Running Title

Controlled ovarian stimulation with hCG

Title Page

Efficacy and safety of hCG for follicular phase stimulation in assisted reproduction: a systematic review and meta-analysis

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Conflicts of interest: Antonio Requena is Board membership of Ferrin Spain.

Financial support: None.

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Capsule (word count 30)

The administration of hCG in the early and late follicular phase in controlled ovarian stimulation shows similar results than conventional regimens, with the advantage of decreasing the doses of FSH.

Abstract (word count 200)

Objective

To assess the efficacy and safety of hCG to induce follicular stimulation.

Design

Systematic literature searches of PubMed, EMBASE, CENTRAL, and SciSearch databases. Randomized controlled trials (RCTs) using hCG in early or late follicular phases were included.

Setting

Three reproductive medicine Services of Gynecology in Spain and two Universities

Patients

A total of 1,068 women treated in 11 RCTs were included.

Interventions

Use of hCG versus other hormonal treatments, no administration, or placebo during the period of follicular stimulation.

Main Outcome Measures

Live birth, clinical pregnancy, mature oocytes, miscarriage, ovarian hyperstimulation syndrome (OHHS), and FSH doses.

Results

No differences in live birth, miscarriage, and OHHS between hCG (given at either early or late follicular phases) and different control regimens were found. Pooled analysis for clinical pregnancy showed significant differences in favor of hCG at late follicular phase. The doses of FSH were lower in women treated with hCG either at early or late follicular phases than in those treated with FSH alone.

Conclusions

The use hCG in the early and late follicular phase in controlled ovarian stimulation has advantage of decreasing the doses of FSH.

Key words: hCG, follicular maturation, controlled ovarian stimulation, FSH doses; systematic review; meta-analysis.

Introduction

Controlled ovarian hyperstimulation for in vitro fertilization (IVF) is usually achieved follicular stimulation hormone (FSH) or luteinising hormone (LH). Human chorionic gonadotropin (hCG) purified from the urine of pregnant women (u-hCG) or produced by recombinant DNA technology (r-hCG) is one of the hormones used to trigger ovulation. Usually one injection of 5000-10,000 IU of u-hCG or 250 μg of r-hCG is administered when one or more follicles measuring > 17 mm in diameter are observed on ultrasound. Various hormonal treatment protocols include the administration of 5000 UI of hCG for luteal phase support (1). However, the use of hCG in more initial phases of the stimulated cycle to enhance follicular growth is infrequent and poorly characterized.

The main objective of this systematic review was to assess the efficacy of hCG to induce follicular development during controlled ovarian hyperstimulation. A secondary objective was to evaluate the safety of this approach according to data provided in the studies included in the review.

Materials and Methods

The study was exempt from Institutional Review Board approval because this was a systematic review and meta-analysis.

Search Strategy

We performed an exhaustive electronic search in the following databases: MEDLINE (from 1950 until March 2011), EMBASE (from 1980 until March 2011), and The Cochrane Central Register of Controlled Trials (CENTRAL) (issue 1, 2011). The search combined terms and descriptors related to IVF, ovarian controlled hyperstimulation, and hCG. The search strategy was modified to fit with the syntaxes used in each database

consulted. We added validated filters to that strategy to retrieve clinical trials (2,3). Moreover, we searched for ongoing trials at the main clinical trials registers, including www.controlled-trials.com, www.clinicaltrials.gov and the WHO International Clinical Trials Registry Platform (www.who.int/trialsearch). No language limits were placed. Reference lists of all identified articles and overviews, and a Science Citation Index Search (SciSearch, ISI Web of Knowledge) of relevant articles, provided additional sources of potentially eligible clinical trials.

Eligibility Criteria

The review included randomized controlled clinical trials (RCTs) of women undergoing treatment for infertility of sub-infertility independently of whether the cause of infertility lies on the male or the female partner. The type of intervention evaluated was the administration of u-hCG versus other hormonal treatments, no administration, or the administration of placebo during the period of follicular stimulation. Parallel or crossover RCTs were included in the study. In order to avoid the potential of a carry-over bias, crossover designs were eligible if data for the first treatment before assignment to the second treatment being tested was available.

Outcome Measures

Main outcomes of interest for the review were live birth (per women randomized), clinical pregnancy (per women randomized), number of mature oocytes, doses of FSH, adverse events, including miscarriage (per women randomized) and ovarian hyperstimulation syndrome (OHHS) as defined by International Committee Monitoring Assisted Reproductive Technologies (ICMART) (4).

Data Extraction

Data were collected using standard forms in which characteristics of the study design, participants, interventions, comparisons, and main results were recorded. Two independent authors (M.A.C., A.R.M.) judged study eligibility, assessed quality and extracted data solving discrepancies by agreement, and if needed, reaching consensus with a third author (J.J.E.). Agreement between reviewers was analyzed using the weighted kappa for each inclusion criterion (5) and kappa with quadratic weighting for the quality components (6).

Assessment of Risk of Bias

The risk of bias of the studies included in the review was evaluated according to the Cochrane Collaboration's recommended tool (7), which is a two-part tool, addressing six specific domains (sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting and 'other issues') and answering a pre-specified question about the adequacy of the study in relation to the entry. A judgment of "yes" for all domains indicates a low risk of bias, a judgement of "no" for one or more domains indicates a high risk of bias, and a judgment of "unclear" for at least one domain indicates an unclear risk of bias.

Analysis

To determine the pooled effect of each variable, a random effects model was used. The relative risk (RR) for dichotomous data and the mean difference (MD) for continuous data accompanied by the 95% confidence intervals (CIs) were calculated. Statistical significance was set at a *P* value <.01. We evaluated the degree of variation across studies attributable to heterogeneity with the I^2 statistic (8). We conducted meta-analyses using Review Manager software.

Results

A total of 1865 studies were retrieved in the initial electronic search but 1786 were excluded at title/abstract screening. The remaining 80 studies were considered eligible by one or both reviewers. During the second phase of the inclusion process, 69 were excluded because the inclusion criteria were not fulfilled (n = 49) or administration of hCG for indications other than follicular stimulation (n = 20). The flow chart of the 11 trials included in the meta-analysis is shown in Figure 1. The two reviewers achieved good agreement in the selection of trials for inclusion (weighted kappa 0.73, 95% CI 0.58-0.89).

Description of Included Studies

Eleven RCTs evaluating hCG during the period of follicular stimulation in women undergoing assisted reproduction met the inclusion criteria (9-19). The sample size ranged from 20 to 300 women, with a total of 1,068 women. Ages of the participants ranged between 20 and 40 years. A total of 283 (four studies) women participated in studies in which hCG was administered in the early follicular phase and 785 (seven studies) in which hCG was administered in the late follicular phase. In seven studies, one control group was included (9-13,15,19), whereas in the remaining four studies, two controls groups were included (14,16-18). Main causes of infertility were male factor, tubal factor, ovulatory factor, endometriosis and unexplained etiology. Characteristics of the studies included in the meta-analysis are shown in Table 1.

Internal Validity of Included Studies

As shown in Figure 2, the risk of bias was important in most of them, particularly because blinding of the randomization process and the interventions was not mentioned. Except for the clinical trial of Lossl et al. (11), the risk of bias was high or unclear for the majority of studies.

Main outcomes

Live Birth

Only the study of Lossl et al. (10) provided data on the use of hCG in the early phase of the cycle (103 women) for the outcome of live birth rate. As shown in Figure 3, there were no significant differences in the number of live births between the intervention and the control group (RR = 0.78, 95% CI 0.43-1.41). Four studies assessing the administration of hCG in the late follicular phase (13,17-19) reported quantitative information on the number of live births, with percentages ranging between 10% and 46%. The pooled analysis with all four trials did not show statistically significant differences between hCG and control treatments (RR = 1.42, 95% CI 0.97-2.08, I^2 = 0%) (Figure 3).

Clinical Pregnancy

Three studies (9,11,12) with a total of 263 women reported data for the outcome of clinical pregnancy with the use of hCG at early follicular phase. The pooled analysis with these three trials did not show significant differences between hCG and control groups (RR = 1.30, 95% CI 0.89-1.91, I^2 = 52%) (Figure 4). Five studies have assessed the administration of hCG at the late follicular phase (13,16-19) for the outcome of clinical pregnancy. The pooled analysis showed statistically significant differences in favor of the hCG group (RR = 1.32, 95% CI 1.06-1.64) (P = .01, I^2 = 0%) (Figure 4).

The stratified analysis according to treatment of the control group showed no significant differences when hCG was compared highly purified FSH (HP-FSH) or human menopausal gonadotropin (hMG). However, the comparison of hCG with recombinant human FSH (rFSH), showed a statistically significant difference for the hCG group (P = .04) (Figure 4).

Retrived Metaphase II oocytes

Pooled analysis of three studies (13,15,16) that evaluated the administration of hCG in the late follicular phase versus FSH, in association with agonists and antagonists of gonadotropin-releasing hormone (GnRH) in both study groups, with a total of 313 women, there were significant differences in the number of mature oocytes retrieved for the FSH versus hCG protocols (MD -0.30, 95% CI -0.44 to -0.16, I^2 = 0%).

Doses of FSH

The doses of FSH were lower for women treated with hCG than for those treated with FSH alone both in the two studies in which hCG was administered in the early follicular phase (9,11) (MD -1094.34, 95% CI -1374.73 to -813.96) as well as in the four studies (12,13,14,16) in which hCG was given in the late follicular phase (MD -460.22, 95% CI -541.51 to -378.93).

Adverse Events

Miscarriage

Only the study of Filicori et al. (10) provided data on the number of miscarriages when hCG was administered at early follicular phase, and significant differences between the study groups were not found (MD 0.20, 95% CI 0.01-3.70). When hCG was

administered in the late follicular phase (16-18), differences in the miscarriage rate between the hCG protocol and either urinary FSH (uFSH) or rFSH protocols were not observed (RR = 1.40, 95% CI 0.51-3.82, I^2 = 0%).

Ovarian Hyperstimulation Syndrome

Only two studies reported data on development of OHSS for the use of hCG in the early follicular phase (11,12). In the study of Lossl et al. (11), no case of OHSS was observed, whereas in the study of Drakakis et al. (12), 7 patients in each group (hCG and rLH) developed OHSS (RR = 0.97, 95% CI 0.36-2.58). Seven studies (13-19) provided data on OHSS for the administration of hCG in the late follicular phase. Pooled analysis showed no statistically significant differences for the comparison of hCG with either hMG, rFSH or HP-hMG (RR = 0.57, 95% CI 0.31-1.04, I^2 = 0%).

Discussion

This is the first meta-analysis that evaluates the efficacy and safety of the administration of hCG for the stimulation of follicles. According to the present findings, the use of hCG to induce follicular stimulation seems to be associated with similar results than those obtained with standards FSH regimens. Usually, hCG has been used as a substitute for the mid-cycle LH surge because of the degree of homology between the two hormones (20). hCG has a slower plasma metabolic clearance, which consists of a rapid phase in the first 5-9 h following intramuscular administration and a slower phase in the first 1- days. Both LH and hCG are complex heterodimeric glycoproteins with a molecular weight of ~30 kD for LH and 40 kD for hCG (20). These two hormones have identical α-subunits and a high cysteine content. Most importantly, they have the same natural function, --to cause ovulation and support lutein cells. The major differences

between hCG and LH include the sequence of the β-subunit, the regulation of the secretion of the two hormones, and the pharmacokinetics of clearance of hCG as opposed to LH (22). The longer half-life and greater affinity for the LH/hCG receptor of hCG account for a potency ratio estimate of hCG-to-LH of around 1:6 (23).

The theoretical possibility of the use of hCG refers to two specific situations, the first in ovarian priming prior to the use of FSH at the beginning of the cycle, and the second, in the late follicular phase at the time of appearance of LH receptors. These two possibilities were analyzed in this study. The dose of hCG used for priming ranged between 200 and 1250 IU. The action of LH/hCG in preantral and small antral follicles is limited to its stimulatory action on theca cells in conjunction with local growth factors, to induce the formation of the androgen substrate needed for granulosa cells conversion into estradiol helping follicular differentiation. Moreover, after 14 days of pituitary desensitization, LH can be abnormally low, so LH or hCG is normally added to prevent this and to ensure adequate androgen production in theca cells (24). Although in this meta-analysis, an increase in the number of live births was not found, a higher number of mature oocytes was observed probably induced by previous androgenization induced by hCG.

At the late follicular phase, hCG was administered at doses of 200 IU/day when follicles reached 12-14 mm of diameter both in GnRH agonist and antagonist cycles. In physiological conditions, in this second part of the follicular phase, there is a decrease in FSH levels and LH plays a critical role in the control of folliculogenesis (25). Low dose hCG may promote the growth of intermediate-sized follicles which have LH/hCG receptors expressed in the granulosa cells, potentially resulting in effective and safe induction of ovulation until the final stages without the presence of FSH (26). The clinical pregnancy rate in the hCG group was significantly higher than in the standard

FSH group. Although a lower number of metaphase II oocytes was obtained, a better oocyte quality may be the reason for a higher number of clinical pregnancies. hCG activity is present in HP-FSH and in the MERIT clinical trial, better embryo quality in patients treated with HP-FSH was also reported (27).

In relation to OHHS, which is particularly associated with hCG for triggering ovulation (28), in the present meta-analysis a higher number of cases of OHHS in the hCG group was not detected. This is in contrast to data reported in a systematic review of luteal support in assisted reproduction cycles, in which it was concluded that hCG was associated with a greater risk of OHSS when used with GnRH agonists (29). We observed a lower number of metaphase II oocytes in the group of women treated with hCG. In our opinion, the doses of hCG used for oocytes maturation are lower than those used for triggering ovulation and therefore would trigger fewer hyperesponses; also hCG-mediated LH effect would allow the growth of larger follicles and the atresia of small-sized follicles without expression of LH receptors (26). Moreover, low responders were included in some of the studies, which would justify a lower risk of ovarian hyperstimulation.

The use of hCG may have the advantage of a reduction in the FSH doses used in the cycles of controlled ovarian hyperstimulation, and in this meta-analysis a reduction of the total dose of FSH in the groups given hCG as a supplementation of treatment was observed. According to these data both hCG and FSH protocols for follicular stimulation are equally effective and safe, but low cost of hCG stimulation makes this alternative more efficient.

The current study has several potential limitations but also some strengths. Systematic reviews have become standard practice in medical research to synthesize the best available evidence but a potential risk for publication bias remains. However,

publication bias seems unlikely given that no single study included in the meta-analysis concluded in favor of use of hCG to increase live birth. In making health care management decisions, patients and clinicians must trade off the benefits and downsides of alternative strategies, thus they need to know how much confidence they can place in the estimates of effect. In this systematic review the most relevant limitations are those related to the lack of information regarding the design and execution (methodological limitations) of the included studies, some degree of heterogeneity and more importantly a low number of events and hence a low degree of precision. Except for the study of Lossl et al. (11), the risk of bias was high or unclear for the majority of studies. The overall quality of the evidence was therefore considered low, which decreases our confidence in the estimate of effect showed by adding hCG in controlled ovarian hyperstimulation.

In summary, the administration of hCG in the initial follicular phase appears to be associated with similar results than those obtained with conventional regimens used in controlled ovarian stimulation, but has the advantage of decreasing the doses of FSH. In terms of safety, the incidence of OHSS is similar to standard protocols without hCG. For this reasons, hCG may be a valid alternative to decrease the dose of FSH in the treatment of infertility. This possibility, however, is based on a limited number of studies with different protocols for follicular stimulation. Due to methodological limitations of the studies and different ovarian stimulation regimens, well designed clinical trials are necessary to define better whether hCG is useful for ovarian growth stimulation at early follicular phase.

Acknowledgments

We thank Marta Pulido, MD, for editing the manuscript and editorial assistance.

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Legends

Figure 1. Flow chart for the trial identification and selection process.

Figure 2. Risk of bias for the 11 randomized controlled clinical trials included in the meta-analysis.

Figure 3. Effectiveness of hCG supplementation versus standard stimulation protocols for the outcome of live birth.

Figure 4. Effectiveness of hCG supplementation versus standard stimulation protocols for the outcome of clinical pregnancy.