J Assist Reprod Genet (2012) 29:1343–1351
DOI 10.1007/s10815-012-9863-2

ASSISTED REPRODUCTION TECHNOLOGIES

MILD ovarian stimulation with GnRH-antagonist vs. long protocol with low dose FSH for non-PCO high responders undergoing IVF: a prospective, randomized study including thawing cycles

Simona Casano · Daniela Guidetti · Ambra Patriarca · Giulia Pittatore · Gianluca Gennarelli · Alberto Revelli

Received: 24 July 2012 / Accepted: 12 September 2012 / Published online: 20 October 2012

Abstract
Objective To compare the effectiveness of two stimulation protocols in non-polycystic ovary (PCO) high responders undergoing in vitro fertilization (IVF).
Design Prospective randomized trial.
Setting A Reproductive Medicine and IVF Unit of a University Hospital and a private IVF Clinic.
Methods Four hundred-and-twelve normoovulatory women with good ovarian responsiveness were randomized to receive either the "mild" (FSH 150 IU/day from day 4 of a spontaneous cycle followed by GnRH-antagonist from day 8; n=205) or the "long" (FSH 150 IU/day; n=207) stimulation protocol. The outcome of these two regimens was compared including "fresh" and thawing cycles.
Results The total FSH dose and the peak estradiol level were significantly lower in the "mild" protocol, whereas the retrieved oocytes, fertilization rate, number and quality of embryos, pregnancy and implantation rates, cumulative "fresh plus thaw" success rate, and incidence of severe ovarian hyperstimulation syndrome were comparable with the two regimens.
Conclusions In young, normoovulatory patients with good ovarian responsiveness undergoing IVF the "mild" stimulation protocol has effectiveness and risks comparable to the "long" protocol with low FSH starting dose, even when thawing cycles are included in the comparison.

Keywords Mild ovarian stimulation · IVF · GnRH antagonist · FSH

Capsule Mild stimulation vs. long protocol in non-PCO high responders.

S. Casano · A. Patriarca · G. Pittatore · G. Gennarelli · A. Revelli (✉)
Physiopathology of Reproduction and IVF Unit,
Department of Obstetrical and Gynecological Sciences,
University of Torino,
via Ventimiglia 3,
10126 Torino, Italy
e-mail: AERRE99@YAHOO.COM

D. Guidetti · G. Gennarelli · A. Revelli
LIVET infertility and IVF Clinic,
via Tiziano 3,
10126 Torino, Italy

Introduction

In human IVF, the protocol of ovarian stimulation in which gonadotropin administration is started at a low dose (100–150 IU/d) on day 4–7 of the menstrual cycle, thus allowing a "natural" recruitment of follicles by endogenous FSH in the early follicular phase, has been addressed as "mild" [1–6]. The "mild" stimulation strategy has been proposed as an alternative to the standard "long" stimulation protocol, in which pituitary suppression is obtained in the luteal phase of the run-in cycle and the recruitment of ovarian follicles is achieved by exogenous gonadotropins.

The "mild" protocol has several advantages in comparison with the classical one: it is more patient-friendly [7], has less frequent side-effects and it is better tolerated, it bears a very low risk of ovarian hyperstimulation syndrome (OHSS), it is cheaper as much less medications are used, and it is quicker as it lasts for only 10–14 days [4]. On the other side, the "mild" stimulation strategy has been criticized because the retrieval of less oocytes is traditionally considered a negative issue for IVF effectiveness [8]: less oocytes means less embryos available for selection, transfer, and cryostorage.

The use of "mild" stimulation regimens in human IVF has been compared with the standard "long" protocol in a few randomized controlled trials (RCTs) [9–11]; taken singularly, they showed that the two regimens were substantially equivalent in terms of effectiveness, but the "mild" one caused less undesired effects and complications.

Unfortunately, however, pooling together the results of these RCTs, a noticeably lower effectiveness of the "mild" strategy appeared [12]. These RCTs [9–11], moreover, did not include the results deriving from frozen/thawed material (embryos and/or oocytes), a lack that did not allow to properly estimate the relative effectiveness of the two different stimulation approaches.

The present randomized prospective trial was designed to compare the outcome and the complication rate of a "mild" protocol with late start of FSH administration with a classical "long" protocol using a low FSH dose. A specific, properly numbered subset of patients was chosen: young women with prognostic indexes of high responsiveness to ovarian stimulation, without polycystic ovary (PCO), at their first IVF attempt. The results obtained with fresh embryos were considered together with those of the subsequent oocyte thawing cycles, and the fresh-plus-thaw cumulative success rates were calculated and compared.

Materials and methods

Patients

A total number of 463 IVF patients younger than 38 years, with basal (day 3) follicle-stimulating hormone (FSH) <8 U/l, anti-Mullerian hormone (AMH) >2 ng/ml and antral follicle count (AFC) >16, at their first IVF attempt, were identified as eligible for the study. Among them, 48 refused randomization and were excluded; other three were excluded because a spontaneous pregnancy occurred just before beginning ovarian stimulation. Among the selected patients, 412 were randomly assigned to receive either the late start, "mild" protocol (Mild Group, $n=205$) or the classical "long" protocol (Long Group, $n=207$) (Fig. 1).

The study was authorized by the local ethical committee and all patients signed a detailed informed consent.

Stimulation protocols

The "mild" protocol was performed administering 150 IU/d of recombinant FSH (Gonal F, Merck-Serono, Switzerland) from day 4 of a spontaneous menstrual cycle; the GnRH antagonist cetrorelix (Cetrotide, Merck-Serono, Switzerland; 0.25 mg/d) was then given from the fifth day of stimulation (day 8 of the cycle). When required, rFSH dose was adjusted according to the individual ovarian response from day 5 of stimulation (day 8 of the cycle).

The classical "long" protocol was performed administering the GnRH agonist buserelin (Suprefact, Hoechst, Germany; 900 mcg/d intranasally) from day 21 of the run-in cycle. After approximately two weeks, pituitary suppression was verified (appearance of a menstrual bleeding, serum estradiol <50 pg/ml, endometrial thickness <3 mm) and rFSH (Gonal F, Merck-Serono, Switzerland) was started at a daily dose of 150 IU, that was then adjusted, when required by the individual response, from day 5 of stimulation.

IVF cycle management

The ovarian response to FSH was monitored by transvaginal US plus serum estradiol (E2) measurement every third day from stimulation day 5. The cycle was cancelled when no more than one follicle >10 mm diameter was seen at US and serum E2 was <80 pg/ml the day of the first checkpoint.

Ovulation was triggered by injecting subcutaneously 10,000 IU of hCG (Gonasi HP, Ibsa, Switzerland) when at least two leading follicles reached 18 mm, with appropriate serum E2 levels.

Transvaginal ultrasound-guided oocyte aspiration (OPU) was performed approximately 36–37 hours after hCG injection under local anaesthesia (paracervical block).

Either IVF or ICSI was performed according to the clinical indication. According to Italian rules on assisted reproduction, that recommend to limit embryo overproduction, 3-8 oocytes/cycle were inseminated, deciding their number on the basis of semen quality after in vitro preparation. Mature (MII) spare oocytes with good morphological quality were frozen using the previously described slow freezing technique [13] and kept in liquid nitrogen until further use.

After two days of in vitro culture, embryos were scored according to Holte et al. [14] and transferred in utero using a soft catheter (Sydney, Cook, Australia). No more than two embryos were transferred in order to avoid triplet pregnancies, that could occur rather frequently replacing three embryos in good prognosis patients. If more than two good scoring embryos were obtained, spare embryos were frozen and kept in liquid nitrogen until further use.

The luteal phase was supported administering 180 mg/d natural progesterone (Crinone 8, Merck-Serono, Switzerland) for 15 days.

Pregnancy was assessed by serum hCG assay after 15 days from embryo transfer and then confirmed when a gestational sac was visualized at vaginal US after two further weeks. Only cases with US confirmation of pregnancy were counted in the calculation of pregnancy and implantation rates, whereas biochemical pregnancies were not considered.

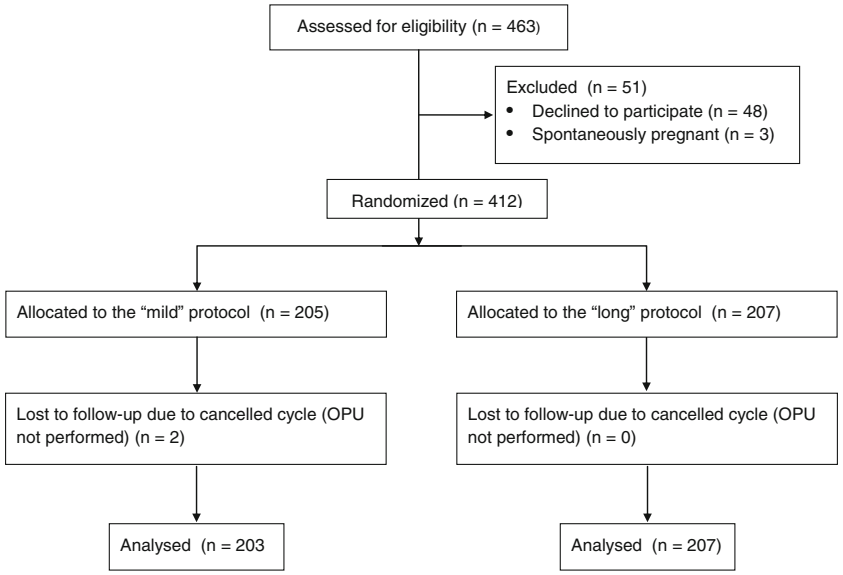

Fig. 1 Flow diagram of the study according to CONSORT guidelines 2010

Thawing cycle management

All thawing cycles were performed on a spontaneous cycle: transvaginal US was performed every seconddday from cycleday 8, and a urinary LH detection method (Clearblue) was used every day from the day following the visualization of a dominant follicle reaching 16 mm diameter. A maximal number of two thawed embryos were replaced two days after the occurrence of a positive LH detection test; oocytes were thawed and inseminated by ICSI the day after the positive LH detection test, and the derived embryos were transferred two days later. In thawing cycles, the luteal phase was supported administering 90 mg/die natural progesterone (Crinone 8, Merck-Serono, Switzerland) for 15 days.

Power calculation

The power of the study was calculated according to the primary outcome, that was the number of oocytes retrieved and available for fertilization or freezing. The calculation showed that 200 cases per study arm was the minimum number needed to detect a difference of 15 % in the primary outcome with 85 % statistical power (beta error 15 %) and a significance level at $p<0.05$. Since the study population was composed of women with prognostic variables that suggested a high responsiveness to ovarian stimulation, a very low cancellation rate (1.5 %) was a priori estimated. Secondary outcomes were the following: total gonadotropin dose, follicular phase length, ovarian sensitivity index (OSI=exogenous FSH/retrieved oocyte) [15], fertilization rate (FR), pregnancy rate (PR) per started cycle, per OPU and per embryo transfer (ET), implantation rate (IR) and live birth rate (LBR) with fresh embryos; pregnancy rate per started cycle, per OPU and per ET, implantation rate and live birth rate with thawed oocytes; cumulative fresh-plus-thaw pregnancy rate (CPR).

Randomization

Randomization was performed using a computerized algorhythm without any restriction. No blocks were used since the size of the study group was estimated to be large enough to ensure a balanced distribution of patients between groups. Allocation concealment was obtained using sequentially-numbered dark envelopes: until they were opened at the time of allocation, both physicians and patients were blinded to the study. Patients whose ovarian stimulation cycle was cancelled and did not undergo OPU were considered as lost to follow-up and not included in the analysis of the primary outcome (number of retrieved oocytes).

Data analysis

Finally, the data analysis was performed on 205 patients vs. 207. Data were expressed as mean±SD or as percentages when required. Statistical comparison between groups was performed using the Fisher exact test, Yeats' corrected Chi square test, Wilcoxon's test or Student's *t* test, as appropriate. The JMP software was used for statistical elaboration. Significance was defined as a p value<0.05.

The Jones' formula was used to calculate the cumulative fresh-plus-thaw pregnancy rate. Since thawed embryos and

thawed oocytes give the same chance of pregnancy in our hands, and both procedures were equally distributed in the two study groups, they were considered together in the calculation of CPR.

Results

The "Mild" and "Long" groups resulted to be properly balanced for the main clinical variables influencing IVF outcome (age, BMI, smoke habit, years of infertility) and for the frequency of associated male factor (Table 1). Also basal FSH, AMH and AFC were comparable between groups (Table 1). The clinical and endocrine characteristics confirm that the study population was composed of young, normally weighting women with good ovarian follicular reserve and good prognosis.

The follicular phase length was similar between groups, whereas the total FSH dose and the peak estradiol level were significantly higher in patients using the "long" protocol (Table 2). Only two cycles (1 %) were cancelled for insufficient, monofollicular response, both in the "mild" stimulation group. The number of retrieved oocytes was similar in the two groups, as well as the fertilization rate, the number of embryos available for transfer and the proportion of top-scoring embryos (Table 2). The ovarian sensitivity index (OSI) was comparable in the two groups, confirming a homogeneous ovarian responsiveness in the study population.

Overall, 178 embryo transfers were performed in the "mild" stimulation group and 192 in the "long" protocol group (Table 2), leading to 134 clinical pregnancies, 64 in the "mild" and 70 in the "long" groups, respectively (Table 2). One pregnancy in the "mild" group and two in the "long" group were twin pregnancies. The pregnancy rates per started cycle, per OPU and per ET were similar in both groups; also the implantation rate and the live birth rate were similar (Table 2). The rate of severe OHSS (requiring hospitalization) was overall low considering that the patients included herein were high responders, and was comparable between study groups (Table 2).

In about one third of the cycles (67 in the "mild" stimulation group and 73 in the "long" protocol group), embryo and/or oocyte freezing was performed (Table 3). A total number of 80 patients (42 in the "mild" group and 38 in the "long" group) underwent a thawing cycle, resulting in 12 and 10 clinical pregnancies, respectively (Table 3). The pregnancy and implantation rates with

Table 1 Clinical and hormonal characteristics of patients in the two study groups. AMH=anti-Mullerian hormone; AFC=antral follicle count. Data are expressed as mean±SD

	Mild (n=205)	Long (n=207)	p
Age (years)	34.1±3.1	34.8±3.1	ns
BMI	22±3.3	22.4 ±3.4	ns
Smoke habit (%)	13.6	17.6	ns
Associated male factor (%)	67	70	ns
Infertility duration (years)	3.4±2.1	3.4±2.0	ns
Day 3 FSH (IU/l)	6.8±1.7	6.4±1.5	ns
AMH (ng/ml)	4.3±1.6	4.4±1.9	ns
AFC	24.2±7.0	22.8±6.2	ns

Table 2 Fresh IVF cycle outcome in the two study groups. Data are expressed as mean±SD

	Mild	Long	P
Started cycles	205	207	
Cancelled cycles (%)	1	–	ns
Total FSH dose (IU)	1315±447	1709±456	< 0.001
Follicular phase length (days)	13.3±1.6	12.5±1.7	ns
Peak estradiol level (pg/ml)	2045±829	2642±1008	<0.001
OPUs	203	207	
Retrieved oocytes	9.9±5.1	10.3±4.6	ns
OSI (IU)	207±161	202±176	ns
Fertilization rate (%)	71.7	73	ns
Transferred embryos/ET	1.6±0.4	1.7±0.4	ns
Top scoring embryos (%)	46.7	42.1	ns
ETs	178	192	
Clinical pregnancies	64	70	
PR/started cycle (%)	31.2	33.8	ns
PR/OPU (%)	31.5	33.8	ns
PR/ET (%)	35.9	36.4	ns
IR (%)	21.0	23.8	ns
Abortions	14	17	
AR (%)	21.9	24.3	ns
Live births	51*	55**	
LBR/started cycle (%)	24.9	26.6	ns
LBR/OPU (%)	25.1	26.6	ns
LBR/ET (%)	28.6	28.6	ns
OHSS (%)	1.6	2.0	ns

*one twin birth

**two twin births

Table 3 Outcome of the IVF cycles with thawed eggs in the two study groups. Data are expressed as mean±SD

	Mild	Long	P
Cycles with embryo/oocyte freezing	67	73	
Cycles with embryo/oocyte freezing/OPU (%)	36.2	35.3	ns
ETs with thawed embryos/oocytes	42	38	
Clinical pregnancies	12	10	
Pregnancy rate/ET (%)	28.6	26.3	ns
IR (%)	17.1	14.1	ns
Live births	9	8	
LBR/ET (%)	21.4	21	ns
Cumulative fresh-plus-thaw PR/ET (%)	42.7	41.7	ns

thawed embryos/oocytes were similar in the two groups. The cumulative pregnancy rate considering fresh plus thaw cycles was also similar between groups (42.7 % in the "mild" group vs. 41.7 % in the "long" group) (Table 3).

Discussion

The "mild" ovarian stimulation strategy, in which a low dose of exogenous gonadotropins is administered from day 4–7 of the menstrual cycle, allows initial follicle recruitment by endogenous FSH [1–6]. In women with abundant ovarian follicular reserve, starting FSH on cycleday 4 with a fixed daily dose of 150 IU is sufficient to overcome dominance and finally obtain an average number of 5-12 oocytes [16]. As ovarian stimulation is initiated without any control over pituitary function, a GnRH-antagonist is given as soon as the circulating estradiol rise approaches the threshold level at which endogenous LH surge may be generated.

The more conventional "long" protocol, instead, is accomplished giving a GnRH-agonist from the luteal phase of the run-in cycle, and follicle recruitment is performed by exogenous gonadotropins after pituitary suppression has been achieved [17]. The "long" protocol has been the most widely used in IVF throughout the world until recent times, and has been reported to be quite effective, although prone to cause a relevant incidence of side effects and complications, such as the severe ovarian hyperstimulation syndrome (OHSS) [17]. Severe OHSS may occur particularly when the classical "long" protocol is applied to young, highly responsive women, its incidence ranging between 2 and 6 % of the IVF cycles in this kind of women [17].

The "mild" stimulation approach is considered more patient-friendly, bears less frequent side-effects, a lower risk of severe OHSS, and a lower impact of daily life. Some published reports compared the effectiveness of "mild" and "long" stimulation regimens in women with good ovarian reserve [9–11]. Taken singularly, all three RCTs found comparable results in terms of IVF effectiveness; however, if their results are pooled together—reaching a total number of 592 fresh IVF cycles, 313 performed with the "mild" regimen and 279 with the "long" regimen—it may be seen that relevantly poorer results were obtained with the "mild" protocol [12]. In addition, none of the three RCTs included freeze-thaw cycles, that relevantly contribute to the overall cumulative pregnancy rate; theorically, the "mild" approach should produce less oocytes and embryos available for freezing than the "classical" one, and therefore the inclusion of thawing cycles could widen the gap in favour of the "classical" strategy.

The present trial is, to the best of our knowledge, the first prospective randomized trial comparing a "mild" and a "long" stimulation regimen that includes in the calculation of IVF outcome both "fresh" cycles and cycles in which thawed material (embryos or embryos derived from thawed oocytes) was transferred. We studied young, lean women with normal ovaries and good prognostic indexes of ovarian responsiveness to exogenous FSH; patients with polycystic ovaries (PCO) were excluded because we wanted to study an homogeneous group and, in our opinion, PCO women represent a specific patients' population with an ovarian responsiveness which is totally different from that of normally cycling women.

In the present study, the FSH daily dose was intended to be the same in the two groups: 150 IU/d was chosen for the following reasons; a) it was considered the proper dose for the subset of patients studied herein; b) it was the dose used in two of the previous RCTs comparing "mild" and "long" protocols in normoovulatory young patients [9, 10]; c) a retrospective study showed that it may be used in a "long" protocol to get a mild stimulation in young patients [18]. FSH administration in the "mild" protocol was started on day 4 of the menstrual cycle because according to our preliminary experience, a later start (from day 5 onward) is associated with an unacceptably high cancellation rate due to monofollicular response (around 15 %

starting FSH on day 5). Indeed we observed herein a very low incidence of cycle cancellation (1 %) in the "mild" group, and no cycle was cancelled in the "long" group. We do suggest, therefore, that day 4 of the cycle is a good time to start ovarian stimulation when multifollicular development for IVF is required and a "mild" stimulation approach is chosen.

Overall, we observed a significantly lower FSH consumption in the "mild" group; this was not surprising since in this group no exogenous FSH was used in the first four days of the follicular phase; a careful analysis of economical costs was not the aim of the present study, but our data suggest that "mild" stimulation is probably associated with a lower cost per IVF cycle. Indeed some previous studies showed the convenience of the "mild" stimulation strategy over the "long" approach in terms of economical costs [19].

Rather surprisingly, we did not observe a significant difference between groups in terms of retrieved oocytes (primary outcome); also the fertilization rate was comparable between groups, and, as a consequence, the number of embryos available for transfer and the proportion of top-scoring embryos. We did not observe any difference in the implantation rate either; despite some previous studies claimed that the "long" protocol could worsen embryo implantation chance for the negative effect of high peak E2 levels on the endometrium [20, 21], herein, although E2 peak level was significantly higher in the "long" group, the implantation rate was similar to that observed in the "mild" group. The E2 levels that we observed were probably not as much higher in the "classical" group as requested to affect clinical results.

Overall, all variables that could affect the outcome of "fresh" IVF cycle were comparable in the two groups, and this was reflected in an equal, final effectiveness. This observation agrees with previous studies that reported that the outcome of IVF treatment is not affected by the use of a "mild" stimulation if at least five oocytes are obtained at follicular aspiration [22, 23]. Since both stimulation strategies allowed to obtain a similar number of mature oocytes, even the proportion of cycles in which spare embryos and oocytes were available for freezing was similar. When the outcome of embryo/oocyte freeze/thaw cycles were included in calculations, the effectiveness of IVF remained very similar in the two study groups.

The incidence of the most severe side-effect, severe OHSS, was comparable in both study groups; excluding from this trial PCO patients, that are known to have a high risk of developing severe OHSS (6–9 %), the OHSS frequency observed herein was the same reported by Heijnen [9] with the "mild" protocol. We are aware that this frequency could become even lower in the "mild" group if a GnRH-agonist would be used to trigger ovulation instead of hCG [24], but in the present study we preferred to stitch to hCG in order to limit the number of differences between the studied groups to a minimum. As a result, the "mild" and "long" stimulation regimens with a low FSH daily dose resulted to be equally safe. The minor side effects were not precisely recorded in the present study; our subjective impression is that the "mild" protocol is more tolerable than the "long" one, being shorter it has a lower impact on daily life organization, and almost all patients did not complain of any unpleasant symptom that could be related to ovarian stimulation (e.g. abdominal tension, nausea, etc.).

In conclusion, the present study shows that when young, normally ovulating good responders undergo IVF, starting ovarian stimulation on day 4 of the cycle according to the "mild" strategy has the same effectiveness than the classical "long" protocol with a low start FSH dose. With the limitations due to the relatively low number of thawing cycles, our results show that the "mild" regimen with FSH plus GnRH-antagonist and the classical "long" regimen with GnRH-agonists have a comparable effectiveness not only in "fresh" IVF cycles, but also when the results of subsequent thawing cycles are additionally considered. Also the risk of developing a severe OHSS is very similar (and quite low) with both regimens in this subset of patients. The only real difference between the two regimens studied herein could be in terms of economical costs, but a careful cost analysis should be accomplished before drawing definitive conclusions. Thus, which kind of ovarian stimulation may be defined "mild"? Our results suggest that not only a stimulation with late FSH administration plus GnRH antagonist is "mild", but also a classical "long" protocol can be "mild", provided a low dose of exogenous gonadotropins is used.

Competing interests The authors declare that they have no competing interests.

Authors' contribution SC, GP and AP collected the data. SC provided the first draft of the manuscript. GG and DG participated in the design of the study and performed the statistical analysis. AR conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Appendix

Table 4 CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	1
	2b	Specific objectives or hypotheses	2
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	2
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	2
Participants	4a	Eligibility criteria for participants	2
	4b	Settings and locations where the data were collected	2
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	2–3
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	3
	6b	Any changes to trial outcomes after the trial commenced, with reasons	–
Sample size	7a	How sample size was determined	3
	7b	When applicable, explanation of any interim analyses and stopping guidelines	–
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	3
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	3
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	3
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	3
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	3
	11b	If relevant, description of the similarity of interventions	–
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	3
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	–
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Flow diagram
	13b	For each group, losses and exclusions after randomisation, together with reasons	Flow diagram
Recruitment	14a	Dates defining the periods of recruitment and follow-up	–
	14b	Why the trial ended or was stopped	–
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	table
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	–
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	–

Table 4 (continued)

Section/Topic	Item No	Checklist item	Reported on page No
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	–
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	5
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	5
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	5
Other information			
Registration	23	Registration number and name of trial registry	–
Protocol	24	Where the full trial protocol can be accessed, if available	–
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	–

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

References

1. Nargund J, Fauser BCJM, Macklon NS, Ombelet W, Nygren K, Frydman R. The ISMAAR proposal on terminology for ovarian stimulation for IVF. Hum Reprod. 2007;11:2801–4.
2. Macklon NS, Fauser BCJM. Mild stimulation in in vitro fertilization. Ann N Y Acad Sci. 2003;997:105–11.
3. Check JH. Mild ovarian stimulation. J Assist Reprod Genet. 2007;24:621–7.
4. Fauser BC, Nargund G, Andersen AN, Norman R, Tarlatzis B, Boivin J, Ledger W. Mild ovarian stimulation for IVF: 10 years later. Hum Reprod. 2010;25:2678–84.
5. Ubaldi F, Rienzi L, Baroni E, Ferrero S, Lacobelli M, Minasi MG, Sapienza F, Romano S, Colasant A, Litwicka K, Greco E. Hopes and facts about mild ovarian stimulation. Reprod BioMed Online. 2007;14:675–81.
6. Verberg MF, Macklon NS, Nargund G, Frydman R, Devroey P, Broekmans FJ, Fauser BC. Mild ovarian stimulation for IVF. Hum Reprod Update. 2009;15:13–29.
7. De Klerk C, Macklon NS, Hejinen EMEW, Eijkemans MJC, Fauser BCJM, Passchier J, Hunfeld JAM. The psychological impact of IVF failure after two or more cycles of IVF with a mild versus standard treatment strategy. Hum Reprod. 2007;22:2554–8.
8. van der Gaast MH, Eijkemans MJ, van der Net JB, de Boer EJ, Burger CW, van Leeuwen FE, Fauser BC, Macklon NS. Optimum number of oocytes for a successful first IVF treatment cycle. Reprod Biomed Online. 2006;13:476–80.
9. Hejinen EMEW, Eijkemans MJC, De Klerk C, Polinder S, Beckers NGM, Klinkert ER, Broekmans FJ, Passchier J, Te Velde ER, Macklon NS, Fauser BCJM. A mild treatment strategy for in vitro fertilization: a randomised non inferiority trial. Lancet. 2007;369:743–9.
10. Hohmann FP, Macklon NS, Fauser BC. A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist co-treatment for in vitro fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. J Clin Endocrinol Metab. 2003;88:166–73.
11. Baart EB, Martini E, Eijkemans MJ, Van Ostal D, Beckers NG, Verhoeff A, Macklon NS, Fauser BC. Milder Ovarian stimulation for in vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomised controlled trial. Hum Reprod. 2007;22:980–8.
12. Revelli A, Casano S, Salvagno F, Delle Piane L. Milder is better? Advantages and disadvantages of "mild" ovarian stimulation for human in vitro fertilization. Reprod Biol Endocrinol. 2011;9:25–37.
13. De Santis L, Coticchio G. Theoretical and experimental basis of slow freezing. Reprod Biomed Online. 2011;22:125–32.
14. Holte J, Berglund L, Milton K, Garello C, Gennarelli G, Revelli A, Bergh T. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum Reprod. 2007;22:548–57.
15. Biasoni V, Patriarca A, Dalmasso P, Bertagna A, Manieri C, Benedetto C, Revelli A. Ovarian sensitivity index is strongly related to circulating AMH and may be used to predict ovarian response to exogenous gonadotropins in IVF. Reprod Biol Endocrinol. 2011;9:112–18.
16. Hohmann FP, Laven JSE, de Jong FH, Eijkemans MJ, Fauser BCJ. Low-dose exogenous FSH initiated during the early, mid or late follicular phase can induce multiple dominant follicle development. Hum Reprod. 2001;16:846–54.
17. Macklon NS, Stouffer RL, Giudice LC, Fauser BCJM. The science behind 25 years of ovarian stimulation for in vitro fertilization. Endocr Rev. 2006;27:170–207.
18. Fernandez-Shaw S, Pèrez Esturo N, Cercas Dunque R, Pons Mallol I. Mild IVF using GNRH agonist long protocol is possible: comparing stimulations with 100 UI vs. 150 UI recombinant FSH at starting dose. J Assisted Reprod Genet. 2009;26:75–82.
19. Polinder S, Heijnen EM, Macklon NS, Habbema JD, Fauser BJ, Eijkemans MJ. Cost-effectiveness of a mild compared with a standard strategy for IVF: a randomized comparison using cumulative term live birth as the primary endpoint. Hum Reprod. 2008;23:316–23.
20. Horcajadas JA, Díaz-Gimeno P, Pellicer A, Simón C. Uterine receptivity and the ramifications of ovarian stimulation on endometrial function. Semin Reprod Med. 2007;25:454–60.

21. Valbuena D, Jasper M, Remohi J, Pellicer A, Simon C. Ovarian stimulation and endometrial receptivity. Hum Reprod. 1999;14:107–11.
22. Barri PN, Tur R, Martinez F, Coroleu B. Mild stimulation in assisted reproduction. Gynecol Endocrinol. 2010;26:261–4.
23. Verberg MFG, Eijkemans MJC, Macklon NS, Heijen EMEW, Baart EB, Hohmann FP, Fauser BCJM, Broekmans FJ. The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: a meta-analysis. Hum Reprod Update. 2009;15:5–12.
24. Humaidan P, Papanikolaou EG, Tarlatzis BC. GnRHa to trigger final oocyte maturation: a time to reconsider. Hum Reprod. 2009;24:2389–94.