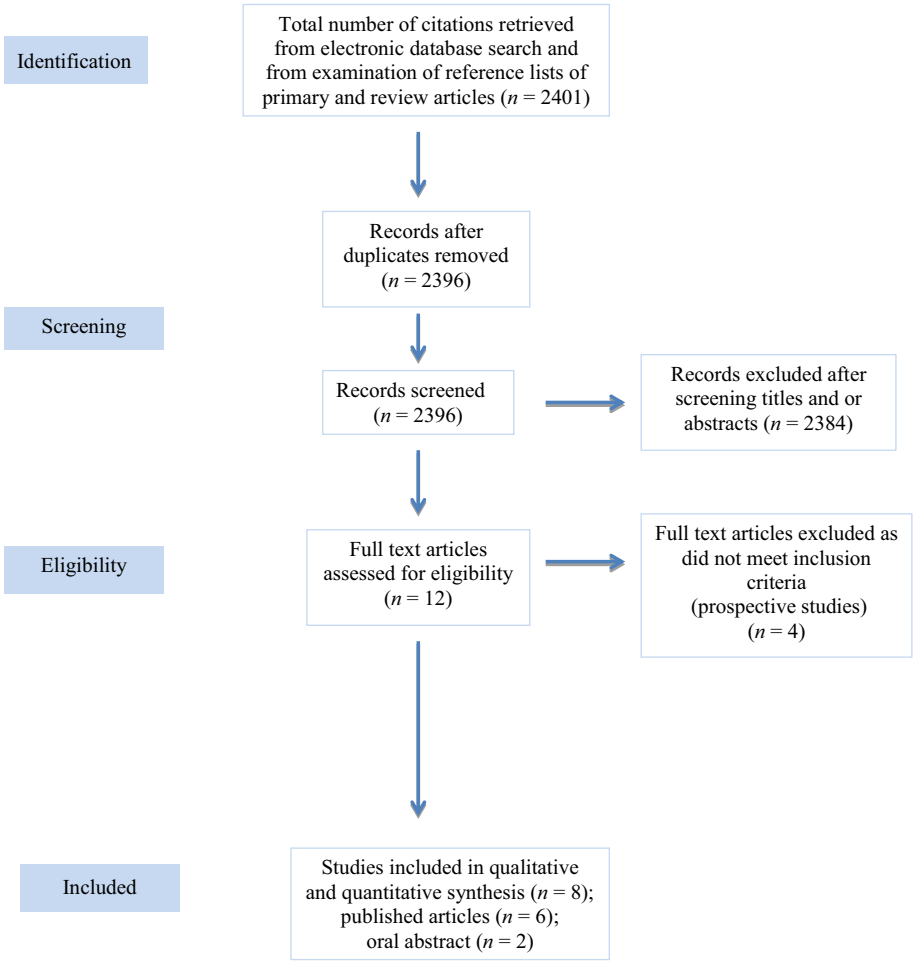

Figure 1 Study selection process for the systematic review.

Table 1 Characteristics of the studies included in the systematic review.

Author/number of cases and controls	*Inclusion criteria*	*Exclusion criteria*	*Cases protocol*	*Control protocol*	*Variables accounted for*	*Embryo stage*	*Outcomes*
Aaleyasin et al., 2015: *n* = 483; cases: *n* = 240; control: *n* = 243	All infertile women <40 years undergoing first IVF–ICSI	Age >40 years, percutaneous epididymal sperm aspiration, testicular sperm extraction, myomectomy, hydrosalpinges, uterine fibroid with press effect on endometrium, endometriosis and azoospermia	500 IU HCG (urinary) in 0.05 ml culture media given 5–7 min before the embryo transfer	0.05 ml culture media without HCG	Age, antral follicle count, anti-Müllerian hormone, type and duration of infertility, oocyte number, 2PN, number of embryo transfer	Day 2–3	Implantation rate plus clinical pregnancy rate plus spontaneous abortion plus live birth rate
Cambiaghi et al., 2013 (abstract): *n* = 44; cases = 22; control = 22	Fresh donor blastocyst and endometrial thickness >7 mm in recipient	Not mentioned	500 IU HCG 6 h before embryo transfer (volume and type not mentioned)	Straight to transfer	Not mentioned	Day 5 blastocyst	Implantation rate plus clinical pregnancy rate
Hong et al., 2014: *n* = 300; cases *n* = 148; control *n* = 152	Women's age <43 years, all patients with fresh or frozen	Not simultaneously participating in other prospective trial	500 IU HCG (purified urinary) in 0.02 ml culture media given less than 3 min before embryo transfer.	0.02 ml culture media without HCG	Age, number of embryos transferred. Patients of advanced age or previous failed implantation were offered comprehensive chromosome screening.	Day 6 blastocyst fresh or frozen	Sustained implantation rate (transferred embryo reaching ≥24 weeks gestation) plus clinical pregnancy rate plus spontaneous abortion
Janati et al., 2014 (abstract): *n* = 159; cases: *n* = 106; control *n* = 53	Women undergoing IVF–ICSI	Not mentioned	500 IU and 1000 IU (volume, type, duration not mentioned)	No HCG	Not mentioned	Not mentioned	Implantation rate plus clinical pregnancy rate

(*continued on next page*)

Table 1 (*continued*)

Author/number of cases and controls	*Inclusion criteria*	*Exclusion criteria*	*Cases protocol*	*Control protocol*	*Variables accounted for*	*Embryo stage*	*Outcomes*
Mansour et al., 2011: *n* = 495; cases: *n* = 293; control: *n* = 202	Women's age <40 years; male factor	Previous IVF–ICSI, azoospermia, uterine myoma/ myomectomy, endometriosis, hydrosalpinges	100, 200 and 500 IU HCG in 0.04 ml culture medium given IU 7 min before embryo transfer.	No HCG or culture media	Age, duration of infertility, oocyte number, 2PN, number of embry transfers	Cleavage stage	Implantation rate plus clinical pregnancy rate plus spontaneous abortion plus live birth rate
Santibáñez et al., 2014; *n* = 210; cases *n* = 101; control: *n* = 109	Women's age <40 years; donors and non donors, recurrent implantation failure	Azoospermia	500 IU HCG (urinary) in 0.02 ml culture media given 4 min before transfer	0.02 ml culture media without HCG	Age, oocyte number, 2PN, embryo transfer number	Day 3 fresh or frozen	Biochemical pregnancy, clinical pregnancy rate
Wirleitner et al., 2015: *n* = 1186; cases = 599; control = 587	Women's age <43 years	Recurrent implantation failure, donors cycles	500 IU HCG (urinary) in 0.04 ml of culture media, cohort An infusion carried out 48 h before transfer, cohort B infusion 3 min before transfer	0.04 ml culture media without HCG	Age, cause of infertility, oocyte number, 2PN, embryo transfer number, embryo quality	Day 5 blastocyst	Pregnancy rates; clinical pregnancy rate; spontaneous abortion rate; live birth rate
Zarei et al., 2014: *n* = 210; cases: *n* = 105; control: *n* = 105	Women's age 18–40 years, normal day 3 hormonal profile, thyroid stimulating hormone, prolactin and HSG	Autoimmune disorder, endocrinopathies, previous IVF–ICSI, endometriosis, azoospermia and hydrosalpinges.	250 μg of HCG (recombinant), equivalent to 6500 IU, 12 min before embryo transfer.	0.5 ml normal saline instead of HCG.	Age, duration of infertility, oocyte number, 2PN, embryo transfer number	Day 3	Implantation rate plus clinical pregnancy rate plus spontaneous abortion rate

2PN, two-pronuclear zygote.

Table 2 Quality of studies included in the systematic review.

Author	*Method of randomization*	*Allocation concealment*	*Blinding*	*Intention-to-treat analysis*	*Follow-up rate (%)*	*Design*
Aaleyasin et al., 2015	Computer generated	Not documented	Double	Yes	100	RCT
Cambiaghi et al., 2013 (oral abstract)	Computer generated	Not documented	ND	Yes	100	RCT
Hong et al., 2014	Random number function used to create variable blocks of four to eight.	Yes (sealed opaque envelope)	Double	Yes	100	RCT
Janati et al., 2014 (oral abstract)	Computer generated	Not documented	ND	Yes	100	RCT
Mansour et al., 2011	Random allocation	Yes (dark sealed envelope)	ND	Yes	92 (total) Cases: 90 (29 dropouts) Control 95 (11 dropouts)	RCT
Santibáñez et al., 2014	Computer generated	Not documented	Double	Yes	100	RCT
Wirleitner et al., 2015	Computer generated	Not documented	YES	Yes	98 (23 dropouts)[a]	RCT
Zarei et al., 2014	Computer generated	Not documented	Double	Yes	86 Cases: 78 (23 dropouts) Control: 93 (seven dropouts)	RCT

RCT, randomized controlled trial.
[a]Dropouts not included in the analysis as number of cases and controls was not indicated.

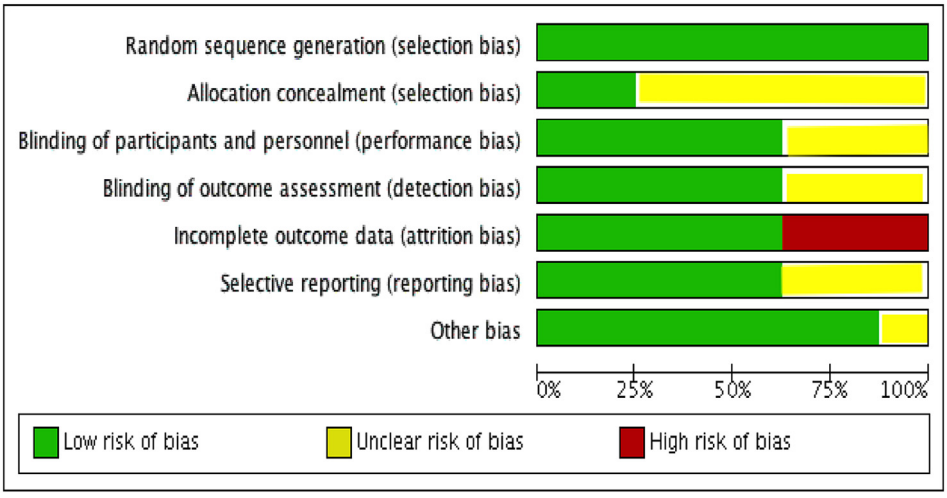

Figure 2 Risk of bias for studies included.

Secondary outcome measures

Clinical pregnancy rate

All eight studies reported CPR. Significant heterogeneity was found between the studies ($I^2 = 71\%$; $P = 0.001$); therefore, a random effects model was used. Pooling of the results of the eight studies ($n = 3087$) showed that the HCG group had significantly higher CPR compared with the control group (RR 1.18; 95% CI 1.00 to 1.39; $P = 0.04$) (**Figure 5**).

Spontaneous abortion rate

Five studies (Aaleyasin et al., 2015; Hong et al., 2014; Mansour et al., 2011; Wirleitner et al., 2015; Zarei et al., 2014) reported spontaneous abortion rate. No significant heterogeneity was found between the studies ($I^2 = 0\%$); therefore, a fixed effects model was used. Pooling of the results of these studies ($n = 1216$) showed that no significant difference in spontaneous abortion rate was found between the two groups (RR 1.00; 95% CI 0.74 to 1.34) (**Figure 6**).

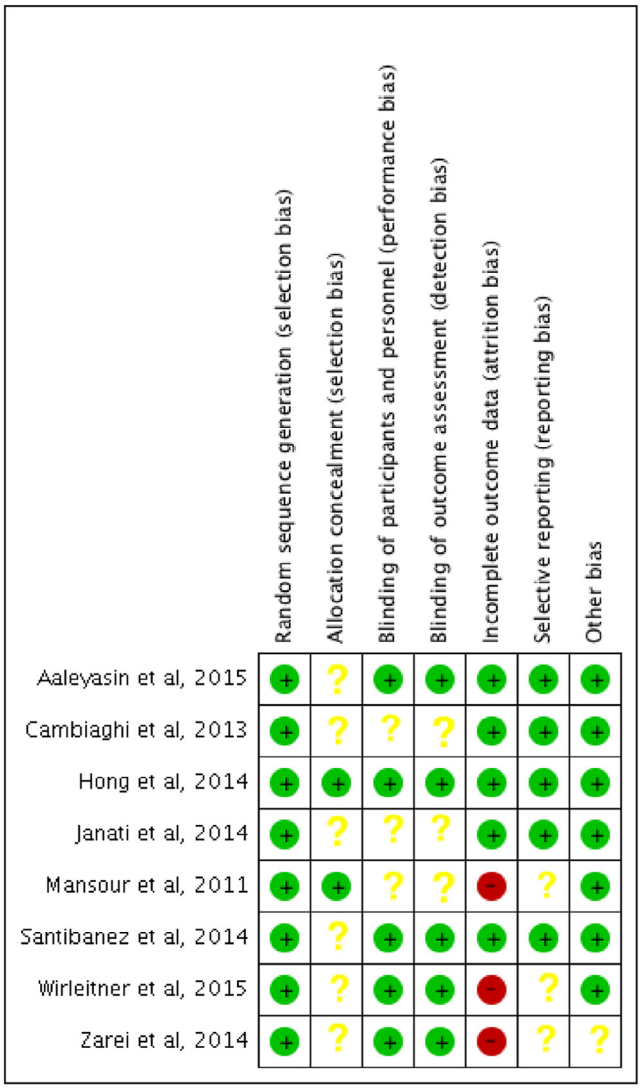

Figure 3 Risk of bias for studies included.

A sensitivity analysis was conducted to examine the effect of a fixed dose of HCG (500 IU regimens). The LBR results of three studies (Aaleyasin et al., 2015; Mansour et al., 2011; Wirleitner et al., 2015) ($n = 1884$) were pooled. No significant difference between the two groups was observed (RR 1.28; 95% CI 0.87 to 1.87) (**Figure 7**). A significant heterogeneity between the studies was found ($I^2 = 89\%$; $P = 0.0001$); therefore, a random effects model was used.

Seven studies (Aaleyasin et al., 2015; Cambiaghi et al., 2013; Hong et al., 2014; Janati et al., 2014; Mansour et al., 2011; Santibáñez et al., 2014; Wirleitner et al., 2015) used a dose of 500 IU of HCG and reported CPR. Pooling of the results of these studies ($n = 2544$) showed a statistically significant difference in CPR with the use of intrauterine HCG compared with no HCG (RR 1.21; 95% CI 1.01 to 1.46; $P = 0.04$) (**Figure 8**). No significant heterogeneity was found between the studies ($I^2 = 75\%$; $P = 0.0006$); therefore, a random effects model was used.

The spontaneous abortion rate results of four studies (Aaleyasin et al., 2015; Hong et al., 2014; Mansour et al., 2011; Wirleitner et al., 2015) ($n = 1018$), used a dose of 500 IU of HCG, were pooled. No significant difference between the two groups was observed (RR 0.93; 95% CI 0.68 to 1.27) (**Figure 9**). No significant heterogeneity between the studies was observed ($I^2 = 0\%$); therefore, a fixed effects model was used.

Discussion

This systematic review and meta-analysis of reported randomized controlled trials of intrauterine HCG infusion before embryo transfer suggest that this intervention does not improve the LBR after IVF treatment. Although the CPR in patients who received intrauterine HCG significantly increased compared with patients who did not receive HCG, the confidence interval approached the line of unity, suggesting that a significant benefit on IVF outcome could not be confirmed. Studies that used 500 IU of HCG were then analysed separately to determine if this certain dose has an effect on the treatment outcome. Similar results were reproduced.

To the best of our knowledge, this meta-analysis is the first to evaluate the effect of intrauterine HCG infusion before embryo transfer on LBR. Our results are in disagreement with a recently published meta-analysis (Ye et al., 2015). Our study used the LBR as the primary outcome, and included additional studies of which one has the largest number of participants to date (Wirleitner et al., 2015).

It is well established that the early developing embryo secretes HCG. The level of HCG in the culture media of developing embryos is positively correlated with the number of the blastomeres as well as embryo grade (Wang et al., 2014). Therefore, it has been suggested that embryos secreting higher levels of HCG, reflecting a good quality embryo, have higher

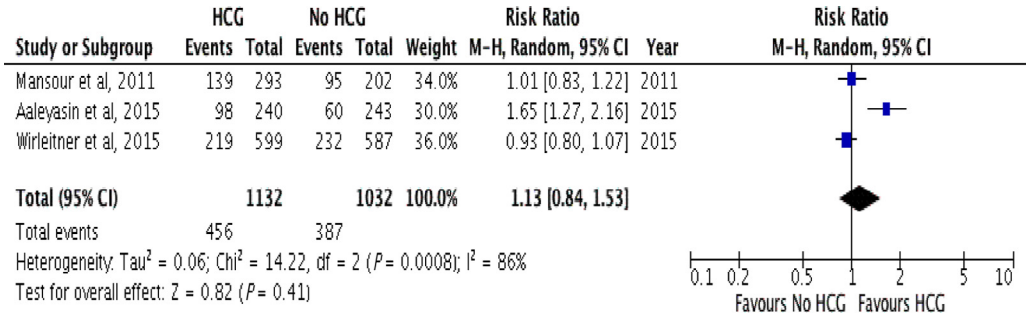

Figure 4 Live birth rates for intrauterine HCG administration versus no HCG.

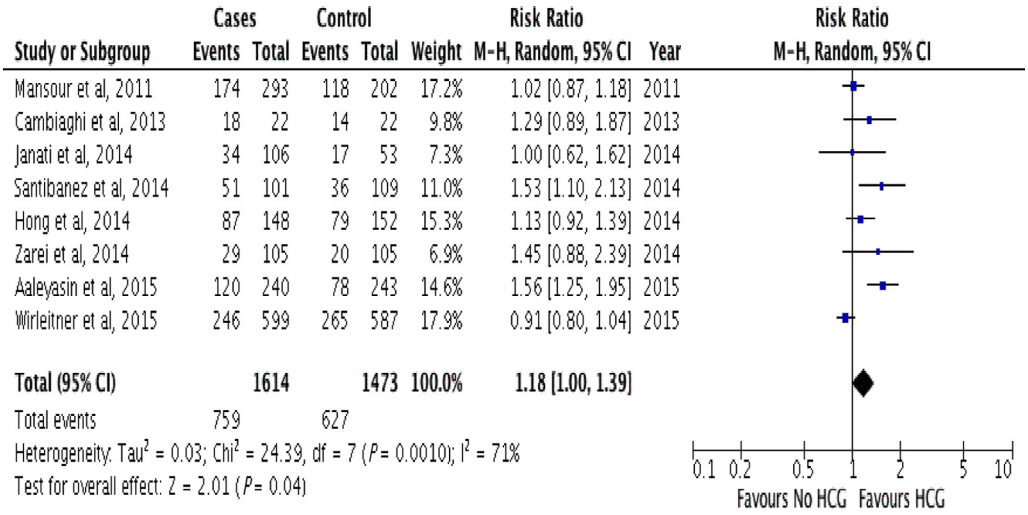

Figure 5 Clinical pregnancy rates for intrauterine HCG administration versus no HCG.

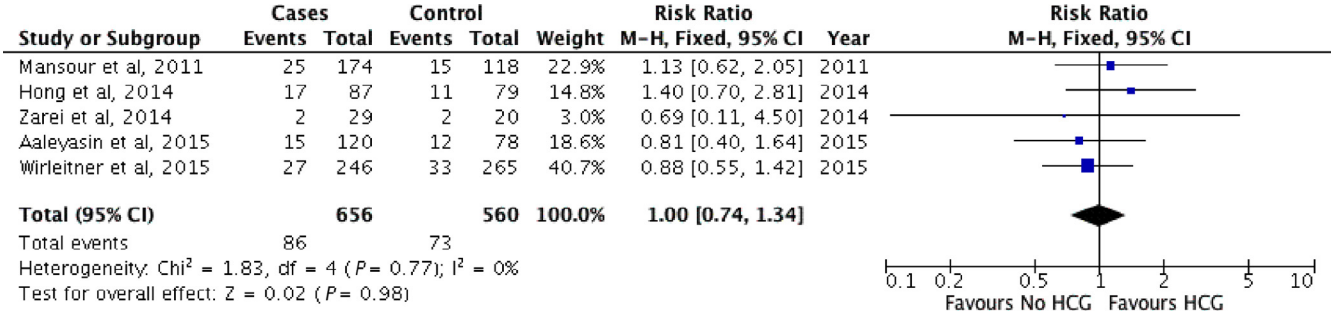

Figure 6 Spontaneous abortion rates for intrauterine HCG administration versus no HCG.

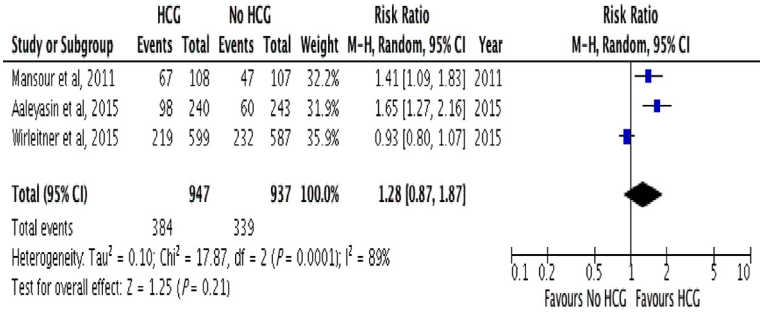

Figure 7 Live birth rates for administration of 500 IU HCG versus no HCG.

chances of implantation. Only one study included in our meta-analysis accounted for the embryo quality with intrauterine HCG injection (Wirleitner et al., 2015). They reported no difference in the pregnancy rates when HCG was added to top or poor quality embryos.

Despite the known biological role of HCG secreted by the developing blastocyst and endometrial cells in facilitating implantation (Paiva et al., 2011; Racicot et al., 2014), our study results are in agreement with the recently published Cochrane review which suggests that adding HCG in the luteal phase whether in addition to progesterone or with placebo did not show benefit for IVF outcome (van der Linden et al., 2015).

It is possible that the physiological effects of HCG are only achieved at certain physiological doses produced in a timely fashion that takes into account the stage of embryo development and stage of endometrial receptivity. The different isoforms of HCG, including hyperglycosylated HCG, HCG and beta HCG are produced by the developing embryo, cytotrophoblast and syncytiotrophoblast in different dominance levels depending on the stage of embryo, implantation and pregnancy (Butler et al., 2013; Cole, 2012; Sasaki et al., 2008). This fine-tuning of the different isoforms may not be achieved by administering a high dose of HCG (urinary, purified or recombinant). Also, it is possible that the favourable physiological function of HCG is only achieved when it is produced

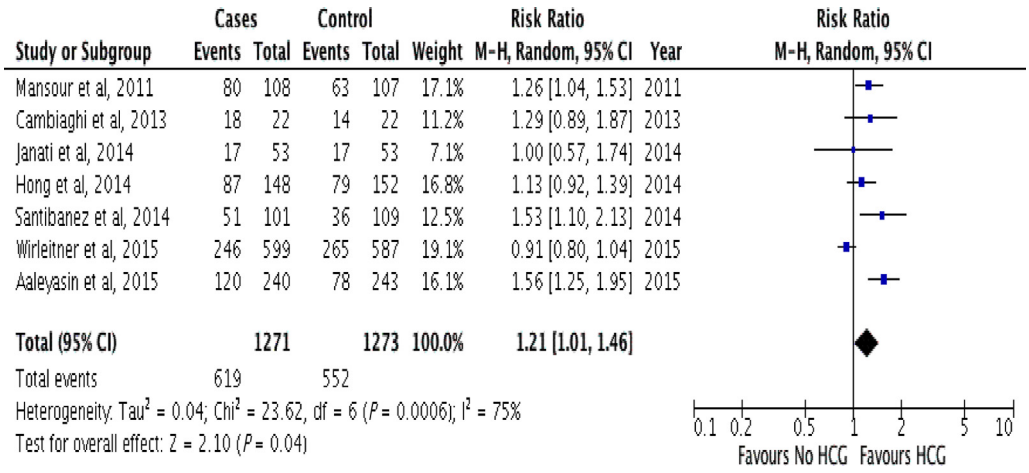

Figure 8 Forest plot of clinical pregnancy rates for administration of 500 IU HCG versus no HCG.

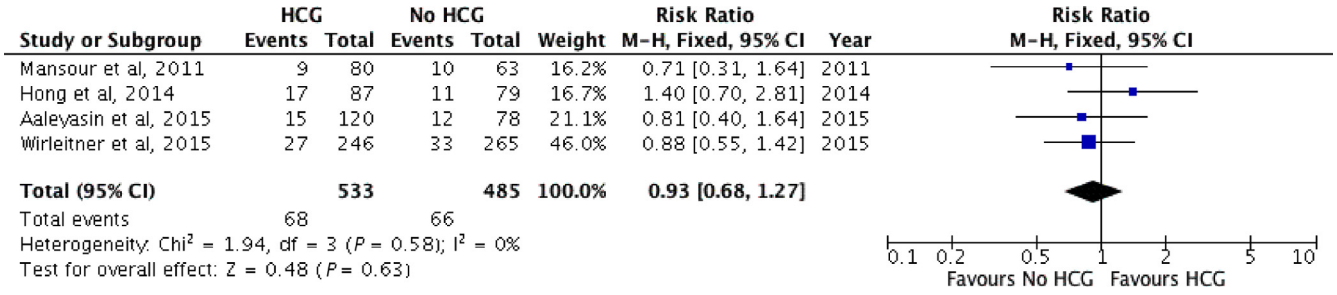

Figure 9 Comparison of spontaneous abortion rates for administration of 500 IU HCG versus no HCG.

by the actual embryo representing a normally developing embryo.

Although the current analysis does not demonstrate that HCG infusion before embryo transfer confers benefit in the LBR or in reducing the spontaneous abortion rate, there is a concern for potential harm stemming from the fact that chronic exposure of the endometrium to HCG may down-regulate its receptors rendering them unresponsive to subsequent HCG produced by the blastocyst (Evans and Salamonsen, 2013).

The results of the current study should be interpreted with caution because of the considerable clinical and statistical heterogeneity detected among the studies. For example, two studies included donor cycles in the inclusion criteria (Cambiaghi et al., 2013; Santibáñez et al., 2014), whreas other studies have combined fresh and frozen embryo transfers in their analysis (Hong et al., 2014; Santibáñez et al., 2014). Other factors that also warrant caution are the variation in the type of HCG used (urinary or recombinant), the volume of fluid infused (0.02–0.5 ml) as well as the time interval between HCG infusion and embryo transfer (which ranged between 3 min to 48 h). Furthermore, the embryonic (cleavage or blastocyst) stage achieved at the time of embryo transfer was inconsistent. Additionally, the inclusion and exclusion criteria of the population recruited in these studies were variable.

In conclusion, current evidence does not support the use of intrauterine HCG administration before embryo transfer. Further evidence gathered through a well-designed and well-conducted multicentre trial to address this issue and providing robust evidence of benefit is warranted.

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