Ovulation induction with clomiphene citrate + gonadotropin + GnRH antagonist versus ovulation induction without clomiphene citrate in women submitted to assisted reproductive techniques

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**Introduction**

Assisted reproductive techniques (ART) are widely used for the treatment of infertility/subfertility. They frequently include the recruitment of multiple follicles through a controlled ovarian hyperstimulation. The estimated pregnancy rate per started cycle with standard ovarian stimulation is 33% (Gnoth *et al.*, 2011). However, when minimal ovarian stimulation is performed the achieved pregnancy rate per cycle is much lower, around 10% (Pelinck *et al.*, 2006). Such difference highlights the relevance of ovarian stimulation for the success of ARTs.

Clomiphene citrate (CC) - a derivative of trifenilethilene steroids - was widely used in the 80’s due to the simplicity of its administration (oral route), low cost and acceptable success rate (Lopata, 1983; Quigley *et al.*, 1985; Trounson *et al.*, 1981; Vargyas *et al.*, 1984). However, the introduction of GnRH agonists for the induction of ovulation enabled the prevention of premature LH surge resulting in lower cancellation rates, improved follicular recruitment with a larger number of oocytes recovered, and improvement in routine organization of assisted reproduction (Zorn *et al.*, 1987) overwhelming the advantages in CC use. In an attempt to increase the number of oocytes retrieved aiming embryo cryopreservation and a consequent improvement in ART success, a generalized rise in the daily dosage of gonadotropins was introduced in the late 80s and early 90s. Such practice led to an increase in ART costs and, more alarming, an elevation of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS); which are considered to be the major negative consequences of ART (Nastri *et al.*, 2010). On the other hand, evidence favoring mild ovarian stimulation is accumulating recently (Fauser *et al.*, 2010; Verberg *et al.*, 2009; Zarek and Muasher, 2011).

After GnRH antagonists were introduced, the use of CC regained attention since it could be used without the premature LH surge. The combined use of CC, gonadotropins and GnRH antagonists (CC+Ant) was first published a few years ago in women with previous poor response or with polycystic ovarian syndrome (Craft *et al.*, 1999). Such protocol seems to
reduce the number of injections, the total cost (Hwang et al., 2003) and the risk of OHSS (Lin et al., 2007). However, there are some concerns regarding a possible worsen in endometrial receptivity, since CC has an anti-estrogenic effect on the endometrium (Gerli et al., 2000; Mansour et al., 2003).

The small studies that have been performed so far point out the need for a systematic meta-analysis of the best available evidence to facilitate a more robust conclusion. The objective of this study is to compare the effectiveness and safety of CC+Ant protocols with protocols that do not include CC for ovulation induction (non-CC), by using only gonadotropins associated with either GnRH agonists or antagonists: the non-CC protocols are currently the mainstream for ovulation induction.

Methods

Eligibility criteria

Only truly randomized clinical trials will be considered eligible for inclusion. Quasi and pseudo-randomized clinical trials will be excluded. Cross-over trials would be included for completeness, but only the data from the first phase would be pooled in the meta-analysis because the design is not valid in the context of subfertility trials (Vail and Gardener, 2003). We will include studies that compared CC+Ant protocols vs. Non-CC protocols for ovulation induction in women undergoing ART requiring oocyte retrieval and embryo transfer. The number of allocated women will be used as the denominator and data will be analysed according to the intention-to-treat (ITT) principle. The only exception is to miscarriage, when the number of clinical pregnancies in each group will be considered as the denominator because miscarriage is a harm that can only occur in pregnant women. Trials evaluating only per cycle outcomes will not be included because they potentially lead to a unit-of-analysis error.
**Information sources and search**

We will search the following electronic databases: Cochrane Central Register of Controlled Trials (CENTRAL), Database of Abstracts of Reviews of Effects (DARE), Medical Literature Analysis and Retrieval System Online (MEDLINE), Embase, PsycINFO; Cumulative Index to Nursing and Allied Health Literature (CINAHL); and *Literatura Latino-Americana e do Caribe em Ciências da Saúde* (LILACS). We will search for ongoing trials on Current Controlled Trials (www.controlled-trials.com), ClinicalTrials.gov (http://clinicalTrials.gov), and the World Health Organization International Trials Registry Platform search portal (www.who.int/trialsearch/Default.aspx); and for conference abstracts on the ISI Web of Knowledge. The following search terms will be used, adjusting for each database as necessary: ((clomiphene) or (clomifene) or (milophene) or (serophene) or (clomid) or (omifin)) and ((antagonist) or (cetrorelix) or (ganirelix) or (abarelix) or (degarelix) or (cetrotide) or (orgalutran) or (plenaxis) or (firmagon)).

**Study selection**

Two authors (ADDV and JBPF) will independently scan the retrieved titles and abstracts selecting those who clearly do not meet the eligibility criteria; disagreements between reviewers will be resolved by consulting a third author (WPM), who will obtain full copy of trials. Full articles will be examined for eligibility independently by two reviewers (ADDV and JBPF) and disagreements between reviewers will again be resolved by consulting a third author (WPM).

**Data collection process**

Two authors (ADDV and JBPF) will independently extract the following data from included studies using a data extraction form designed and pilot-tested by the authors. Disagreements between reviewers will be resolved by consulting a third author (WPM). We will contact the study corresponding author in order to resolve any data queries as required. In order to analyze data according to the intention-to-treat principle, the number of women randomized
will be used as the denominator. The names of article authors and titles of the included studies will be juxtaposed seeking for duplicate publication; if any duplicated publication is found we will consider both articles as being part of a unique study.

Data items

The following data will be extracted from included studies: (1) methods: aim of study, method of recruitment of participants, inclusion/exclusion criteria, and if informed consent was obtained as well as ethical approval; (2) participants characteristics: number, age; (3) intervention: type of protocols used for ovulation induction; (4) outcomes of effectiveness: live birth per allocated woman, and clinical pregnancy per allocated woman; (5) adverse events: miscarriage per clinical pregnancy, OHSS per allocated woman; (6) other outcomes: endometrial thickness, total oocytes retrieved, MII oocytes retrieved, total gonadotropin used, and days of ovulation induction. Although the implantation rate could not be included in the quantitative meta-analysis due to different denominator (transferred embryos instead of allocated women), we have also acquired data for the implantation rate observed in the studies as an additional outcome.

Risk of bias in individual studies

We will use Cochrane risk of bias assessment tool to judge the risk of bias (Higgins and Green, 2011): trials will be classified as being of low, high or unclear risk of bias. Two reviewers (JBPF and ADDV) will independently assess the risk of selection bias (random sequence generation and allocation concealment); performance bias (blinding of participants and personnel); detection bias (blinding of outcome assessors); attrition bias (incomplete outcome data); reporting bias (selective outcome reporting); and other potential sources of bias (e.g. number of embryos transferred, age of participants). Disagreements between these authors will be resolved by consulting a third author (WPM).

Summary measures
All results will be combined for meta-analysis with Review Manager 5.1 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). For dichotomous data we will use the numbers of events in the control and intervention groups of each study to calculate Mantel-Haenszel risk ratio. For continuous data (e.g. total gonadotropin used) we will calculate mean differences between treatment groups, using the inverse variance random-effects method. We will present 95% confidence intervals for all outcomes.

We choose to use Mantel-Haenszel methods because they have better statistical properties when there are few events (Higgins and Green, 2011). If, for any outcome, we observe a zero cell count or prevalence < 1% we will use Peto fixed-effect model because this method was found to be the least biased and most powerful, providing the best confidence interval coverage in these situations (Higgins and Green, 2011). We also prefer the risk ratio (RR) because odds ratio (OR) is the hardest summary statistic to understand and to apply in practice, and many practicing clinicians report difficulties in using them; additionally there is some concern that routine presentation of the results of systematic reviews as odds ratios will lead to frequent overestimation of the benefits and harms of treatments when the results are applied in clinical practice (Higgins and Green, 2011).

We will consider as appreciable harm or benefit for the dichotomous variables a RR ≤ 0.80 or RR ≥ 1.20 (Martins et al., 2011b). For endometrial thickness, we will arbitrarily defined a difference greater than 2mm as appreciable harm or benefit, since 2mm is the expected intra- and inter-observer agreement (Martins et al., 2011a). For the number of oocytes retrieved (both total and MII) we will consider ≥ 2 oocytes as appreciable harm or benefit (arbitrary choice). We will not stipulate any value for the change in total gonadotropin used or for duration of ovulation induction as appreciable harm or benefit.

Presence of statistical heterogeneity of treatment effect among studies will be examined using $I^2$. The following broad classifications of heterogeneity will be used (Higgins and Green, 2011): 0% to 40%, might not be important; 30% to 60%, may represent moderate
heterogeneity; 50% to 90%, may represent substantial heterogeneity; 75% to 100%, considerable heterogeneity present. Substantial heterogeneity (I² > 50%) among the studies will be addressed firstly by checking again that the data is correct and, secondly, by performing subgroup analysis and searching for its causes.

*Risk of bias across studies*

If 10 or more studies are included in an analysis, a funnel plot will be used.

*Additional analysis (subgroup analysis)*

If substantial heterogeneity is observed we will stratify the results by the type of participants: 1. Unselected or non poor prognosis women; 2. Women with previous poor response or low ovarian reserve; 3. Women with polycystic ovary syndrome (PCOS) or at high risk for OHSS. By unselected woman we mean women evaluated by studies that did not specify inclusion criteria based on prognosis; by non poor prognosis women, we mean women evaluated by studies that used as inclusion criteria at least one of the following: a maximum age; a maximum FSH value; a maximum number of previous unsuccessful IVF attempts; and absence of uterine abnormalities. If significant heterogeneity persists, we will perform subgroup analysis separating studies by the Non-CC protocols: GnRH agonists or GnRH antagonists.

**Authors’ roles**

Wellington P. Martins: Drafted the protocol and developed a search strategy. This author will also obtain copies of trials, select which trials to include, carry out the analysis, interpret the analysis, and draft the final review.

Jaqueline B. P. Figueiredo: Drafted the protocol and developed a search strategy. This author will also search for trials, select which trials to include, and draft the final review.

Andréa D. D. Vieira: Drafted the protocol and developed a search strategy. This author will
also search for trials, select which trials to include, and draft the final review.

Carolina O. Nastri: Drafted the protocol. This authors will also interpret the analysis, and draft the final review.

**Sources of support**

**Internal sources**

CAPES, Brazil: PhD scholarship; CNPq, Brazil: Post-doctoral fellowship; FMRP-USP, Brazil: Authors' salary.

**External sources**

This study will not receive any outside funding.

**Conflict of interest**

None declared.
References


Zarek SM, Muasher SJ. Mild/minimal stimulation for in vitro fertilization: an old idea that needs to be revisited. *Fertility and Sterility* 2011;95:2449-2455.