Prospective, randomized comparison between raloxifene and clomiphene citrate for ovulation induction in polycystic ovary syndrome

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Objective: To compare the ovulation rate between raloxifene and clomiphene citrate (CC) in patients with polycystic ovary syndrome (PCOS).
Design: Double-blind, randomized, superiority clinical trial.
Setting: Tertiary university hospital.
Patient(s): Women with ovulatory dysfunction and PCOS based on the Rotterdam criteria.
Intervention(s): One of two oral treatments: 5 days of 100 mg/day of CC or R.
Main Outcome Measure(s): Ovulation, based on follicle collapse on serial ultrasound and midsecretory serum progesterone concentration ($\geq$3 ng/dL).
Result(s): The women with PCOS (n = 82) were randomized to receive CC (n = 40) or raloxifene (n = 42). From these, 68 patients finished the trial according to the protocol (CC: n = 37; raloxifene: n = 31). There were no statistically significant differences between the groups in ovulation rates per an intention-to-treat analysis based on ultrasound alone (CC: 21 of 40 vs. raloxifene: 17 of 42) or on progesterone levels (CC: 16 of 40 vs. raloxifene: 11 of 42). No serious adverse events were observed in either group.
Conclusion(s): No statistically significant difference in ovulation was observed between raloxifene and clomiphene citrate in patients with PCOS with ovulatory dysfunction. (Fertil Steril® 2011;96:769–73. ©2011 by American Society for Reproductive Medicine.)

Key Words: Clomiphene citrate, ovulation, polycystic ovary syndrome, progesterone, raloxifene, ultrasound

Polycystic ovary syndrome (PCOS), the most common endocrine disorder affecting reproductive-age women, is a major cause of infertility due to anovulation and hormonal imbalances. According to the 2003 (Rotterdam) criteria of the European Society for Human Reproduction (ESHRE) and the American Society of Reproductive Medicine (ASRM), PCOS is defined as a syndrome of ovarian dysfunction along with the cardinal features of hyperandrogenism and polycystic ovary morphology (1). Increased risk of miscarriage after spontaneous or assisted conception has been reported up to 50% in PCOS patients, with rates that are threefold higher than in healthy women (2–4).

Clomiphene citrate (CC) is a selective estrogen-receptor modulator (SERM), and it presumably works to induce ovulation by inhibiting negative endogenous estrogen feedback on the hypothalamic-pituitary axis, resulting in an increase in follicle-stimulating hormone (FSH) secretion, follicular growth, and ovulation (5). In 2008, the consensus on infertility treatment related to PCOS recommended CC as the first line of treatment (1); however, miscarriage rates remain high with this medication (6). Some investigators have proposed that the antiestrogenic effects of CC on endometrium could be related to the lower implantation rates or higher rates of early pregnancy loss (7, 8). In a recent study, CC has been implicated as a risk factor for birth defects as well (9).

Alternatives to CC are limited. By use of a DNA microarray, we recently showed that the endometrium of women with PCOS who are receiving CC may have reduced expression of certain implantation-specific biomarkers, including leukemia inhibitory factor (10). Given the relative differences in antiestrogenic characteristics of SERMs, there may be opportunities to optimize endometrial receptivity when inducing ovulation in women with PCOS. Tamoxifen is a SERM that appears to be as effective as CC for induction of ovulation, though it is not licensed in the United States for that purpose (11, 12) and has been shown to have excessive estrogen-promoting activity in endometrial cells (13). Raloxifene is a SERM approved for the treatment of osteoporosis in postmenopausal women (14), with antiestrogenic effects at the level of the hypothalamus and/or pituitary similar to CC and tamoxifen. Raloxifene may have a favorable impact on markers of endometrial receptivity compared with CC and tamoxifen (15), and it has been shown to increase FSH levels in premenopausal women (16). To date, there are no published reports on the use of raloxifene for inducing ovulation in PCOS. Our randomized clinical trial gained early evidence of the effectiveness of raloxifene to

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induce ovulation and compared the ovulation rate with raloxifene and CC as ovulation agents in patients with ovulatory dysfunction and PCOS based on Rotterdam criteria.

MATERIALS AND METHODS

Participants and Trial Design

Our randomized, superiority trial was performed between September 2008 and October 2009 at the Hospital de Clínicas de Porto Alegre, a tertiary teaching hospital. Women with PCOS, according to the Rotterdam criteria (17), were administered a standard questionnaire and underwent a physical examination. Of note, all women under consideration had oligomenorrhea (fewer than six menses/year) as one of the Rotterdam criteria. Patient enrollment was performed by two of the authors (GSM, RAC). Women were excluded if they had elevated levels of thyroid-stimulating hormone, prolactin, or 17α-hydroxyprogesterone, if they had used oral contraceptives in the previous 2 months, or if they had a history of endometriosis.

Intervention, Randomization, and Allocation

Eligible patients were randomized to one of two treatment groups: 5 days of 100 mg/day of CC or raloxifene, each starting on day 3 after initiation of menses. The 100 mg/day dosage for raloxifene was chosen based on a previous study by Baker et al. (16). The randomization sequence was generated by computer using blocks of 4 in a 1:1 ratio. The allocation was concealed from the trial group and was performed by use of opaque, sequentially numbered, sealed envelopes by one of the authors (RFS).

The medications were prepared by a local pharmacy in identically coded packets and capsules to ensure blinding. Menstruation was induced with 7 days of oral medroxyprogesterone acetate, 10 mg per day, with the first day of menses considered to be day 1. The participants took the assigned medication on days 3 to 7. On day 10, the participants returned their medication packets to document their compliance, and they underwent an ultrasound scan every other day for up to 21 days to monitor their ovulation. Staff physicians performed the ultrasound scans. Serum was obtained to measure the levels of progesterone 8 to 10 days after presumed ovulation or on days 22 to 24 if ovulation was not detected.

Outcomes

The primary outcome was ovulation, detected either by ultrasound and/or by progesterone values on days 8 to 10 after ovulation. Ovulation detected by ultrasound was defined as the presence of a dominant follicle and its subsequent collapse. If a dominant follicle was not observed by day 21 after menses, the ovulation induction was considered to be a failure. The level of serum progesterone that indicated ovulation was considered to be 3 ng/mL or greater.

The progesterone assay was performed by a single diagnostic PNCQ-approved (Programa Nacional do Controle da Qualidade, the Brazilian organization equivalent to the Clinical Laboratory Improvement Amendments) endocrine laboratory using electrochemiluminescence immunoassay (Elecsys Progesterone II Immunoassay, cat. no. 2145383. Roche Diagnostics GmbH, D-68298), with a sensitivity of 0.03 ng/mL and an interassay and intra-assay variability of <0.2. The serum samples were obtained in the morning, between 9 AM and noon.

Patients, health-care providers, data collectors, and analysts were blind to the group allocation. Ovulation recorded by ultrasound and progesterone was a combination of both stated definitions. As a secondary outcome, participants were questioned about any side effects related to their medication.

Sample Size, Statistics, and Ethics Approval

A sample size of 40 women per arm was calculated for this superiority trial, considering an alpha and beta error of 0.05 and 0.2, respectively, to find an absolute difference of 30% in the ovulation rate between groups, based on a previous study published by Mitwally and Casper (18) where the ovulation rate with CC was approximately 45%. The statistical analysis was performed per protocol and by intention to treat using the ovulation rate (%) and a 95% confidence interval (95% CI). Cases that were lost to follow-up observation, dropped out of the study, failed to collect progesterone on days 22 to 24, and lacked menses after medroxyprogesterone acetate treatment were considered as failures according to the intention-to-treat analysis. The women who became pregnant after randomization but did not take the medication were considered as ovulatory successes in the intention-to-treat analysis. The per-protocol analysis was considered in all who had exposure to treatment and no protocol violations. The study was approved by the national institutional review board and was registered at ClinicalTrials.gov (NCT00427700).

Student's *t*-test and Fisher's exact test were used for statistical analysis. $P<.05$ was considered statistically significant. If a normal distribution of data was not found, a Mann-Whitney *U* test was used instead of Student's *t*-test.

RESULTS

During the study, 115 patients were evaluated for the trial, and 82 patients were selected (clomiphene: n = 40; raloxifene: n = 42) (Fig. 1). Three participants in the CC group were lost to follow-up, discontinued the study, or failed to induce menses. In the raloxifene group, five participants dropped out, four lacked menses, and two became pregnant after medroxyprogesterone acetate treatment. All participants returned the empty packets.

Demographic characteristics are shown in Table 1. No statistically significant differences were found in age, body mass index (BMI), hormone levels, waist-hip ratio, Ferriman-Gallwey score, or fasting glycemia (Table 1). As shown in Table 2, the primary outcome as measured by ultrasound was similar between the groups for both intention to treat and per protocol. Serum progesterone levels failed to document ovulation in some cases that were positive by ultrasound using the criteria of 3 ng/mL (Table 2). No difference in ovulation was observed by progesterone levels between the raloxifene and CC groups. As an indirect measure of ovulation, the mean serum progesterone levels in ovulatory cases was 8.6 $\pm$ 3.3 vs. 10.2 $\pm$ 7.1 (mean $\pm$ SD) in the CC and raloxifene groups, respectively; no statistically significant difference was found ($P=.5$, Student's *t*-test). The absolute difference between treatments for intention to treat was 0.12 (−0.09 to 0.33) for ultrasound, and 0.14 (−0.064 to 0.34) for progesterone.

The side effects were mild in both groups. There were two cases in raloxifene group: one woman had nausea, and the other woman had nausea, headache, and pelvic pain. One woman in the CC group had nausea, headache, and abdominal bloating. All symptoms disappeared after cessation of medication. No statistically significant difference was observed in the endometrial lining in either group: 9 $\pm$ 2.9 vs. 8.4 $\pm$ 2.1 (mean $\pm$ standard deviation) for raloxifene and clomiphene, respectively ($P=.33$).

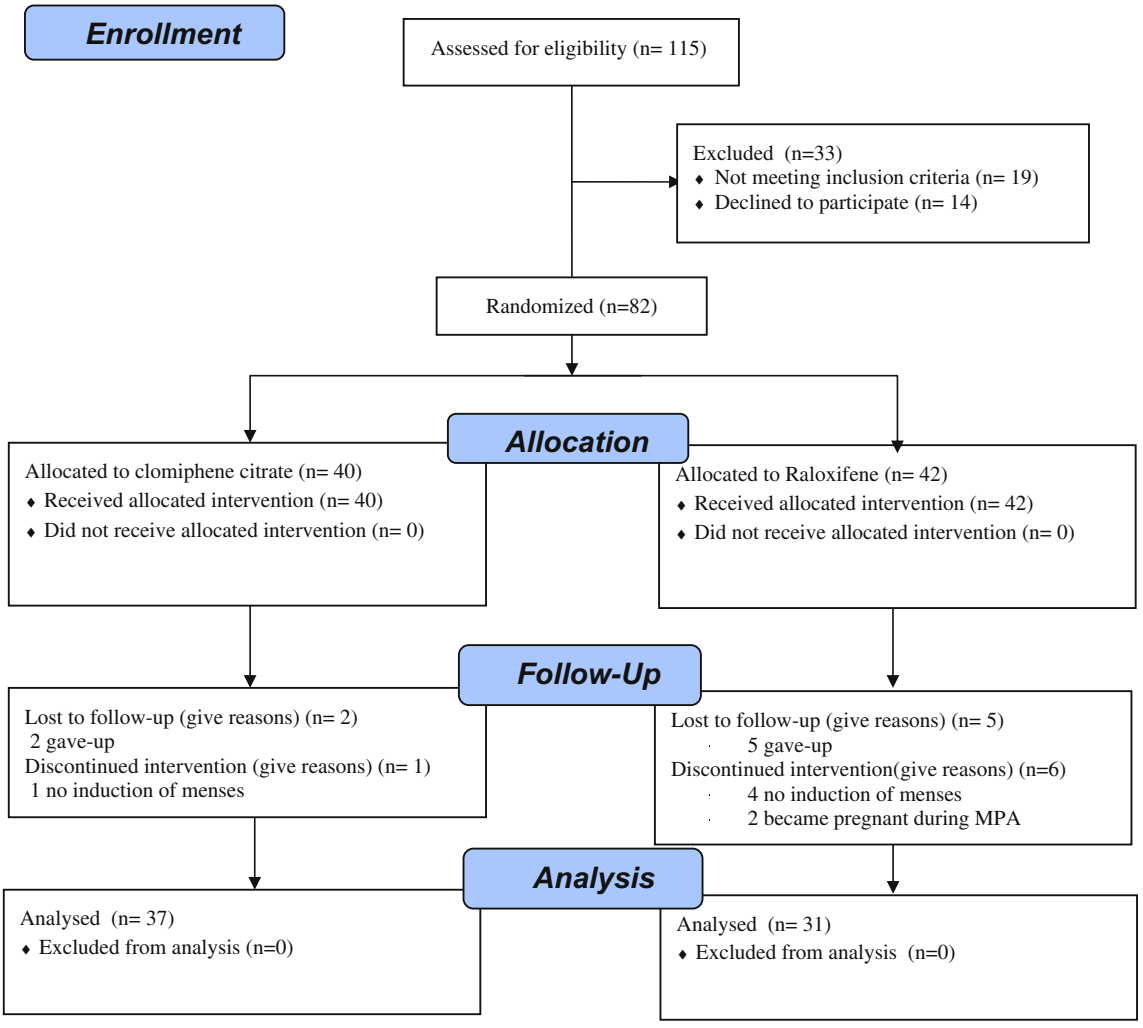
DISCUSSION

In this first randomized clinical trial comparing CC and raloxifene for ovulation induction in women with PCOS, we were unable to demonstrate a statistically significant difference in ovulation rates between the raloxifene and CC groups. Based on the study design, we cannot state that either drug is superior to the other for ovulation induction in PCOS. Actually, the absolute difference between both treatments varied between 8% and 14%, depending on which outcome was analyzed: ultrasound or progesterone levels (Table 2). Importantly, that these data suggest for the first time that raloxifene has potential for inducing ovulation in women with PCOS. Some studies have indirectly associated CC with a higher miscarriage rate (19, 20), birth defects (9), and reduced endometrial receptivity (8). Thus, our study sets the stage for future trials using raloxifene to examine the potential benefits for pregnancy outcomes in this group of women.

The endometrium of women with PCOS appears to be dysfunctional (21). Estrogen receptors (ERα) are normally reduced during

FIGURE 1

Flow Diagram.



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the midsecretory phase in fertile women (22), while the endometrium from PCOS patients exhibits high expression of this steroid receptor (23) and reduced expression of $\alpha v\beta 3$ expression (24), leukemia inhibitor factor, and GRB2-associated binding protein 1 (10). These putative defects in endometrial response and associated progesterone resistance are additional reasons for using antiestrogenic medications, and a benefit for ovulation induction in women with PCOS may be seen. Aside from the effect on pituitary FSH levels, appropriate antiestrogenic compounds might improve endometrial receptivity by suppressing the actions of ERα. Raloxifene, for example, appeared to have a positive impact on endometrial expression of $\alpha v\beta 3$ in an in vitro study model (15). A potential additional advantage of raloxifene is the shorter half-life of 32.5 hours (25) and the lack of persistent systemic accumulation compared with CC (5 to 7 days) (26). The shorter duration of raloxifene might eliminate the refractory period observed in CC treatment along with the potential for adverse effects on the fetus.

We are not aware of other studies that have compared raloxifene with CC for ovulation induction. A meta-analysis comparing CC with tamoxifen, another SERM with antiestrogen action, found no apparent benefit of one medication over the other (12), similar to our results. Tamoxifen, however, has potent estrogenic actions on endometrium, whereas raloxifene had negligible risk for hyperplasia (27). Based on its estrogenicity, tamoxifen is potentially unsuitable for ovulation induction in PCOS because it lacks any apparent benefit for uterine receptivity (15).

The strengths of this study include the randomization and concealed allocation. Few participants were lost to follow-up observation, and compliance with the treatment was high. All personnel involved in the study were blinded to the group allocation. We found that the intention-to-treat and per-protocol results were similar, suggesting that the results were robust. Another strength of the study was the inclusion of only a single month of treatment. The additive effects of CC compared with raloxifene could change the outcomes if more than one cycle were considered. Also, the study used two methods for detecting ovulation; the extended period of time for ultrasound surveillance and its correlation with progesterone levels provided greater confidence that ovulation was accurately detected.

TABLE 1

Characteristics of the patient sample before entering the trial.

Characteristic	Raloxifene (n = 42)	Clomiphene citrate (n = 40)
Age, y	28.21 ± 5.3	28.7 ± 4.8
Oligomenorrhea	42	40
Clinical hirsutism[a]	27	27
Laboratory hyperandrogenism[a]	9	9
Ovary volume >10 cm^3[a]	25	26
Pregnancy		
0	30	29
1	6	8
2	2	2
Missing data	4	1
Para	3	6
Miscarriage	7	6
Prolactin, ng/mL	11.5 ± 4.0	10.3 ± 4.2
TSH, mIU/mL	2.4 ± 1.3	2.0 ± 0.8
17-OHP ng/mL	1.05 ± 0.6	0.9 ± 0.5
Body mass index, kg/m^2	33.2 ± 7.8	32.3 ± 6.3
Waist/hip ratio	0.87 ± 0.09	0.86 ± 0.09
Ferriman-Gallwey score	10.8 ± 7.1	12.3 ± 7.6
Fast glycemia	88.2 ± 14.3	90.8 ± 11.6

Note: Values are n or mean ± SD. 17-OHP = 17α-hydroxyprogesterone; SD = standard deviation; TSH = thyroid-stimulating hormone.
[a] One patient may have more than one criterion.

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Serum progesterone levels had the further advantage of allowing a comparison of the quality of ovulation in the women who responded to the medications.

In this initial examination of raloxifene for ovulation induction in PCOS patients, we did not examine pregnancy rates or pregnancy outcomes. Furthermore, the level of insulin resistance in these patients was also not addressed in this study. Because insulin sensitivity is not a criterion for inclusion, according to the Rotterdam criteria, we did not consider this variable in our evaluation of the results. In addition, our study did not restrict patients with a very high BMI; however, inclusion of a wide range of BMIs enhances the generalizability of our results. The number of patients randomized was based on an appropriate power analysis using a 30% difference in outcome, based on a previous study (18). This difference, however, was much lower than expected, between 8% and 14%, and no statistically significant difference was found. Another limitation of our study was that only one cycle was evaluated, which could have contributed to a lower overall ovulation rate for the CC group. In subsequent cycles, raloxifene (due to its lack of accumulation) might become better than CC, as some patients with PCOS become refractory to CC over subsequent cycles (28), but future studies are necessary to verify this hypothesis. The lower rates of ovulation with raloxifene could also be related to the dosage chosen in addition to the number of cycles. In future studies, we plan to examine both the

TABLE 2

Ovulation outcome between raloxifene and clomiphene citrate according to ultrasound, progesterone levels, and ultrasound + progesterone levels, analyzed by intention to treat and per protocol.

Ovulation outcome	Raloxifene n (%) [95% CI]	Clomiphene n (%) [95% CI]	Absolute difference [95% CI]	*P* value[a]
Intention to treat	n = 42	n = 40		
Ultrasound	17 (40.4) [25.6–56.6]	21 (52.5) [36.1–68.5]	0.12 [−0.09–0.33]	.3
Progesterone[b]	11 (26.1) [13.8–42]	16 (40) [24.8–56.6]	0.13 [−0.06–0.34]	.2
Ultrasound and progesterone	11 (26.1) [13.8–42]	16 (40) [24.8–56.6]	0.13 [−0.06–0.34]	.2
Per protocol	n = 31	n = 37		
Ultrasound	15 (48.3) [30.1–66.9]	21 (56.7) [39.4–72.9]	0.08 [−0.15–0.32]	.6
Progesterone[b]	9 (29) [14.2–48]	16 (43.2) [27–60.5]	0.14 [−0.08–0.37]	.3
Ultrasound and progesterone	9 (29) [14.2–48]	16 (43.2) [27–60.5]	0.14 [−0.08–0.37]	.3

[a] Fisher's exact test.
[b] Ovulation was considered when levels of progesterone were >2.99 ng/mL.

de Paula Guedes Neto. Ovulation induction with raloxifene in PCOS. Fertil Steril 2011.

pregnancy rate and the effect of different dosages on outcome in women seeking fertility treatment.

Little information is currently available regarding the use of raloxifene for ovulation induction in PCOS. Existing studies suggest that this SERM has a favorable antiestrogenic profile for endometrium and functions similar to CC to increase serum FSH levels. In this superiority trial, we conclude that there is insufficient evidence to rule out chance as an explanation for the observed difference between these two SERMs for ovulation induction, based on our sample size and a 30% difference between treatments. However, our results do establish parameters for future studies that will compare conception rates between these SERMs, including comparison of higher dosages of raloxifene and studies on the in vivo effect on biomarkers of endometrial receptivity.

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