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ORIGINAL ARTICLE *Infertility*

Clomifene citrate or low-dose FSH for the first-line treatment of infertile women with anovulation associated with polycystic ovary syndrome: a prospective randomized multinational study

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BACKGROUND: Clomifene citrate (CC) is accepted as the first-line method for ovulation induction (OI) in patients with polycystic ovary syndrome (PCOS) associated with infertility owing to anovulation. Low-dose FSH has been reserved for women failing to conceive with CC. In this RCT, we tested the hypothesis that pregnancy rate (PR) and live birth rates (LBR) are higher after OI with low-dose FSH than with CC as first-line treatment.

METHODS: Infertile women (<40 years old) with PCOS-related anovulation, without prior OI treatment, attending 10 centres in Europe/South America were randomized to OI with either CC (50–150 mg/day for 5 days) or FSH (starting dose 50 IU) for up to three treatment cycles. The primary outcome was clinical PR.

RESULTS: Patients ($n = 302$) were randomized to OI with FSH ($n = 132$ women; 288 cycles) or CC ($n = 123$; 310 cycles). Per protocol analysis revealed that reproductive outcome was superior after OI with FSH than with CC with respect to PR per first cycle [30 versus 14.6%, respectively, 95% confidence interval (CI) 5.3–25.8, $P = 0.003$], PR per woman, (58 versus 44% of women, 95% CI 1.5–25.8, $P = 0.03$), LBR per woman (52 versus 39%, 95% CI 0.4–24.6, $P = 0.04$), cumulative PR (52.1 versus 41.2%, $P = 0.021$) and cumulative LBR (47.4 versus 36.9%, $P = 0.031$), within three cycles of OI.

CONCLUSIONS: Pregnancies and live births are achieved more effectively and faster after OI with low-dose FSH than with CC. This result has to be balanced by convenience and cost in favour of CC. FSH may be an appropriate first-line treatment for some women with PCOS and anovulatory infertility, particularly older patients.

CLINICAL TRIALS REGISTRATION: ISRCTN41865643.

Key words: clomifene citrate / low-dose FSH / polycystic ovary syndrome / ovulation induction

Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent female endocrinopathy affecting at least 7% of all women in the fertile age group (Kousta *et al.*, 1997). The symptoms of PCOS include irregular or absent ovulation, hyperandrogenism manifesting as hirsutism and persistent acne, and characteristic changes in ovarian morphology. The syndrome is often

accompanied by abdominal obesity and insulin resistance and, as such, is frequently associated with the metabolic syndrome. PCOS is diagnosed in 75% of women with anovulatory infertility (Kousta *et al*., 1997): successful treatment by ovulation induction (OI) will result in the delivery of a single healthy baby avoiding the complications of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS).

For almost 50 years, the undisputed first-line medication for OI in PCOS has been clomifene citrate (CC). CC is a simple, cheap and effective method to induce ovulation with minimal side-effects. Acting as an estrogen receptor antagonist in the hypothalamus and anterior pituitary, thus increasing the secretion of FSH, CC induces ovulation in 75–80% of patients but pregnancy is achieved in only 35–40% (Homburg, 2005). The discrepancy between ovulation and pregnancy rates is thought to be related mainly to the anti-estrogenic effect of CC on cervical function and on endometrial receptivity. Increased LH secretion induced by CC, which does not occur with FSH administration, may also reduce the likelihood of conception (Hamilton-Fairley and Franks, 1990).

When CC resistance (failure to ovulate with maximal doses) or CC failure (failure to conceive despite ovulation in six or more cycles of treatment) occurs, the accepted second-line of treatment is direct ovarian stimulation and OI with injectable FSH. In the earlier days of gonadotrophin administration, this was fraught with the complications of multiple pregnancy and OHSS which could even be life-threatening (Hamilton-Fairley and Franks, 1990) owing to the high sensitivity of polycystic ovaries to FSH. Fortunately, these complications have been largely overcome following the introduction of low-dose protocols for the administration of FSH, which achieve mono-ovulation in 70% of cycles (Polson *et al*., 1987; Homburg and Howles, 1999). This has almost completely eliminated the prevalence of severe OHSS and has lowered the incidence of multiple pregnancy from 34 to <6% (Hamilton-Fairley and Franks, 1990; Homburg and Howles, 1999).

These clear benefits of the low-dose FSH protocol as a second-line therapy suggested the possibility of using it as a first-line approach for OI in PCOS, which might result in an increased singleton pregnancy rate and shorter treatment to pregnancy interval. The aim of this RCT was to test the hypothesis that the (cumulative) pregnancy rate (PR) and live birth rates (LBR) in OI are higher with low-dose FSH than with CC as a first-line treatment.

Materials and Methods

A prospective, randomized, multicentre, multinational, controlled trial in 10 centres throughout Europe and South America. This study was reported according to the CONSORT criteria for RCTs comparing medical treatment in two different arms.

Subjects were <40 years old. There were no weight or BMI restrictions. Tubo-peritoneal evaluation was performed only in subjects with a previous history of pregnancy resulting in a spontaneous abortion, a previous history of gynaecological or abdominal surgical intervention or pelvic inflammatory disease. Those admitted to the study all had a normal uterine cavity and tubal patency demonstrated by radiological (hysterosalpingogram), laparoscopic or ultrasonic means before entering the study. Male partners all had a normal semen analysis conforming to World Health Organization criteria (World Health Organization, 1999).

The study was conducted from August 2005 to March 2009.

A power calculation, using Russ Lenth's power and sample size comparing two proportions (Lenth, 2006–2009) demonstrated that 150 subjects were required in each arm to achieve a relative increase in PR of 50% from 35 to 52.5% with 80% power, a significance level of 0.05 and allowing for a total of 50 drop outs from the study.

Treatment-naïve women ($n = 302$) with anovulatory or oligo-ovulatory infertility associated with PCOS (diagnosed according to the Rotterdam European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine criteria, 2004) were randomized by computer-generated randomization in sealed opaque envelopes, to receive three cycles of either CC or FSH. Randomization was stratified by centre in blocks of 20 inclusions per centre. The starting dose of CC was 50 mg/day (oral) for 5 days from Day 4 of a spontaneous or progestin-induced menstruation, rising by 50 mg/day up to 150 mg in subsequent cycles if ovulation was not achieved. Recombinant human FSH (Puregon, Schering-Plough, Houten, The Netherlands) was given s.c. in a low-dose protocol starting with 50 IU on cycle day 4, with weekly increments of 25 IU as necessary to induce a follicular response (Leader, 2006).

Treatment cycles were monitored by ultrasound examination of follicle number and size and endometrial thickness starting on Day 11 of the cycle for both CC and FSH cycles. Repeat examination was performed every 1–3 days as clinically indicated until the criterion for hCG administration

Table I Baseline demographic and clinical characteristics of patients in a prospective, randomized multinational study of CC versus low-dose FSH for the first-line treatment of infertile women with anovulation associated with PCOS.

	CC ($n = 143$)	FSH ($n = 159$)
Age (years)	29.4 (4.0)	29.8 (3.8)
BMI (kg/m^2)	25.7 (6.0)	25.1 (5.2)
Waist circumference (cm)	91.1 (13.9)	91.5 (11.8)
Duration of infertility (years)	2.1 (1.8)	2.1 (1.9)
Primary infertility	107 (75%)	124 (78%)
Secondary infertility	36 (25%)	35 (22%)
*Clinical or biochemical hyperandrogenism	111 (78%)	102 (64%)
Polycystic ovaries on ultrasound	143 (100%)	159 (100%)
Duration of average cycle		
<28 days	2 (1%)	3 (2%)
28–35 days	6 (4%)	10 (6%)
36–90 days	56 (39%)	72 (45%)
91–180 days	40 (28%)	36 (23%)
>180 days	38 (27%)	34 (21%)
LH (IU/l)	10.1 (4.4)	9.8 (5.9)
FSH (IU/l)	5.8 (1.4)	5.5 (1.7)
Testosterone (nmol/l)	2.1 (0.9)	2.1 (0.9)
Androstenedione (nmol/l)	8.7 (3.3)	8.4 (3.5)
**DHEAS (μmol/l)	5.4 (2.4)	4.4 (2.2)
SHBG (nmol/l)	52.5 (28.9)	50.6 (24.6)
17-OH progesterone (nmol/l)	3.8 (1.9)	3.5 (1.5)
Fasting glucose (mg/dl)	82.7 (16.7)	81.7 (20.6)
Fasting insulin (IU/l)	8.6 (5.6)	8.3 (5.9)

DHEAS, dehydroepiandrosterone sulphate; SHBG, sex hormone-binding globulin.
Data are mean (SD) or *n* (%).
*$P < 0.01$.
**$P < 0.04$, multivariate analysis CC versus FSH.

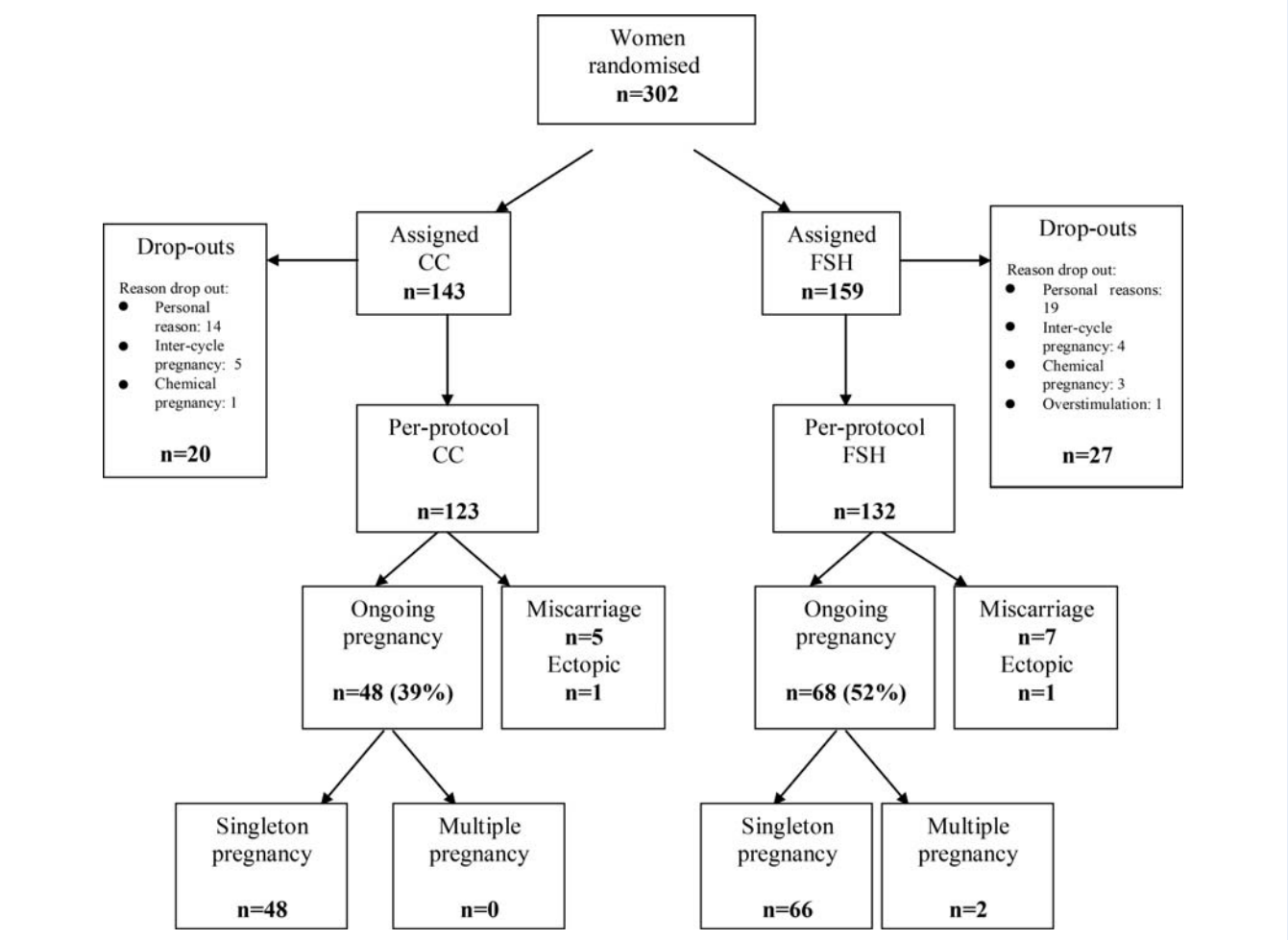

Figure 1 Profile of prospective, randomized multinational study of CC versus low-dose FSH for the first-line treatment of infertile women with anovulation associated with PCOS. FSH, low-dose FSH.

(10 000 IU i.m.) was reached (at least one follicle ≥17 mm diameter). hCG was withheld if three or more follicles of >15 mm diameter were seen on ultrasound. Intercourse was advised on the day of hCG and the following day. Clinical pregnancy was defined as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs with at least one foetus at 6–7 weeks gestation and this pregnancy was deemed as ongoing pregnancy if it continued for more than 20 completed weeks of gestation. A miscarriage was defined as the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestation and a multiple pregnancy as one in which there was more than one foetus. A live birth was defined as the birth of a viable infant, reported on a clinical research form transmitted to the co-ordinating centre.

Each participating centre received appropriate ethical and other regulatory committee approvals (registered trial ISRCTN41865643). An informed consent form was signed by every participant in the study.

All biochemical analyses were performed with commercially available kits. LH and FSH were measured using a two-step immunoassay, total testosterone with a standard immunoassay, androstendione and sex hormone-binding globulin with an enzyme immunoassay, dehydroepiandrosterone sulphate (DHEAS) and 17-hydroxy progesterone with a delayed one-step immunoassay, fasting glucose with hexokinase/glucose-6-phosphate dehydrogenase methodology and insulin by immunoradiometric assay.

Statistical analyses

Descriptive statistical analyses were performed using χ^2 and *t*-tests. Both per protocol analysis and intention-to-treat analysis were carried out. To calculate cumulative PR and LBR, we used the Kaplan–Meier survival analysis. An important advantage of the Kaplan–Meier curve is that the method can take into account 'censored' data, which are losses caused by drop outs from the study before the final outcome (pregnancy) is observed (Daya, 2005). The log-rank test was used to test the probability that there was a difference in survival scores between the CC and FSH groups. Statistical analysis was performed using the Statistical Package for the Social Sciences 15.0 software for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

Results

Of 302 subjects randomized, 143 received CC (340 cycles) and 159 received low-dose FSH (326 cycles). The only demographic or clinical differences between the randomized groups, confirmed by multivariate analysis, were a higher prevalence of clinical hyperandrogenism ($P < 0.01$) and higher serum DHEAS concentrations ($P < 0.04$) in the CC group (Table I). In the CC group, 20 patients did not complete the full study (5 inter-cycle and 1 chemical pregnancy and 14 for

personal reasons) compared with 27 in the FSH group (4 inter-cycle and 3 chemical pregnancies, 19 for personal reasons and 1 following a cycle cancelled because of overstimulation) leaving 123 patients in the CC group and 132 patients in the FSH group evaluable for analysis (310 cycles CC, 288 cycles FSH) (Fig. 1). Thus, only one subject withdrew from the study owing to a treatment-related effect (overstimulation). For analysis, all started treatment cycles were considered.

Intention-to-treat analysis

The intention-to-treat analysis revealed that the PR per cycle was higher after OI with FSH (23.3%) than after OI with CC (15.9%) [95% confidence interval (CI) 1.3–14, $P = 0.016$], and that there was a similar proportion of women achieving a clinical pregnancy (80/159; 50.3 versus 59/143 or 41.3%, 95% CI −2.1 to 20.3, $P = 0.1$) or a live birth (72/159 or 45.3 versus 53/143 or 37%, 95% CI 2.8 to 19.3, $P = 0.12$).

Per protocol analysis

Of the women receiving CC, 63% ovulated on the minimum dose of 50 mg a day resulting in 39 of the 54 pregnancies (72.2%) achieved with CC. Eight women (6.5%) were CC resistant, failing to ovulate on 150 mg/day. Treatment was cancelled in 19 (6.6%) of the 288 cycles of low-dose FSH treatment because of overstimulation and so hCG was withheld to prevent multiple pregnancy and ovarian hyperstimulation. There were no such cases of overstimulation in the CC group.

When compared with OI with CC, OI with FSH was associated with a higher proportion of women achieving a clinical pregnancy (58 or 76/132 versus 44% or 54/123, 95% CI 1.5–25.8, $P = 0.03$) or a live birth (52 or 68/132 versus 39% or 48/123, 95% CI 0.4–24.6, $P = 0.04$) (Table II). When compared with OI with CC, OI with FSH was associated with a higher PR per cycle (26.4 versus 17.4%, 95% CI 2.4–15.6, $P = 0.008$), but with a similar multiple PR, first trimester miscarriage rate and ectopic PR (Table II).

Clinical pregnancies in the CC group were achieved fairly evenly over the three cycles (33, 41 and 26% of the pregnancies, respectively), whereas those from FSH were mainly achieved in cycle 1 (52.6%) with a further 35.5% in cycle 2. The PR after the first cycle of treatment was twice as high after OI with FSH (30%) than after OI with CC (14.6%, 95% CI 5.3–25.8, $P = 0.003$). The PR after the first two cycles of treatment was also higher after OI with FSH (50.7%) than after OI with CC (32.5%, $P = 0.003$).

By survival analysis, using the Kaplan–Meier and log-rank test, the cumulative clinical PR after three cycles was 41.2% for the CC group compared with 52.1% for the FSH group ($P = 0.021$). The cumulative LBR after three cycles was 36.9% for the CC group compared with 47.4% for the FSH group ($P = 0.031$; Fig. 2).

Cost of visits

The monetary costs of follicle tracking and administrative were the same for both treatments (data not shown). The number of ultrasound monitoring visits per cycle was 3.3 for CC and 4.1 for FSH, the number of visits per live birth therefore was 12.3% greater with CC than FSH.

Overall, on per protocol analysis, there was both a relative and an absolute advantage in reproductive outcome after OI with low-dose FSH when compared with OI with CC with respect to clinical PR after the first cycle (100 and 13%, respectively), PR per cycle over the three cycles (77 and 13%), cumulative PR within three cycles (27 and 11%) and cumulative LBR within three cycles (27 and 10%).

Table II Results (per-protocol) comparing treatment with clomifene and low-dose FSH.

	CC	FSH	*P*-value
Number of patients randomized	143	159	
Number of patients per protocol	123	132	
Cycles	310	288	
Clinical pregnancies (per patient)	54 (44%)	76 (58%)	0.03
Ongoing pregnancies (per patient)	48 (39%)	68 (52%)	0.04
Clinical pregnancies (per cycle)	54 (17.4%)	76 (26.4%)	0.008
Ectopic pregnancies	1	1	
Miscarriage rate per pregnancy[a]	5 (9.2%)	7 (9.2%)	
Multiple pregnancies (twins only)	0	2 (3.4%)	
Cumulative pregnancy rate			
Cycle 1	12.9%	25.6%	
Cycle 2	29.3%	44.8%	
Cycle 3	41.2%	52.1%	0.02

Data are *n* (%), χ^2 and *t*-tests (two-sided) used for simple comparisons and Kaplan–Meier survival analysis for cumulative pregnancy rates
[a]In first trimester.

Discussion

Our study demonstrated that in treatment-naive patients with PCOS, the reproductive outcome after OI with low-dose FSH was significantly better than after OI with CC, with respect to clinical PR and LBR per (first) cycle, cumulative clinical PR and LBR over three cycles and time to pregnancy. These results are in line with the trend observed in another similar study but which did not reach significance because of the small numbers involved ($n = 38$ in each group) (Lopez *et al.*, 2004).

Although from the patient perspective CC is more convenient (oral intake) and cheaper than low-dose FSH, this relative advantage for CC is reduced as current guidelines in most countries now recommend the same intensity of monitoring with ultrasound for CC cycles as for low-dose FSH treatment. Furthermore, 7–15% of subjects do not ovulate with maximal doses of CC and 15–50% of those who ovulate demonstrate a suppressed thin endometrium which considerably reduces the chance of pregnancy (Gonen and Casper, 1990; Homburg, 2005). In contrast, OI with low-dose FSH is not associated with an anti-endometrial effect and only rarely associated with ovulation failure (Homburg and Howles, 1999).

While our study confirmed our hypothesis that OI with low-dose FSH results in a high PR and LBR, the reproductive outcome after OI with CC was better than expected (high PR and LBR) and surprisingly was associated with a complete absence of multiple pregnancies (Homburg, 2005). We hypothesize that these positive outcomes after OI with CC can be explained by the low starting dose (50 mg/day), the consistent use of ultrasound monitoring, patient management in

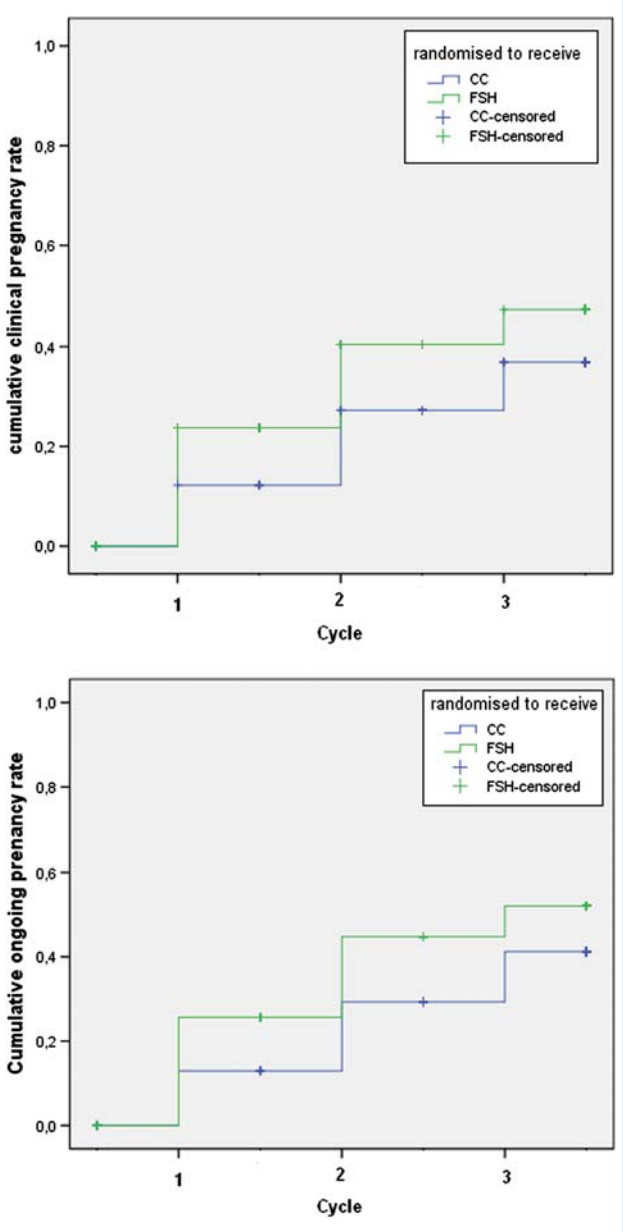

Figure 2 Kaplan–Meier curves for pregnancy and live births.

specialized OI settings and accurate timing of sexual intercourse. The criteria for the timing of hCG administration in both arms were the same. Although it has been common practice to administer hCG in CC cycles at a later stage of follicular growth than in FSH cycles, there is little evidence to support this practice which has also been recently disputed (Farhi *et al.*, 2010). Further, the high PRs following CC in this study suggest that using the same criteria for hCG administration for both arms did not influence results.

Although differences in cost and convenience may limit the choice of FSH as first-line treatment in some settings, our results may justify OI with low-dose FSH as a first line of treatment in other settings where this is financially possible and where there is access to the required level of monitoring expertise. While we agree that the cost of recombinant human FSH is much higher than CC, this cost difference is offset by a number of factors: (i) The likelihood of pregnancy after the first cycle of OI with low-dose FSH is twice as high than after OI with CC; (ii) Follicle tracking and administrative costs are the same for both treatments—the number of visits per live birth therefore is greater with CC than FSH and (iii) The psychological burden on women taking CC who undergo 6–12 cycles without conceiving before progressing to low-dose FSH therapy should also be considered.

In conclusion, our study has, for the first time, shown that reproductive outcome is superior after OI with low-dose FSH when compared with OI with CC. These data may be utilized when balancing the pros and cons of initiating OI with low-dose FSH rather than OI with CC for women with PCOS-associated anovulatory infertility.

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Authors' roles

R.H. devised and supervised the study and was mainly responsible for the writing of the report. All authors were involved in recruitment and played an active role in the conduct of the study. M.-L.H. and T.E.K. organized the data collection and analysis. All authors reviewed and criticized the manuscript prior to submission.

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Conflict of interest

None declared.

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