Efficacy of Follicle-Stimulating Hormone (FSH) Alone, FSH + Luteinizing Hormone, Human Menopausal Gonadotropin or FSH + Human Chorionic Gonadotropin on Assisted Reproductive Technology Outcomes in the “Personalized” Medicine Era: A Meta-analysis

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Setting: Luteinizing hormone (LH) and human chorionic gonadotropin (hCG) act on the same receptor, activating different signal transduction pathways. The role of LH or hCG addition to follicle-stimulating hormone (FSH) as well as menopausal gonadotropins (human menopausal gonadotropin; hMG) in controlled ovarian stimulation (COS) is debated.

Objective: To compare FSH + LH, or FSH + hCG or hMG vs. FSH alone on COS outcomes.

Design: A meta-analysis according to PRISMA statement and Cochrane Collaboration was performed, including prospective, controlled clinical trials published until July 2016, enrolling women treated with FSH alone or combined with other gonadotropins. Trials enrolling women with polycystic ovarian syndrome were excluded (PROSPERO registration no. CRD42016048404).

Results: Considering 70 studies, the administration of FSH alone resulted in higher number of oocytes retrieved than FSH + LH or hMG. The MII oocytes number did not change when FSH alone was compared to FSH + LH, FSH + hCG, or hMG. Embryo number and implantation rate were higher when hMG was used instead of FSH alone. Pregnancy rate was significantly higher in FSH + LH-treated group vs. others. Only 12 studies reported live birth rate, not providing protocol-dependent differences. Patients’ stratification by GnRH agonist/antagonist identified patient subgroups benefiting from specific drug combinations.
**INTRODUCTION**

Luteinizing hormone (LH) and human chorionic gonadotropin (hCG) are heterodimeric glycoprotein hormones, acting on the same receptor (LH-CGR) (1). These gonadotropins were considered equivalent at the molecular level for long time, until the demonstration of specific intracellular-mediated signaling (2). In vitro models of human granulosa cells demonstrated that hCG is more potent than LH in inducing cyclic adenosine monophosphate production (cAMP) production (2), while the latter leads to preferential ERK1/2 and AKT pathways activation (2). Thus, although LH and hCG activate different kinet (2, 3), whether and how they differently influence in vivo response remains unclear (4).

In humans, follicle-stimulating hormone (FSH) and LH act in concert to stimulate folliculogenesis and ovulation. Therefore, these gonadotropins are used in the controlled ovarian stimulation (COS) in order to produce relatively high oocyte number to be used fresh or after cryopreservation (5) to obtain pregnancies. The physician identifies the presumably most appropriate regimen, in terms of gonadotropin-releasing hormone (GnRH) analog protocol, FSH formulation, starting FSH dose, and combination of different gonadotropins, following the evaluation of demographic, anthropometric, and ovarian reserve profiles (6–8). Generally, FSH is selected as standard treatment, and hCG or LH may be added. The knowledge of human physiology provides a rationale for LH activity supplementation during COS. Although in vitro and animal models provided the evidences of hormone-specific actions, the choice of the optimal gonadotropin combination to be used in COS is not well standardized and remains entrusted to clinicians’ decision. Especially, the pregnancy hormone hCG is generally used to obtain LH-like activity and support of multi-follicle growth since decades (9). With this in mind, human menopausal gonadotropin (hMG) is commonly used as preparation with LH-like activity, due to the presence of LH and/or hCG molecules. hMG alone and hCG/LH + FSH were repeatedly proposed (10, 11) but some unfavorable results, in particular in terms of number of oocytes retrieved (12, 13), provided concerns about the usefulness of addition of “LH activity.”

Currently, the gonadotropin market offers a wide choice, including urinary and recombinant preparations of FSH, LH, hCG, and hMG alone or in various combinations, recently further enriched by biosimilars. This palette of competitor drugs, registered for the same indication but biochemically and physiologically different, introduced the concept of “personalized” assisted reproductive technology (ART) schemes, which is very attractive for patients and doctors but not supported by solid evidence and largely industry-promoted. These gonadotropins show different kinetics in in vitro models, but no clear in vivo differences in COS are available so far. Most studies have been tried to answer the question of what is the best gonadotropin combinations, although inconclusive results were achieved, not sufficient to guide a really evidence-based, personalized choice in ART. Indeed, no powerful, properly designed, controlled prospective clinical trials are available to support the rationale of any COS scheme so far. As a matter of fact, the design of randomized clinical trials is challenging in this setting, due to the peculiar emotional situation and heterogeneity of the infertile population together with the time and costs required. Thus, 64 meta-analyses have been published to compare different ART approaches and outcomes (Table 1). However, each review is focused on a specific single comparison (e.g., hMG vs. FSH, GnRH agonist vs. antagonists, etc.) in a peculiar clinical setting. In particular, 25 systematic reviews compared the efficacy of different GnRH analogs, 17 compared urinary and recombinant FSH preparations, and only 6 evaluated the efficacy of LH supplementation to FSH (Table 1). None of these comparisons provided a comprehensive analysis of entire process, from oocyte recruitment to live birth rate, and their conclusions are rarely translated in clinical practice. In fact, no accepted guideline exists in this field of medicine in which registered indications and reimbursability of gonadotropins by the national health care systems are guided by costs rather than scientific evidence/clinical outcome.

Having in mind physiology and the different in vitro effects of LH and hCG, in this work, we addressed the question whether LH, LH-like activity, and hCG could have different results on COS outcomes. To this purpose, we evaluated the efficacy of LH or hCG plus FSH or hMG alone, compared to what is considered the standard care for COS, i.e., the use of FSH alone, using a meta-analytic approach. This is the first meta-analysis in which all gonadotropin combinations are considered. Moreover, a full-spectrum evaluation of all ART endpoints is provided, to recognize when and how LH, LH-activity, and hCG influence ART outcomes.

**MATERIALS AND METHODS**

We performed a meta-analysis according to the Cochrane Collaboration and PRISMA statement. The meta-analysis was accepted in the International Prospective Register of Systematic Reviews (PROSPERO; registration n. CRD42016048404) prior to commencing the study, ensuring transparency and originality of the review process.

**Data Sources and Searches**

We conducted a comprehensive literature search for English-language articles in MEDLINE (PubMed), EMBASE, Cochrane Library, SCOPUS, and UpToDate, published until July 2016.
| First author | Journal                        | Year | Comparison                                                                 | End-points            | Number of studies |
|-------------|--------------------------------|------|-----------------------------------------------------------------------------|-----------------------|------------------|
| Daya        | Fertil Steril                  | 1995 | U-follicle-stimulating hormone (FSH) vs. r-FSH                              | Pregnancy rate        | 8                |
| Daya        | Cochrane Database Syst Rev     | 1996 | U-FSH vs. r-FSH                                                             | Pregnancy rate        | Withdrawn        |
| Daya        | Hum Reprod                     | 1999 | U-FSH vs. r-FSH                                                             | Oocytes retrieved     | 12               |
| Nugent      | Cochrane Database Syst Rev     | 2000 | Different u-FSH in polycystic ovarian syndrome (PCOS)                       | Pregnancy rate        | 23               |
| Daya        | Cochrane Database Syst Rev     | 2000 | U-FSH vs. r-FSH                                                             | Pregnancy rate        | 18               |
| van Wely    | Fertil Steril                  | 2003 | Human menopausal gonadotropin (hMG) vs. r-FSH                               | Pregnancy rate        | 6                |
| Al-Inany    | Hum Reprod                     | 2003 | U-FSH vs. r-FSH                                                             | Oocytes retrieved     | 20               |
| Albuquerque | Cochrane Database Syst Rev     | 2005 | Depot gonadotropin-releasing hormone (GnRH) agonist vs. daily GnRH agonist  | Pregnancy rate        | 6                |
| Pandian     | Cochrane Database Syst Rev     | 2005 | In vitro fertilization (IVF) vs. intrauterine insemination (IUI)            | Pregnancy rate        | 10               |
| Salam       | Cochrane Database Syst Rev     | 2006 | GnRH agonist timing in endometriosis                                        | Pregnancy rate        | 3                |
| Grinesinger | Reprod Biomed Online           | 2006 | GnRH agonist vs. GnRH antagonist in PCOS                                    | Oocytes retrieved     | 13               |
| Franco      | Reprod Biomed Online           | 2006 | GnRH agonist vs. GnRH antagonist in PCOS                                    | Oocytes retrieved     | 6                |
| Sunkara      | Reprod Biomed Online           | 2007 | GnRH agonist vs. GnRH antagonist                                             | Oocytes retrieved     | 9                |
| Modlar      | Cochrane Database Syst Rev     | 2007 | R-hCG plus r-FSH vs. r-FSH in GnRH antagonist                               | Pregnancy rate        | 14               |
| Pandian     | Cochrane Database Syst Rev     | 2007 | GnRH agonist vs. GnRH antagonist                                             | Live birth rate       | 9                |
| Daya        | Cochrane Database Syst Rev     | 2007 | U-FSH vs. r-FSH                                                             | Live birth rate       | Withdrawn        |
| Kolbrianakis | Hum Reprod Update              | 2007 | R-LH plus r-FSH vs. r-FSH in GnRH antagonist                               | Live birth rate       | 5                |
| Baruffi     | Reprod Biomed Online           | 2007 | R-LH plus r-FSH vs. r-FSH in GnRH antagonist                               | Oocytes retrieved     | 5                |
| Al-Inany    | Reprod Biomed Online           | 2008 | hMG vs. r-FSH                                                               | Live birth rate       | 10               |
| Coomarasamy | Hum Reprod                     | 2008 | U-FSH vs. r-FSH                                                             | Live birth rate       | 7                |
| Al-Inany    | Reprod Biomed Online           | 2008 | hMG vs. r-FSH                                                               | Live birth rate       | 5                |
| Al-Inany    | Gyneol Endocrinol             | 2009 | hMG vs. r-FSH                                                               | Pregnancy rate        | 6                |
| Jee         | Gyneol Obstet Invest           | 2010 | hMG vs. r-FSH                                                               | Pregnancy rate        | 10               |
| Lebert      | Reprod Biomed Online           | 2010 | hMG vs. r-FSH                                                               | Oocytes retrieved     | 16               |
| Pandian     | Cochrane Database Syst Rev     | 2010 | GnRH agonist vs. GnRH antagonist                                             | Live birth rate       | 15               |
| Pandian     | Cochrane Database Syst Rev     | 2010 | Different GnRH analog protocols                                            | Live birth rate       | 10               |
| Sterrenburg | Hum Reprod Update             | 2011 | Different r-FSH doses                                                        | Pregnancy rate        | 10               |
| Al-Inany    | Cochrane Database Syst Rev     | 2011 | GnRH agonist vs. GnRH antagonist                                             | Live birth rate       | 45               |
| Youssef     | Cochrane Database Syst Rev     | 2011 | GnRH agonist vs. hCG for trigger                                            | Live birth rate       | 11               |
| van Wely    | Cochrane Database Syst Rev     | 2011 | hMG vs. r-FSH                                                               | Live birth rate       | 42               |
| Youssef     | Cochrane Database Syst Rev     | 2011 | U-hCG vs. r-hCG                                                             | Live birth rate       | 14               |
| Siristatidis| Cochrane Database Syst Rev     | 2011 | Different GnRH agonist protocols                                            | Pregnancy rate        | 29               |
| Maheshwari  | Cochrane Database Syst Rev     | 2011 | Short vs. ultra-short GnRH agonist protocols                               | Pregnancy rate        | 29               |
| Funihi      | Hum Reprod                     | 2011 | GnRH agonist vs. GnRH antagonist                                             | Oocytes retrieved     | 14               |
| Bodri       | Fertil Steril                  | 2011 | GnRH agonist vs. GnRH antagonist                                             | Pregnancy rate        | 8                |
| van Wely    | Hum Reprod Update              | 2012 | hMG vs. r-FSH                                                               | Live birth rate       | 42               |
| Hill        | Fertil Steril                  | 2012 | R-LH plus r-FSH vs. r-FSH in GnRH antagonist                               | Pregnancy rate        | 7                |
| Konig       | Fertil Steril                  | 2012 | R-LH plus r-FSH vs. r-FSH in GnRH antagonist                               | Pregnancy rate        | 9                |
| Mahmoud Youssef | Fertil Steril                  | 2012 | Long acting FSH vs. r-FSH                                                   | Pregnancy rate        | 4                |
| Pandian     | Cochrane Database Syst Rev     | 2012 | IVF vs. IUI                                                                  | Pregnancy rate        | 6                |
| Gibreel     | Cochrane Database Syst Rev     | 2012 | Gonadotropins vs. clomiphene citrate                                        | Live birth rate       | 14               |
| Pouwer      | Cochrane Database Syst Rev     | 2012 | Long acting FSH vs. r-FSH                                                   | Live birth rate       | 4                |
| Funihi      | Reprod Biomed Online           | 2012 | GnRH agonist vs. GnRH antagonist in PCOS                                     | GnRHSS rate           | 9                |
| Albuquerque | Cochrane Database Syst Rev     | 2013 | GnRH agonist vs. GnRH antagonist in PCOS                                     | Pregnancy rate        | 16               |
| Matsuag     | Gyneol Obstet Invest           | 2013 | Mild ovarian stimulations vs. traditional IVF                               | Pregnancy rate        | 5                |
| Xiao        | Fertil Steril                  | 2013 | GnRH agonist vs. GnRH antagonist                                             | Pregnancy rate        | 12               |
|Fan          | Gyneol Endocrinol             | 2013 | rLH supplementation in poor responders                                     | Pregnancy rate        | 3                |
|Fan          | Gyneol Endocrinol             | 2013 | GnRH agonist vs. GnRH antagonist                                             | Oocytes retrieved     | 7                |
| Youssef     | Cochrane Database Syst Rev     | 2014 | GnRH agonist vs. hCG for trigger                                            | Live birth rate       | 17               |
| Xiao        | PlosONE                       | 2014 | GnRH agonist vs. GnRH antagonist                                             | Oocytes retrieved     | 23               |
| Chen        | Gyneol Endocrinol             | 2014 | Timing of hCG administration                                                 | Oocytes retrieved     | 7                |
| Lin         | PlosONE                       | 2014 | GnRH agonist vs. GnRH antagonist                                             | Pregnancy rate        | 9                |
| Hu          | J Int Med Res                 | 2014 | LH priming vs. FSH alone                                                    | Estradiol serum levels| 3                |
| Song        | Gyneol Endocrinol             | 2014 | GnRH agonist vs. letrozole                                                  | GnRHSS rate           | 3                |
| Siristatidis| Cochrane Database Syst Rev     | 2015 | different GnRH agonist protocols                                            | Pregnancy rate        | 37               |
| Weiss       | Cochrane Database Syst Rev     | 2015 | U-FSH vs. r-FSH in PCOS                                                     | Live birth rate       | 14               |
| Nugent      | Cochrane Database Syst Rev     | 2015 | Different u-FSH in PCOS                                                     | Withdrawn             |                  |
| Nahuis      | Cochrane Database Syst Rev     | 2015 | U-FSH vs. r-FSH in PCOS                                                     | Withdrawn             |                  |
| Pandian     | Cochrane Database Syst Rev     | 2015 | Long acting FSH vs. r-FSH                                                   | Pregnancy rate        | 8                |
| Pouwer      | Cochrane Database Syst Rev     | 2015 | GnRH agonist vs. hCG for trigger                                            | Live birth rate       | 6                |
| Youssef     | J Adv Res                      | 2015 | GnRH agonist vs. hCG for trigger                                            | Pregnancy rate        | 19               |
Search key words were as follows: controlled ovarian stimulation (COS), controlled ovarian hyperstimulation (COH), ART, in vitro fertilization (IVF), intracytoplasmatic sperm injection (ICSI), luteinizing hormone (LH), follicle stimulating hormone (FSH), human menopausal gonadotropin (hMG), hCG, follitropin, oocytes retrieved, and pregnancy. The Boolean functions AND and OR were used to combine key words listed above.

Study Selection and Inclusion Criteria

Types of Studies
The inclusion criteria, established before the literature search, were

- Prospective, longitudinal, and controlled clinical trials;
- Enrollment of women without limits of age;
- Treatment with LH or hCG or hMG during the follicular development phase.

Retrospective studies were not included. Similarly, trials enrolling women with polycystic ovarian syndrome (PCOS) were excluded, due to peculiar endocrine features of these patients. The ART methodology chosen was not an inclusion or exclusion criterion. However, each outcome was further evaluated considering the studies on the basis of the ART protocol used. Finally, randomization was not considered a strict inclusion criterion, thus randomized, semirandomized, and non-randomized clinical trials were reviewed. Therefore, all available controlled studies were considered increasing sample size, in spite of the wide range of clinical protocols available.

Type of Participants
Women undergoing COS for ART were considered. No inclusion criteria were applied for the male partner of the infertile couple.

Type of Interventions
All ART stimulation protocols were considered and studies included provided the comparison between LH, hCG, or hMG in the follicular phase with FSH.

Data Collection Process and Quality
Two authors (Santi Daniele and Casarini Livio) extracted the abstracts from all studies found through literature search until July 2016. All abstracts were evaluated for inclusion criteria, and data were extracted from each study considered eligible, with regard to study design, year of publication, number of included/excluded subjects, number of dropped-out patients, and the use of intention to treat or per protocol analysis.

The quality of trials was assessed using the parameters proposed by Jadad et al. (14) and Table 2 summarizes the features of the selected studies.

Although studies considered in the meta-analysis used different endpoints, we performed an overall meta-analysis considering all studies evaluating at least pregnancy rate or number of oocytes retrieved.

The investigators (DS and LC), using Cochrane risk-of-bias algorithm, independently assessed the risk-of-bias for all trials. The following quality criteria and methodological details were evaluated for each trial included in the meta-analysis: (i) method of randomization, even if the randomization was not an inclusion criterion; (ii) concealment of allocation; (iii) presence or absence of blinding to treatment allocation; (iv) duration and type of treatment and follow-up phases; (v) number of participants recruited, analyzed, or lost to follow-up; (vi) timing of trial; (vii) whether an intention to treat analysis was done; (viii) whether a power calculation was done; (ix) source of funding; and (x) criteria for including participants and assessing outcomes.

Summary Measures
The primary outcome was the number of oocytes retrieved, evaluated as mean difference between the two types of treatment compared. The choice of the primary endpoint derived from the consideration that the number of oocytes retrieved is the unique endpoint available in almost all trials in ART setting. Moreover, our meta-analysis aimed at comparing the efficacy in vivo of gonadotropin combinations, and the number of oocytes retrieved best described pathophysiologically the first step influenced by gonadotropin administration, i.e., follicular and oocyte development. The oocytes number remains the first measurable and reproducible parameter to describe gonadotropin action in vivo.

In clinical practice, the main ART outcome remains live birth rate. However, this parameter was not considered as primary endpoint in our meta-analysis, since it is influenced by a large number of unquantifiable biases and variables. Indeed, the vast majority of clinical trials dedicated to ART outcome do not report this parameter. In fact, the step following oocyte collection, i.e., embryo development, is strongly influenced by another important confounding factor, i.e., sperm quality, which is usually (and unexplainably) disregarded. Further, implantation rate follows embryo development and it is, in turn, affected by other factors, such as the endometrium thickness and activity, which are usually not controlled for. Continuing until pregnancy and live birth rate, each step is influenced by a number of factors, not immediately dependent on gonadotropins. Accordingly, the relationship between live birth rate and oocytes retrieved is suggested in the literature (15), but not universally accepted (16, 17). For these reasons, it is not possible to identify a unique endpoint to evaluate COS outcomes. Thus, we considered each available COS outcome after the number of oocytes retrieved as secondary endpoints, i.e., MII oocytes number, embryos, implantation rate, pregnancy.

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**Table 1 | Continued**

| First author | Journal | Year | Comparison | End-points | Number of studies |
|--------------|---------|------|------------|------------|------------------|
| Fensore | J Ovar Res | 2015 | Long acting FSH vs. r-FSH | Oocytes retrieved | 7 |
| Al-Inany | Cochrane Database Syst Rev | 2016 | GnRH agonist vs. GnRH antagonist | Live birth rate | 63 |
| Youssef | Cochrane Database Syst Rev | 2016 | U-hCG vs. r-hCG | Live birth rate | 18 |
| Authors            | Year | Protocol used      | ART Number | Mean age (years) | Drug 1 Name | Startig drug (IU/daily) | Drug 2 Name | Startig drug (IU/daily) | Drop out | Study group |
|-------------------|------|--------------------|------------|------------------|-------------|------------------------|-------------|------------------------|----------|-------------|
| Gerli             | 1993 | Gonadotropin-      | 17         | 30.9             | FSH         | 225                    | hMG        | Pergonal              | 2        | 15          |
|                   |      | releasing hormone (GnRH) agonist |           |                  |             |                        |             |                        |          |             |
| Daya              | 1995 | GnRH agonist       | 115        | 33.5             | FSH         | 150                    | hMG        | Pergonal              | 117      | 33.2        |
| Westergaard       | 1986 | GnRH agonist       | 104        | 31.0             | FSH         | 225                    | hMG        | Pergonal              | 114      | 32.0        |
| Jansen            | 1988 | None               | 47         | 32.0             | FSH         | 150                    | Humegon    | 32.1                   |          |             |
| Filicori          | 1999 | GnRH agonist       | 17         | 36.4             | FSH         | 300                    | hMG        | Pergonal              | 14       | 36.7        |
| Balasch           | 2001 | GnRH agonist       | 14         | 33.6             | FSH         | 150                    | Gonal F    | 150                    | 1        | 16          |
| De Pacicio        | 2001 | GnRH agonist       | 40         | 30.1             | FSH         | 300                    | Gonal F    | 150                    | 0        | 20          |
| Filicori          | 2001 | GnRH agonist       | 25         | 32.0             | FSH         | 150                    | Metrodin   | 150                    | 0        | 25          |
| Gordon            | 2001 | GnRH agonist       | 69         | 33.5             | FSH         | 225                    | Pergonal   | 12                     | 59       | 33.5        |
| Ng                | 2001 | GnRH agonist       | 20         | 33.5             | FSH         | 150                    | Metrodin   | 20                    | 20       | 32.0        |
| Strehler          | 2001 | GnRH antagonist    | 248        | 32.3             | FSH         | 300                    | Metrodin   | 259                   | 259      | 31.8        |
| Westergaard       | 2001 | GnRH agonist       | 190        | 225              | FSH         | 150                    | Metrodin   | 189                   | 2        | 189         |
| Filicori          | 2002 | GnRH agonist       | 30         | 31.9             | FSH         | 150                    | Metrodin   | 90                    | 90       | 32.7        |
| Ismail            | 2002 | GnRH agonist       | 75         | 33.2             | FSH         | 150                    | Metrodin   | 78                    | 78       | 34.3        |
| Lisi              | 2002 | GnRH agonist       | 331        | 34.7             | FSH         | 150                    | Metrodin   | 122                   | 122      | 34.8        |
| Filicori a        | 2003 | GnRH agonist       | 25         | 31.9             | FSH         | 150                    | Metrodin   | 25                    | 25       | 32.6        |
| Filicori b        | 2003 | GnRH agonist       | 50         | 35.9             | FSH         | 150                    | Metrodin   | 14                    | 14       | 27          |
| Ku                | 2003 | GnRH agonist       | 19         | 34.6             | FSH         | 150                    | Metrodin   | 26                    | 26       | 33.0        |
| Maris             | 2003 | GnRH agonist       | 219        | 31.9             | FSH         | 150                    | Metrodin   | 212                   | 212      | 32.4        |
| Acevedo           | 2004 | GnRH antagonist    | 20         | 23.0             | FSH         | 150                    | Metrodin   | 22                    | 22       | 26.0        |
| Cédrin-Durnerin   | 2004 | GnRH antagonist    | 96         | 31.7             | FSH         | 150                    | Metrodin   | 217                   | 2        | 107         |
| De Pacicio        | 2004 | GnRH agonist       | 46         | 30.4             | FSH         | 150                    | Metrodin   | 46                    | 46       | 30.0        |
| Ferraretti        | 2004 | GnRH agonist       | 104        | 31.7             | FSH         | 150                    | Metrodin   | 24                    | 2        | 31.5        |
| Ferraretti        | 2004 | GnRH agonist       | 104        | 31.7             | FSH         | 150                    | Metrodin   | 22                    | 2        | 32.0        |
| Humaidan          | 2004 | GnRH agonist       | 115        | 30.5             | FSH         | 150                    | Metrodin   | 116                   | 116      | 30.8        |
| Loutardis         | 2004 | GnRH agonist       | 106        | 37.3             | FSH         | 150                    | Metrodin   | 98                    | 98       | 38.1        |
| De Pacicio        | 2005 | GnRH agonist       | 58         | 30.4             | FSH         | 150                    | Metrodin   | 57                    | 57       | 31.5        |
| Drákeulis         | 2005 | GnRH agonist       | 22         | 33.0             | FSH         | 150                    | Metrodin   | 24                    | 24       | 32.4        |
| Filicori          | 2005 | GnRH agonist       | 24         | 33.4             | FSH         | 150                    | Metrodin   | 24                    | 24       | 33.8        |
| Gómez-Palomares   | 2005 | GnRH agonist       | 58         | 39.0             | FSH         | 150                    | Metrodin   | 4                    | 4        | 38.8        |
| Griesinger        | 2005 | GnRH antagonist    | 65         | 30.5             | FSH         | 150                    | Metrodin   | 11                    | 11       | 30.3        |
| Hugues            | 2005 | None               | 30         | 29.9             | FSH         | 150                    | Metrodin   | 0                    | 0        | 117         |
| Fabregues         | 2006 | GnRH agonist       | 60         | 38.2             | FSH         | 150                    | Metrodin   | 5                    | 5        | 60          |
| Levi-Setti        | 2006 | GnRH antagonist    | 20         | 32.3             | FSH         | 150                    | Metrodin   | 4                    | 4        | 20          |
| Tarbatos          | 2006 | GnRH antagonist    | 59         | 30.3             | FSH         | 150                    | Metrodin   | 2                    | 2        | 30.5        |
| Berkkanoglu       | 2007 | GnRH agonist       | 51         | 34.8             | FSH         | 600                    | Metrodin   | 46                    | 46       | 36.3        |
| Berkkanoglu       | 2007 | GnRH agonist       | 51         | 34.8             | FSH         | 600                    | Metrodin   | 48                    | 48       | 35.2        |
| Demiroi           | 2007 | None               | 161        | 30.4             | FSH         | 150                    | Metrodin   | 0                    | 0        | 30.8        |
| Ziebe             | 2007 | GnRH agonist       | 368        | 350              | FSH         | 150                    | Metrodin   | 363                   | 363      | 225         |
| BarrenaBatea      | 2008 | GnRH agonist       | 42         | 41.8             | FSH         | 300                    | Metrodin   | 42                    | 42       | 42.1        |
| Bosch             | 2008 | GnRH antagonist    | 140        | 33.4             | FSH         | 150                    | Metrodin   | 20                    | 20       | 33.2        |
| Hompes            | 2008 | GnRH antagonist    | 317        | 32.0             | FSH         | 150                    | Metrodin   | 15                    | 15       | 31.7        |

(Continued)
| Authors     | Year     | Protocol used | ART   | Number | Mean age (years) | Drug 1 name | Drug 2 name | Startig doe (IU/daily) | Drug 1 name | Drug 2 name | Drop out Numr | Mean age (years) | Drug 1 name | Drug 2 name | Drop out | Startig doe (IU/daily) | Drug 1 name | Drug 2 name | Drop out |
|-------------|----------|---------------|-------|--------|----------------|-------------|-------------|------------------------|-------------|-------------|----------------|----------------|-------------|-------------|----------|------------------------|-------------|-------------|----------|
| Nyboeandersen | 2008     | GnRH agonist  | IVF   | 261    | 31.8          | FSH Gonal F | 150         | 0                      | 265         | 31.7        | FSH Gonal F | 150         | LH Luveris | 75          | 0        |                      |              |              |          |
| Blockeel     | 2009     | GnRH antagonist | IVF   | 35     | 30.0         | FSH Puregon | 225         | 3                      | 35          | 29.0        | FSH Puregon | 225         | hCG Pregnyl | 200         | 6        |                      |              |              |          |
| Check        | 2009     | GnRH agonist  | IVF   | 35     | 35.1         | FSH Gonal F | 300         | 1                      | 35          | 33.6        | FSH Gonal F | 300         | hCG Pregnyl | 25          | 3        |                      |              |              |          |
| Drakakis     | 2009     | GnRH agonist  | IVF   | 58     | 36.4         | FSH Gonal F | 200         | 0                      | 200         | 37.3        | FSH Gonal F | 200         | LH Luveris | 150         | 0        |                      |              |              |          |
| Matorras     | 2009     | GnRH agonist  | IVF   | 68     | 36.7         | FSH Gonal F | 300         | 3                      | 63          | 36.6        | FSH Gonal F | 300         | LH Luveris | 150         | 0        |                      |              |              |          |
| Melo         | 2010     | GnRH agonist  | IVF   | 346    | 24.9         | FSH Gonal F | 225         | 2                      | 62          | 23.9        | hCG Menopur | 225         | LH Luveris | 8           |          |                      |              |              |          |
| Pacchiarotti | 2010     | GnRH antagonist | IVF   | 60     | 34.6         | FSH Gonal F | 225         | 50                     | 311         | 34.7        | FSH Gonal F | 150         | LH Luveris | 75          | 56       |                      |              |              |          |
| Bosch        | 2011     | GnRH antagonist | IVF   | 314    | 34.6         | FSH Gonal F | 225         | 498                    | 34.3        | FSH Gonal F | 150         | LH Luveris | 75          | 56       |                      |              |              |          |
| Caserta      | 2011     | GnRH agonist  | IVF   | 501    | 34.8         | FSH Gonal F | 150         | 75                     | 28.8        | HMG Menopur | 75           |            |              |          |                      |              |              |          |
| Kokac        | 2011     | GnRH agonist  | IUI   | 24     | 29.5         | FSH Gonal F | 75          | 40                     | 35.0        | FSH Puregon | 225         | LH Luveris | 75          |          |                      |              |              |          |
| Pezzuto      | 2011     | GnRH agonist  | IUI   | 40     | 34.0         | FSH Gonal F | 225         | 23                    | 281         | 35.0        | HMG Menopur | 75-150     | LH Luveris | 75          | 5        |                      |              |              |          |
| Sagnella     | 2011     | GnRH agonist  | IUI   | 252    | 36.4         | FSH Gonal F | 150         | 10                     | 32.3        | FSH Gonal F | 150         | LH Luveris | 75          | 5        |                      |              |              |          |
| Barberi      | 2012     | GnRH agonist  | IVF   | 11     | 32.3         | FSH Gonal F | 150         | 10                    | 34.1        | FSH Gonal F | 150         | LH Luveris | 75          | 2        |                      |              |              |          |
| Devroy       | 2012     | GnRH antagonist | IVF   | 375    | 30.4         | FSH Gonal F | 150         | 59                    | 374         | 30.8        | HMG Menopur | 150         | LH Luveris | 75          | 56       |                      |              |              |          |
| Lis          | 2012     | GnRH agonist  | IVF   | 75     | 32.8         | FSH Gonal F | 150         | 75                     | 33.6        | FSH Gonal F | 150         | LH Luveris | 75          |          |                      |              |              |          |
| Madori       | 2012     | GnRH antagonist | IVF   | 26     | 39.2         | FSH Gonal F | 300         | 0                      | 47          | 38.9        | FSH Gonal F | 300         | hCG Pregnyl | 200         | 0        |                      |              |              |          |
| Revelle      | 2012     | GnRH antagonist | IVF   | 286    | 39.2         | FSH Gonal F | 300         | 27                    | 284         | 39.4        | FSH Gonal F | 150         | LH Luveris | 150         | 29       |                      |              |              |          |
| Thussen      | 2012     | GnRH agonist  | IVF   | 16     | 31.5         | FSH Gonal F | 150         | 2                      | 46          | 32.6        | FSH Gonal F | 150         | hCG Pregnyl | 100         | 5        |                      |              |              |          |
| Ye           | 2012     | GnRH agonist  | IVF   | 64     | 36.2         | FSH Gonal F | 225         | 63                     | 36.2        | HMG Menopur | 225         | LH Luveris | 150         | 14       |                      |              |              |          |
| Korrig       | 2013     | GnRH antagonist | IVF   | 128    | 37.9         | FSH Gonal F | 225         | 17                    | 125         | 38.0        | FSH Gonal F | 225         | LH Luveris | 150         | 14       |                      |              |              |          |
| Rashidi      | 2013     | GnRH antagonist | IUI   | 132    | 28.7         | FSH Gonal F | 75          | 3                      | 127         | 29.1        | hCG Menopur | 75           | LH Luveris | 75          |          |                      |              |              |          |
| Thussen      | 2013     | GnRH agonist  | IVF   | 16     | 32.3         | FSH Gonal F | 150         | 0                      | 46          | 32.3        | FSH Gonal F | 150         | hCG Pregnyl | 100         | 0        |                      |              |              |          |
| Razi         | 2014     | GnRH agonist  | IVF   | 20     | 31.3         | FSH Gonal F | 150         | 0                      | 20          | 31.8        | FSH Gonal F | 150         | LH Luveris | 75          | 0        |                      |              |              |          |
| Behre        | 2015     | GnRH agonist  | IVF   | 99     | 37.6         | FSH Gonal F | 300         | 1                      | 103         | 37.4        | FSH Gonal F | 300         | LH Luveris | 150         | 2        |                      |              |              |          |
| Moro         | 2015     | none          | IUI   | 289    | 37.9         | hMG Menopur | 150         | 5                      | 290         | 38.4        | FSH Gonal F | 150         | LH Luveris | 150         | 13       |                      |              |              |          |
| Vuong        | 2015     | GnRH antagonist | IVF   | 120    | 38.0         | FSH Gonal F | 300         | 11                    | 120         | 38.0        | FSH Gonal F | 300         | LH Luveris | 150         | 18       |                      |              |              |          |
| Yilmaz       | 2015     | GnRH agonist  | IVF   | 87     | 29.0         | FSH Gonal F | 300         | 50                    | 30.3        | FSH Gonal F | 300         | LH Luveris | 75          |          |                      |              |              |          |
| Younis       | 2016     | GnRH antagonist | IVF   | 30     | 38.6         | FSH Gonal F | 300         | 6                      | 32          | 38.9        | FSH Gonal F | 300         | LH Luveris | 150         | 5        |                      |              |              |          |
rate, and live birth rate. Moreover, FSH dosage used and the ratio FSH dosage/number of oocytes retrieved were evaluated in order to describe the amount of gonadotropin needed to obtain each oocyte.”

**Data Synthesis and Analysis**

The meta-analysis was conducted using the Review Manager (RevMan) software (Version 5.3.1 Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014). Data were combined using the fixed effect model and weighted mean differences, and 95% confidence intervals were estimated for each endpoint. The random effect model was used when high heterogeneity resulted among studies, as evaluated by I² statistics. Meta-regression analyses were performed to evaluate the relationship between continuous variables.

Values of $p < 0.05$ were considered statistically significant.

**Risk-of-Bias across Studies**

Two authors (Santi Daniele and Casarini Livio) independently evaluated risk-of-bias. Although randomization is not a strict inclusion criterion, it was evaluated as source of biases following the suggestions provided by the Cochrane collaboration.

**RESULTS**

Of the 2,117 publications initially identified, 1,602 remained after duplicates removal. According to the strategy research, we identified 196 potentially relevant studies, based on the information given in the abstract. All trials were thoroughly appraised for eligibility in the meta-analysis and methodological quality. Seventy studies were included in the final analysis (Table 2; Figure 1).

**Considerations on Study Design**

The mean age of all patients was $33.21 \pm 3.43$ years. Considering the wide heterogeneity in clinical trials included in the analysis, regarding inclusion criteria, FSH starting dose chosen and ART approaches, several subgroup analyses were performed (Table 3).

In a subgroup analyses, studies were divided according to the GnRH analog used, agonist or antagonist, respectively. In subgroup analyses, three studies were excluded considering that hMG was administered together with FSH (18–20). An insufficient number of studies were available on the comparison between FSH alone vs. FSH + hCG and between FSH + LH vs. FSH + hCG, limiting the possibility to subgroup studies. Finally, considering the whole group of studies included in the meta-analysis, the ART approaches chosen after COS were different, ranging from intruterine insemination (IUI) to intracytoplasmatic sperm injection (ICSI). However, only four studies evaluated IUI (21–24), thus the vast majority of trials included in the analysis considered IVF/ICSI. Moreover, of these four studies, three compared hMG to FSH alone (21–23) and one LH + FSH to FSH + hCG alone (24). Thus, a subgroup analysis, excluding studies performing IUI, was performed.

**Number of Oocytes Retrieved**

Twenty-nine studies evaluated the comparison of FSH alone vs. FSH + LH, for a total of 5,840 patients. Studies using FSH alone retrieved a significantly higher number of oocytes compared to FSH + LH treatment ($p = 0.010$) (Figure 2A; Table 4). However, different results were found depending on COS protocol. In

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**Table 3**

|                | FSH + LH vs. FSH alone | FSH + hCG vs. FSH alone | hMG vs. FSH alone |
|----------------|------------------------|-------------------------|-------------------|
| Overall analyses | 34                     | 9                       | 29                |

**Subgroup analyses**

- GnRH antagonists: 10, 3, 5
- GnRH agonists: 22, 6, 20
- GnRH analogs missing data: 2, 0, 4
- In vitro fertilization/intracytoplasmatic sperm injection: 33, 9, 26
- Intruterine insemination: 1, 0, 3
- ART schemes missing information: 0, 0, 0

**ART**, assisted reproductive technology; **FSH**, follicle-stimulating hormone; **GnRH**, gonadotropin-releasing hormone; **hCG**, human chorionic gonadotropin; **LH**, luteinizing hormone.
particular, higher oocyte numbers were retrieved when FSH was administered alone in a GnRH agonist protocol (p = 0.010), while no differences were observed in GnRH antagonist protocol (p = 0.840) (Table 4).

Seven studies using FSH alone vs. FSH + hCG were compared, for a total of 948 patients. The overall analysis did not find significant differences in the number of oocytes retrieved between groups (p = 0.850) (Figure 2B; Table 4).

Twenty studies compared hMG with FSH for COS, for a total of 5,512 patients. Number of oocytes retrieved was significantly higher in FSH than hMG group (p < 0.001) (Figure 2C; Table 4). Four of these studies used a GnRH antagonist protocol, confirming the significant increase of oocytes retrieved (p < 0.001), but no difference was found in the 16 studies using GnRH agonist protocol (p = 0.110) (Table 4).

Finally, 5 studies evaluated the oocytes number comparing FSH plus LH to FSH plus hCG, for a total of 538 women. The analysis did not find significant difference between groups (p = 0.530) (Table 4).

**FSH Dose/Retrieved Oocyte Ratio**

The FSH/retrieved oocyte ratio was significantly lower when LH was added to FSH (p < 0.001) (Table 4), as evaluated in 26 studies.
for a total of 5,404 women enrolled. However, different results were found considering the protocol of COS used. In particular, no significant difference was observed in GnRH agonist protocol ($p = 0.080$) (Table 4). On the contrary, a lower ratio was obtained when LH was added to FSH in the GnRH antagonist protocol ($p < 0.001$) (Table 4).

On the other hand, 6 studies compared the use of FSH alone with FSH plus hCG, for a total of 893 patients. The overall analysis did not find significant differences in the ratio between FSH dose and oocytes retrieved between groups ($p = 0.550$) (Table 4).

Fifteen studies compared hMG with FSH for COS, for a total of 4,436 patients. The ratio between FSH dose and number of oocytes retrieved was significantly lower in the FSH group compared to hMG group ($p < 0.001$) (Table 4). This significant difference was lost in the 12 studies using a GnRH agonist protocol ($p = 0.090$), while remained in the three studies using a GnRH antagonist protocol ($p < 0.001$) (Table 4).

Finally, 4 studies evaluated the ratio comparing FSH plus LH to FSH plus hCG, for a total of 382 women. No differences in the FSH/retrieved oocyte ratio were found between groups ($p = 0.480$) (Table 4).

**MII Oocytes**

Twenty studies reported the MII oocytes number, comparing FSH alone and FSH + LH. The two groups did not differ considering the mean MII oocytes number ($p = 0.050$), even when GnRH agonist or antagonist protocols were considered separately ($p = 0.050$ and $p = 0.540$, respectively) (Table 4).

Five studies compared FSH alone vs. FSH + hCG, without finding differences in the mean MII oocytes number ($p = 0.730$) (Table 4).

Eleven studies compared FSH vs. hMG, finding no differences in the mean difference of MII oocytes ($p = 0.100$) (Table 4). Although this result remained also considering GnRH agonist protocols ($p = 0.840$), the MII oocytes number was significantly higher when FSH was used rather than hMG ($p < 0.001$) (Table 4).

Four studies compared directly FSH + LH vs. FSH + hMG, finding no difference in the MII oocytes number ($p = 0.070$) (Table 4).

**Embryos**

Twenty-six studies reported the embryo number in the comparison between FSH alone vs. FSH + LH, without significant differences ($p = 0.540$) (Table 4). Similarly, no differences were observed in the GnRH agonist ($p = 0.430$) and antagonist group ($p = 0.640$).

Seven studies demonstrated a similar embryo number in the comparison of FSH alone vs. FSH + hCG ($p = 0.770$) (Table 4).

Sixteen studies described the embryo number in the comparison between FSH and hMG. In this subgroup, hMG showed a higher embryo number ($p = 0.001$), maintained when GnRH agonist was used ($p < 0.001$), but not in the GnRH antagonist group ($p = 0.860$) (Table 4).

The direct comparison between FSH + LH and FSH + hMG demonstrated a higher embryo number when FSH was used combined to LH ($p < 0.001$) (Table 4).
### Table 4 | Main results of meta-analyses subgroups.

| Oocytes Retrieved (Mean Difference) | Luteinizing hormone (LH) + follicle-stimulating hormone (FSH) vs. FSH | Human chorionic gonadotropin (hCG) + FSH vs. FSH | Human menopausal gonadotropin (hMG) vs. FSH | LH + FSH vs. hCG + FSH |
|-------------------------------------|---------------------------------------------------------------|-----------------------------|---------------------------------|---------------------|
| Overall analysis                    | −0.20 (−0.36, −0.04)                                          | 0.24 (−2.27, 2.75)          | −0.92 (−1.45, −0.39)            | 0.39 (−0.83, 1.61)  |
|                                     | p = 0.01                                                     | p = 0.850                   | p < 0.001                       | p = 0.530           |
|                                     | F = 88%                                                      | F = 99%                     | F = 94%                         | F = 96%             |
| 29 studies                          | 8,840 patients                                              | 7 studies                   | 20 studies                      | 5 studies           |
|                                     | 948 patients                                                | 946 patients                | 6,512 patients                  | 538 patients        |
| Gonadotropin-releasing hormone (GnRH) agonist | −0.35 (−0.63, −0.08)                                         | −0.43 (−0.96, 0.10)         | p = 0.11                        | p = 0.001           |
|                                     | p = 0.01                                                     | F = 93%                     | F = 93%                         | F = 93%             |
| 17 studies                          | 3,677 patients                                              | 16 studies                  | 3,347 patients                  |                     |
|                                     | 1,347 patients                                              |                            |                                |                     |
| GnRH antagonist                     | 0.01 (−0.13, 0.16)                                           | −2.38 (−3.10, −1.66)        | p < 0.001                       | p < 0.001           |
|                                     | p = 0.840                                                    | F = 54%                     | F = 42%                         | F = 90%             |
| 10 studies                          | 2,163 patients                                              | 4 studies                   | 2,165 patients                  | 382 patients        |
| FSH/Oocytes (Mean Difference)       | −0.16 (−0.21, −0.11)                                         | −0.04 (−0.17, 0.09)         | 0.17 (0.11, 0.23)               | −0.25 (−0.94, 0.44) |
|                                     | p < 0.001                                                   | p = 0.550                   | p < 0.001                       | p = 0.480           |
|                                     | F = 92%                                                      | F = 84%                     | F = 86%                         | F = 90%             |
| 26 studies                          | 5,404 patients                                              | 6 studies                   | 15 studies                      | 4 studies           |
|                                     | 893 patients                                                | 893 patients                | 4,436 patients                  | 382 patients        |
| GnRH agonist                        | −0.06 (−0.13, 0.01)                                          | −0.07 (−0.01, 0.14)         | p = 0.090                       | p = 0.090           |
|                                     | p = 0.080                                                   | F = 90%                     | F = 84%                         | F = 94%             |
| 18 studies                          | 3,613 patients                                              | 12 studies                  | 2,800 patients                  |                     |
|                                     | 1,791 patients                                              |                            |                                |                     |
| GnRH antagonist                     | −0.36 (−0.45, −0.26)                                         | 0.35 (0.25, 0.45)           | p < 0.001                       | p < 0.001           |
|                                     | p < 0.001                                                   | F = 95%                     | F = 74%                         | F = 90%             |
| 8 studies                           | 1,791 patients                                              | 3 studies                   | 1,536 patients                  | 382 patients        |
| MII Oocytes (Mean Difference)       | −0.27 (−0.56, 0.02)                                          | −0.37 (−2.45, 1.71)         | −0.60 (−1.31, 0.12)             | −0.54 (−1.13, 0.05) |
|                                     | p = 0.07                                                    | p = 0.730                   | p = 0.10                        | p = 0.07            |
|                                     | F = 94%                                                      | F = 91%                     | F = 89%                         | F = 92%             |
| 20 studies                          | 3,544 patients                                              | 5 studies                   | 11 studies                      | 4 studies           |
|                                     | 352 patients                                                | 352 patients                | 2,871 patients                  | 424 patients        |
| GnRH agonist                        | −0.50 (−1.01, 0.01)                                          | −0.15 (−1.30, 1.60)         | p = 0.84                        | p = 0.07            |
|                                     | p = 0.05                                                    | F = 96%                     | F = 86%                         | F = 92%             |
| 13 studies                          | 1,915 patients                                              | 7 studies                   | 706 patients                    |                     |
|                                     | 1,629 patients                                              |                            |                                |                     |
| GnRH antagonist                     | 0.04 (−0.08, 0.15)                                           | −1.36 (−1.51, −1.21)        | p < 0.001                       | p < 0.001           |
|                                     | p = 0.54                                                    | F = 0%                      | F = 0%                          | F = 0%              |
| 7 studies                           | 1,629 patients                                              | 4 studies                   | 2,165 patients                  |                     |
| EMBRYOS (Mean Difference)           | −0.04 (−0.17, 0.10)                                          | 0.07 (−0.39, 0.53)          | 0.19 (0.07, 0.30)               | −0.12 (−0.19, −0.06) |
|                                     | p = 0.54                                                    | p = 0.77                    | p = 0.001                       | p = 0.001           |
|                                     | F = 83%                                                      | F = 74%                     | F = 94%                         | F = 83%             |
| 26 studies                          | 4,721 patients                                              | 7 studies                   | 16 studies                      | 4 studies           |
|                                     | 918 patients                                                | 918 patients                | 3,321 patients                  | 500 patients        |

(Continued)
Table 4 | Continued

| Luteinizing hormone (LH) + follicle-stimulating hormone (FSH) vs. FSH | Human chorionic gonadotropin (hCG) + FSH vs. FSH | Human menopausal gonadotropin (hMG) vs. FSH | LH + FSH vs. hCG + FSH |
|--------------------------|---------------------------------------------|-------------------------------------------|-----------------------|
| **GnRH agonist** | | | |
| | $-0.07 (-0.25, 0.11)$ | | $0.23 (0.10, 0.35)$ | |
| | $p = 0.43$ | | $p < 0.001$ | |
| | $I^2 = 88\%$ | | $F = 95\%$ | |
| 17 studies | 2,890 patients | 13 studies | 2,889 patients |
| **GnRH antagonist** | | | |
| | $0.03 (-0.11, 0.18)$ | | $-0.02 (-0.19, 0.16)$ | |
| | $p = 0.64$ | | $p = 0.86$ | |
| | $I^2 = 36\%$ | | $F = 74\%$ | |
| 9 studies | 1,831 patients | 3 studies | 732 patients |

**Implantation Rate (Mean Difference)**

| Overall analysis | $0.11 (0.00, 0.21)$ | $-0.06 (-0.03, 0.01)$ | $0.22 (0.02, 0.23)$ | $-0.00 (-0.16, 0.15)$ |
| $p = 0.05$ | $p = 0.59$ | $p = 0.03$ | $p = 0.98$ |
| $I^2 = 88\%$ | $F = 0\%$ | $F = 100\%$ | $F = 96\%$ |
| 15 studies | 2,669 patients | 5 studies | 749 patients |
| | 3,208 patients | 10 studies | 430 patients |

| Overall analysis | $0.16 (0.00, 0.31)$ | | $0.25 (-0.01, 0.51)$ | | |
| $p = 0.05$ | | $p = 0.06$ | | |
| $I^2 = 36\%$ | | $F = 100\%$ | | |
| 10 studies | 1,256 patients | | 8 studies | |
| | 2,299 patients | | 2,299 patients | |

| Overall analysis | $0.01 (-0.08, 0.10)$ | | $0.15 (0.13, 0.17)$ | |
| $p = 0.83$ | | $p < 0.001$ | |
| $I^2 = 85\%$ | | $F = 0\%$ | |
| 6 studies | 1,393 patients | | 2 studies | |
| | 909 patients | | 909 patients | |

**Pregnancy Rate (Odds Ratio)**

| Overall analysis | $1.20 (1.06, 1.37)$ | $0.96 (0.72, 1.26)$ | $1.10 (0.98, 1.22)$ | $1.73 (1.26, 2.38)$ |
| $p = 0.004$ | $p = 0.590$ | $p = 0.100$ | $p < 0.001$ |
| $I^2 = 10\%$ | $F = 0\%$ | $F = 0\%$ | $F = 48\%$ |
| 29 studies | 8 studies | 25 studies | 5 studies |
| | 968 patients | | 6,894 patients |
| | 17 studies | | 989 patients |

| Overall analysis | $1.27 (1.09, 1.48)$ | | $1.17 (1.01, 1.36)$ | |
| $p = 0.002$ | | $p = 0.030$ | |
| $I^2 = 9\%$ | | $F = 0\%$ | |
| 22 studies | 3,834 patients | | 17 studies | |
| | 3,627 patients | | 3,627 patients | |

| Overall analysis | $1.08 (0.87, 1.35)$ | | $1.10 (0.90, 1.34)$ | |
| $p = 0.480$ | | $p = 0.370$ | |
| $I^2 = 0\%$ | | $F = 0\%$ | |
| 9 studies | 1,831 patients | | 4 studies | |
| | 1,865 patients | | 4 studies | |

| Overall analysis | $1.29 (0.91, 1.84)$ | | $1.13 (0.95, 1.33)$ | |
| $p = 0.15$ | | $p = 0.17$ | |
| $I^2 = 45\%$ | | $F = 10\%$ | |
| 5 studies | 164 patients | | 7 studies | |
| | 747 patients | | 747 patients | |

**Live Birth Rate (Odds Ratio)**

**Bold character indicates significant results.**

**Implantation Rate**

The implantation rate was calculated as the ratio between number of gestational sacs and the number of transferred embryos. This was reported in 15 studies comparing FSH alone vs. FSH + LH, demonstrating a similar rate ($p = 0.050$), maintained both in GnRH agonist ($p = 0.050$) and antagonist protocols ($p = 0.830$) (Table 4). Five studies demonstrated an equal implantation rate in the comparison FSH alone vs. FSH + hCG ($p = 0.590$) (Table 4).
Ten studies showed a higher implantation rate when hMG was used instead of FSH \((p = 0.030)\) (Table 4). This result remained in the GnRH antagonist group \((p < 0.001)\), but not in the GnRH agonist group \((p = 0.060)\) (Table 4).

No different implantation rate was found when FSH + LH was directly compared to FSH + hMG \((p = 0.980)\) (Table 4).

**Pregnancy Rate**

The pregnancy rate was significantly higher when LH was added to FSH \((p = 0.004)\), as evaluated in 29 studies for a total of 5,565 women enrolled (Figure 3A; Table 4).

Similarly, the higher pregnancy rate for the FSH plus LH group was maintained only when a GnRH agonist was used \((p = 0.002)\), not with GnRH antagonist \((p = 0.480)\) (Table 4).

Eight studies compared the use of FSH alone vs. FSH + hCG, for a total of 968 patients. The overall analysis did not find significant differences in pregnancy rate between groups \((p = 0.750)\) (Figure 3B; Table 4).

Twenty-five studies compared hMG vs. FSH during COS, for a total of 6,894 patients. Pregnancy rate did not differ between groups \((p = 0.100)\) (Figure 3C; Table 4). However, pregnancy rate was significantly higher when hMG was used in a GnRH agonist protocol \((p = 0.030)\), while it did not change in a GnRH antagonist protocol.
antagonist regimen \((p = 0.370)\) (Table 4). In the comparison between hMG vs. FSH alone, considering only IVF/ICSI cycles, 22 studies remained in the analysis, for a total of 6,354 patients. Pregnancy rate did not differ between groups \((p = 0.070)\) (Figure S1 in Supplementary Material). Considering only GnRH agonist protocols, 18 studies remained in the analysis, confirming the improved pregnancy rate in hMG group vs. FSH alone \((p = 0.003)\) (Figure S2 in Supplementary Material).

Finally, five studies evaluated pregnancy rate comparing FSH + LH vs. FSH + hCG, for a total of 989 women. A higher pregnancy rate was observed when LH was added to FSH, rather than hCG \((p < 0.001)\) (Table 4).

### Risk of Bias

The risk-of-bias was evaluated and summarized in Figure 4.

### Overall Model

The main concepts found by our data analysis were graphically summarized by a plot (Figure 5), representing the means and 95% confidence intervals of each fertilization step and gonadotropin regimen as extensively detailed in the subchapters above. In this overall model, COS served as an example of gonadotropins efficacy *in vivo* illustrating LH and hCG action on the ovary (Figure 5). Second-order polynomial functions were used as a fitting model of the standard mean differences (on the Y axis) calculated for each endpoint of the meta-analysis, considering FSH + LH vs. FSH alone, FSH + hCG vs. FSH alone and hMG vs. FSH (Figure 5). The number of oocytes retrieved is higher when FSH is used alone in all comparison, but the addition of LH or LH activity (such as in the case of hMG) progressively improves the ART outcomes, suggesting a positive effect of LH on oocyte quality. Especially, MII oocytes, embryos, implantation rate, and pregnancy rate improve progressively and linearly when LH is
used (red line), an effect attenuated when hMG is used (blue line) (Figure 5). On the contrary, hCG addition does not improve ART outcome (black line) (Figure 5).

**DISCUSSION**

This is the first meta-analysis comparing comprehensively the efficacy of the mostly used gonadotropin combinations in ART. We find that the administration of FSH alone during COS retrieves higher oocyte number than either LH supplementation or hMG use. However, the combined use of FSH + LH reduces the FSH dose required for oocyte retrieved, while hMG leads to higher FSH dose needed. Interestingly, FSH + LH increases the pregnancy rate of about 1.20 fold, in spite of lower number of oocyte retrieved compared to FSH alone, whereas hMG does not. On the contrary, FSH + hCG treatment does neither change final oocytes number, nor FSH dose required for each oocyte, nor pregnancy rate. Although live birth rate is usually considered a

**FIGURE 4** | Risk-of-bias graph: the authors’ judgment about each risk-of-bias item is presented as percentages across all included studies.

**FIGURE 5** | Overall model of meta-analysis results. Each scatter plot represents the mean differences with related confidence interval (95%) for each of assisted reproductive technology outcomes evaluated. The three lines represent the polynomial trend line. Red line shows the results with luteinizing hormone supplementation, blue line with human menopausal gonadotropin and black line with human chorionic gonadotropin.
better endpoint than pregnancy rate to evaluate ART outcome, it is not reported in many studies included and our meta-analysis does not show significant difference in live birth rate. All these differences are modest but, although apparently not clinically relevant, they are useful to better understand in vivo the overall effects of the different gonadotropin regimens.

These results suggest that gonadotropin preparations differently influence COS outcome, providing some evidence for ART personalization and improvement and leading to different results compared to those of previous meta-analyses. This difference could be due to the wide range of studies evaluated, which are focused on different endpoints and patient characteristics. FSH + LH treatment is linked to a relatively lower number of oocytes retrieved but higher pregnancy rate. The addition of LH or LH-activity might increase the selective pressure exerted on follicular selection exerted by the two gonadotropins together, compensated by improved oocyte quality. Indeed, the differences between FSH alone and FSH + LH or LH activity are lost, at least in terms of MII oocyte number. Moreover, the use of hMG leads to a higher embryos number and implantation rate compared to FSH alone. These results confirmed that the higher pregnancy rate found when FSH + LH or hMG are used together with GnRH agonist protocol, instead of FSH alone, is due to a positive effect of better oocyte quality on fertilization and embryo implantation. On the contrary, FSH + hCG treatment does not change ART outcomes compared to FSH alone, suggesting that LH and hCG result in different actions in vivo in the presence of FSH, reflecting in vitro observations (3). The overall model (Figure 5) shows a progressively better outcome when FSH is used together with LH or LH-activity (such as hMG). Thus, LH and hCG action in vivo is different in women undergoing COS, with LH improving oocyte maturation and quality, and therefore pregnancy rate, more than hCG, reflecting previous in vitro data.

Luteinizing hormone and hCG are characterized by specific molecular and biochemical features; they interact with distinct binding sites of the same receptor (25–27), resulting in lower dissociation rate by hCG than LH binding (28). Gonadotropin-specific ligand-receptor features imply different gene expression and intracellular signaling in vitro, whereby LH triggers higher levels of ERK1/2- and AKT-pathway activation than hCG, which, in turn, mediates more potent cAMP increase in human primary granulosa cells (2). Downstream effects of gonadotropins’ signaling consist in LH-related proliferative and anti-apoptotic signals, vs. high steroidogenic potential and pro-apoptotic activity of hCG in vitro, in both human and goat primary granulosa cells (3, 29). In particular, cell death was described as a result of the intracellular cross-talk among cAMP/protein kinase A (PKA)-mediated steroidogenic and pro-apoptotic pathways (30) preferentially activated by FSH and hCG, in steroidogenic cells in vitro (31).

Interestingly, our analysis of the literature reveals that LH addition to FSH treatment for ART provides lower oocyte numbers than other treatments, probably as a result of higher follicular selection (which is apoptosis-mediated). In this regard, few speculative considerations should be done. First, COS cycles are far from being a physiologic hormonal regimen; they are optimized for multi-follicular maturation in order to obtain the highest number of healthy oocytes (32), subjecting ovaries to treatments with pre-designed, high doses of exogenous hormones, which change the natural endocrine milieu of the woman. As a result, a mono-ovulatory species becomes multi-ovulatory, deviating from the natural, cyclic balance between gonadotropins and steroid hormones (33) and, thereby, life/death signals, a situation clearly different from ovarian physiology. On the other hand, FSH and LH are naturally produced to regulate mono-follicular selection, growth and maturation. The message provided by in vitro studies is that highly steroidogenic gonadotropins, i.e., FSH and hCG, mediate apoptotic stimuli in granulosa cells via cAMP/PKA-pathway (2, 3, 29–31). In the ovarian setting of a multi-follicular maturation as in COS, stimulation is a potent signal for early tertiary follicle recruitment (34) and triggering steroidogenesis, results in estrogen over-production which, in turn, induces more pronounced multi-follicular survival and maturation (35) than that inductive by LH treatment.

The ART outcome obtained with hMG reflects the heterogeneity typical of this compound. hMG derives from post-menopausal or pregnant women and contains both FSH and LH activities (36). LH activity is provided by residual LH molecules and by hCG supplementation, leading to high variability of the product (37). Moreover, given the high steroidogenic potential of hCG demonstrated in vitro (2, 31), which is more similar to that of FSH rather than LH (31), it is not surprising that ART outcome does not change whether hMG is used instead of FSH, except in GnRH agonist protocols, where high oocyte numbers might possibly occur as a positive effect of the flare-up phase on follicle recruitment. The discrepancy provided by GnRH-agonist and -antagonist protocols was not demonstrated by previous meta-analyses, likely due to strict inclusion criteria focused specifically on the evaluation of the analog instead of gonadotropins combination. The most recent meta-analysis on this field suggests only a significant adverse events occurrence reduction when GnRH antagonists are used (38).

This study suggests that GnRH antagonist protocol may be disadvantageous for oocytes quality, although the addition of LH seems to compensate, at least in part, this negative effect. FSH alone allows higher number of oocytes retrieved than FSH + LH, in GnRH agonist, but not antagonist protocols. GnRH antagonist is linked to lower FSH doses required for each oocyte retrieved, in the presence of LH. Moreover, pregnancy rate is higher by hMG than FSH treatment in GnRH agonist, but not antagonist protocols. GnRH antagonist reflects the different mechanism of action and possibly different effects among GnRH analogs, which was hypothesized, although largely debated (39). GnRH analogs are differently used in clinical practice. In particular, GnRH agonists are generally proposed in women with BMI <25 kg/m² (40), in poor responders (38, 41), and/or as a final trigger to minimize the ovarian hyperstimulation syndrome (OHSS) occurrence (42). Overall, GnRH antagonist is linked to reduced COS duration and overall medical costs of the stimulation phase and is recommended when a mild stimulation is required, such as for hyper-responder women (38, 43) or PCOS patients (44). These results support the hypothetical difference between agonists and antagonists, which was never demonstrated by previous meta-analyses (Table 1).

With this in mind, the cost-effectiveness evaluation currently remains the main variable useful to guide the clinician choice in the
setting of the personalized therapy (45). However, the assessment of ART costs is particularly challenging, and the consideration of both COS-related and pregnancy/infant-associated medical costs is mandatory. Several studies evaluated the ART medical costs alone, considering the cumulative gonadotropin dosages used, the cycle cancelation rate and the risk of adverse events. The FASTT study suggested that IUI was the cheapest/efficient first-line treatment (46), while the FORT-T trial suggested better cost-effectiveness results when sequential traditional embryo transfer is selected (47). Crawford et al. (48) recently evaluated the overall ART costs in 14,398 cycles, suggesting that sequential embryo transfer is more expensive, concerning the procedure costs, but markedly cheaper overall, reducing multiple live births and total, final expenses. Although each study seems to be conclusive, these results remain challenging, and international or national consensus on the best COS approach is not reached so far. Moreover, the gonadotropin combination is not generally considered in this cost-effectiveness evaluation, limiting the strength of these suggestions. Our results suggest a reduced FSH dose needed for each oocyte retrieved when the combination of FSH + LH was used for COS. Thus, the gonadotropin combination should be considered in the cost-saving evaluation of a specific ART procedure. The overall charge, even when LH, hCG, or hMG are used in addition/substitution to FSH, must be considered according to the local reimbursement system. Finally, no study so far evaluated the “weight” of gonadotropin-producing companies on the clinician’s decision.

The main limit of this meta-analysis is the heterogeneity of studies included as suggested by the elevated I² score. Couple infertility represents a challenging clinical condition, difficult to define according to strict clinical criteria. Indeed, different inclusion and exclusion criteria are used in each trial, making the comprehensive comparison of these results difficult. As a confirmation, a recent phase III single-blind, randomized, parallel-group clinical trial performed on 939 poor responder women did not find any safety and efficacy differences between FSH alone and FSH + LH (49). This reinforces the knowledge of a high heterogeneity of studies in ART setting, in which also the women classification as poor responders could mask the different gonadotropin effects in vivo. The relative high risk-of-bias of the studies included, as shown in Figure 4, represents an important limit that should be carefully considered to design further appropriate studies. However, although the pharmacological approach to ART is evaluated, no publication biases are evident at funnel plots analyses (data not shown). As highlighted by previous meta-analyses, we found high selection and allocation biases, confirming the finding that more than 80% of clinical trials did not apply any blinding technique (50). This high percentage is probably due to the difficulty in applying these procedures to ART, in which over 30 therapeutic complex approaches are currently available.

In conclusion, we found that different performance in ART is depending on gonadotropin combination used for COS, reflecting the physiological role of these molecules as previously indicated by in vitro data. This leads to important implication for clinical practice, where pregnancy rate or oocyte numbers might be the preferentially selected outcome. Especially, LH addition to FSH decreases FSH need and progressively improves ART outcomes and pregnancy rate. In GnRH agonist protocols, a better pregnancy rate is obtained by FSH + LH and hMG treatment. FSH + hCG or hMG alone are equally effective compared to FSH alone on pregnancy rate.

Author Contributions
DS and LC searched and evaluated separately the studies. All authors participated to the analysis, discussion of the results, and manuscript preparation.

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Supplementary Material
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Figure S1 | Forrest plot evaluating the pregnancy rate comparing follicle-stimulating hormone to human menopausal gonadotropin in in vitro fertilization/intracytoplasmic injection protocols alone.

Figure S2 | Forrest plot evaluating the pregnancy rate comparing follicle-stimulating hormone to human menopausal gonadotropin in in vitro fertilization/intracytoplasmic injection protocols alone, using gonadotropin-releasing hormone agonist.

Table S1 | PRISMA 2009 Checklist.

Data Sheet 1 | Results.

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