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ORIGINAL ARTICLE *Infertility*

How long should we continue clomiphene citrate in anovulatory women?

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STUDY QUESTION: What is the effectiveness of continued treatment with clomiphene citrate (CC) in women with World Health Organization (WHO) type II anovulation who have had at least six ovulatory cycles with CC but did not conceive?

SUMMARY ANSWER: When women continued CC after six treatment cycles, the cumulative incidence rate of the ongoing pregnancy rate was 54% (95% CI 37–78%) for cycles 7–12.

WHAT IS KNOWN ALREADY: If women with WHO type II anovulation fail to conceive with CC within six ovulatory cycles, guidelines advise switching to gonadotrophins, which have a high risk of multiple gestation and are expensive. It is however not clear what success rate could be achieved by continued treatment with CC.

STUDY DESIGN, SIZE, DURATION: We performed a retrospective cohort study of women with WHO II anovulation who visited the fertility clinics of five hospitals in the Netherlands between 1994 and 2010. We included women treated with CC who had had at least six ovulatory cycles without successful conception ($n = 114$) after which CC was continued using dosages varying from 50 to 150 mg per day for 5 days.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Follow-up was a total of 12 treatment cycles. Primary outcome was the cumulative incidence rate of an ongoing pregnancy at the end of treatment.

MAIN RESULTS AND THE ROLE OF CHANCE: We recruited 114 women that had ovulated on CC for at least six cycles but had not conceived. Of these 114 women, 35 (31%) had an ongoing pregnancy resulting in a cumulative incidence rate of an ongoing pregnancy of 54% after 7–12 treatment cycles with CC.

LIMITATIONS, REASONS FOR CAUTION: Limitations of our study are its retrospective approach.

WIDER IMPLICATIONS OF THE FINDINGS: Randomized trials comparing continued treatment with CC with the relatively established second line treatment with gonadotrophins are justified. In the meantime, we suggest to only begin this less convenient and more expensive treatment for women who do not conceive after 12 ovulatory cycles with CC.

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Introduction

Anovulation is a common cause of subfertility and is diagnosed in ~20% of all subfertile couples (Hull *et al.*, 1985). Eighty-five percent of these women have serum concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) within the normal range. This type of anovulation is classified as World Health Organization (WHO) type II. It results in irregularities in the pattern of menstrual bleeding or in amenorrhea (Brown *et al.*, 2009; The ESHRE Capri Workshop Group, 2012). In the majority of cases the anovulation is based on the polycystic ovary syndrome (PCOS). PCOS is characterized by oligo-anovulation, clinical or biochemical hyperandrogenism and polycystic ovaries (The Rotterdam ESHRE/ASRM PCOS Consensus Workshop Group, 2004).

If women with PCOS or anovulation type II wish to conceive, guidelines state that clomiphene citrate (CC) is first line treatment. CC was first described in 1961. Before this time, women with anovulation due to polycystic ovaries could only be treated by a wedge resection of the ovaries. CC is a non-steroidal compound that resembles estrogen and blocks hypothalamic estrogen receptors, signaling a lack of circulating estrogen to the hypothalamus. This process changes the pattern of pulsatile release of GnRH which results in inducing a discharge of FSH from the pituitary gland and thereby folliculogenesis. Treatment results in a 70–85% ovulation rate, and a 40–70% conception rate after six cycles (Homburg 2005; Balen 2013). The Thessaloniki ESHRE/ASRMPCOS Consensus Workshop Group advices to limit treatment with CC to six (ovulatory) cycles, but to consider a maximum of 12 cycles on an individual basis. The NICE Fertility guideline of 2013 suggests to continue CC up to a maximum of six cycles. Both guidelines state that the next step in treating these women is ovulation induction with gonadotrophins (FSH) (The Thessaloniki ESHRE/ASRM PCOS Workshop Group, 2008; NICE Fertility Clinical Guideline 156). Cumulative live birth rates of 50% after second line ovulation induction with gonadotrophins have been reported. Ovulation induction with gonadotrophins involves subcutaneous injections, requires close sonographic monitoring, is expensive and has a high risk of multiple pregnancy (14%) (Eijkemans *et al.*, 2003). In view of this; it might be preferable to continue CC for more than six cycles, but whether this continued treatment with CC for more than six ovulatory cycles is effective, is unknown.

The aim of this study was to investigate the effectiveness of continued treatment with CC for up to 12 ovulatory cycles in women with WHO type II anovulation.

Materials and Methods

Subjects

We performed a retrospective cohort study of a series of consecutive women attending the fertility clinics of five hospitals in the Netherlands between 1994 and 2010 using the patient databases of each individual hospital. We included women aged between 18 and 41 years who were diagnosed with WHO type II anovulation. Serum prolactin and thyroid-stimulating hormone levels were within normal range. All women had been ovulatory for at least six cycles on CC treatment, with a maximum of 150 mg daily for 5 days, but did not conceive. In the five participating hospitals it was standard policy to proceed with CC treatment up to 12 cycles in women that ovulated on CC. Ovulation was proved by ultrasound, basic body temperature, midluteal progesterone level or LH test. Tubal patency had been demonstrated based on hysterosalpingography, diagnostic laparoscopy with tubal testing or hydrolaparoscopy. Women were excluded if they were treated with a combination of CC and metformin or CC and intrauterine inseminations (IUI). Endometriosis proved by laparoscopy was also an exclusion criterion.

We studied charts from all women to confirm the number of ovulatory treatment cycles and to obtain data about the outcome of treatment. Follow-up was a total of 12 treatment cycles.

Primary outcome was the cumulative incidence rate of an ongoing pregnancy after 12 cycles. An ongoing pregnancy was defined as a fetal heartbeat seen on ultrasound by 12 weeks of gestation. Secondary outcomes were number of treatment cycles, miscarriages and multiple pregnancies.

Data analysis

A cumulative hazard function was used to estimate the cumulative hazard or incidence rate of an ongoing pregnancy over time where time was expressed as number of cycles. The cumulative incidence rate estimates the probability of having an ongoing pregnancy for women undergoing ovulation induction with of CC. Conceptions that ended in miscarriage before 12 weeks of gestation were ignored in this analysis, and in these cases follow-up continued until an ongoing pregnancy occurred. Women who did not become pregnant were censored at the time of last treatment. Second, the cumulative incidence rate of all pregnancies was calculated, i.e. including the conceptions ending in a miscarriage before 12 weeks of gestation.

Statistical analyses were performed with SPSS for Windows (version 20).

Results

We analyzed 114 women that had ovulated on CC for at least six cycles but had not conceived. The baseline characteristics of these women are detailed in Table I. From these 114 women, 35 had an ongoing pregnancy (31%) within cycles 7–12. The number of women and ongoing pregnancies per cycle is displayed in Table II. Of the 35 ongoing pregnancies, 32 were singleton pregnancies and 3 (9%) were twin pregnancies. Eight women conceived but had at least one miscarriage before 12 weeks of gestation. One of these eight women had an ongoing pregnancy with CC at a subsequent time. The average ongoing pregnancy rate per cycle was 8.3%. Twenty-nine women (25%) completed 12 treatment cycles and 3 of these 29 women conceived. Fifty-five women (48%) dropped out before reaching 12 cycles, mainly because of a treatment switch to ovulation induction with gonadotrophins despite regular ovulation with CC ($n = 34$). Seven of the dropped out women (13%) changed treatment to CC combined with IUI and four (7%) to IVF or ICSI. Finally, six women (11%) stopped treatment because of personal

Table I Baseline characteristics.

	114 women recruited
Age years (mean ± SD)	30.4 ± 4.8
BMI kg/m^2 (mean ± SD)	25.0 ± 5.4
Primary subfertile n (%)	76 (67)
Duration of subfertility years (mean ± SD)	1.4 ± 0.9
LH IU/l (mean ± SD)	8.8 ± 5.0
FSH IU/l (mean ± SD)	5.8 ± 1.7
Total motile sperm count × 10^6 (median, min–max)	63 (3–557)

Table II Ongoing pregnancies per cycle.

Cycle number	Women	Ongoing pregnancies per cycle
7	114	8 (7%)
8	94	3 (3%)
9	80	12 (15%)
10	62	3 (5%)
11	45	6 (13%)
12	29	3 (10%)

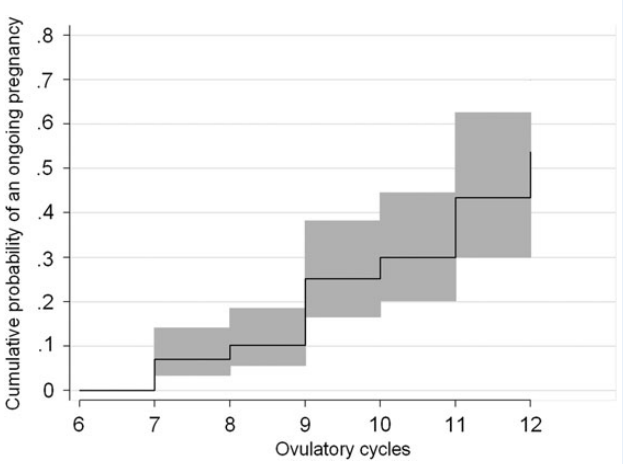

Figure 1 Cumulative probability of an ongoing pregnancy.

reasons, two (4%) because of anovulation, one experienced severe side effects and for one woman the reason for dropout was unknown. We chose to include all these women in our analyses to follow the intention to treat principle.

The treatment cycles were evaluated in a hazard curve (Fig. 1). The cumulative incidence rate of an ongoing pregnancy was 54% (95% CI 37–78%) after 7–12 treatment cycles with CC. Pregnancy rates continued to rise until 12 cycles. The cumulative incidence rate of any pregnancy (including the early miscarriages) was 69% (95% CI 49–96%).

Discussion

Guidelines and reviews (Wolf, 2000; The Thessaloniki ESHRE/ASRM PCOS Workshop Group, 2008; Brown *et al.*, 2009; Balen, 2013; NICE Fertility Clinical Guideline 156) agree on the effectiveness of CC in therapy naïve women with anovulation WHO type II and PCOS. All state that CC should be first in line after lifestyle changes in case of obesity. A recent multicenter randomized controlled trial that compared three cycles of ovulation induction with CC with three cycles of low-dose recombinant FSH in 255 therapy naïve women with PCOS found that cumulative live birth rates are higher with FSH than with CC (47 versus 37%, $P = 0.031$) (Homburg *et al.*, 2011). However, the authors state that this result should be balanced against convenience and costs which are in favor of CC. Another similar but smaller RCT (76 women randomized to either CC or FSH) showed no significant difference for both treatments after three cycles (López *et al.*, 2004). A prospective cohort study found a cumulative singleton live birth rate of 78% within 2 years after treatment with CC for a maximum of six to nine cycles followed by ovulation induction with gonadotrophins of 108 therapy naïve women with PCOS (Veltman-Verhulst *et al.*, 2012).

There are no randomized studies that have focused on women who do ovulate on CC but do not conceive within six cycles. Only two small cohort studies performed a follow-up of women with PCOS that were treated with CC for 10 and 12 cycles (Hammond *et al.*, 1983; Kousta *et al.*, 1997). In these limited studies, cumulative pregnancy rates were, respectively, 80 and 67% in women who ovulated on CC.

Our study shows that continued treatment up to 12 ovulatory cycles with CC in women with WHO type II anovulation results in a cumulative incidence rate of 54% ongoing pregnancies in women not pregnant after six cycles. Some women however dropped out before reaching 12 treatment cycles. Reasons for dropping out were mostly based on reasons not related to pregnancy chances. Main reasons were the wish of patients to change treatment or personal issues like divorces or moving elsewhere. Therefore, we assumed the women who dropped out had the same chances of conception with CC as the women remaining in the cohort. Cumulative incidence rate of an ongoing pregnancy represents the ongoing pregnancy chance of only those women who remained in the study. Though possibly overestimating real practice we find this estimate very informative. If a woman is prepared to undergo another six cycles of CC, the estimate is likely to represent her chances to reach an ongoing pregnancy. We acknowledge the number of women that dropped out and the retrospective design as limitations of our study. A further limitation of this study was its non-comparative nature. We do not know how CC in this group of women compares to ovulation induction with gonadotrophins.

Considering our results and the lack of large (randomized) trials with a focus on women who ovulate with CC but fail to conceive within a certain period of time, guidelines that state that there is no place for CC after six ovulatory cycles may reconsider this advice. Possible carcinogenic effects of extended use of CC are still debated but have never been proved (Gadducci *et al.*, 2013). A large cohort study of 3837 infertile women identified 11 women with a borderline or invasive malignant ovarian tumor, nine of which had used CC. Five of these women had taken CC for 12 cycles or more (Rossing *et al.*, 1994). The authors of a histopathological study reviewing 35 cases of oophorectomies and cystectomies in women treated with IVF suggested a possible relationship between ovarian hyperstimulation and developing ovarian dysplasia. Two of the 35 women were treated with CC for more than six cycles (Chene *et al.*, 2009). Whether this ovarian dysplasia is clinically relevant is unclear since it has been shown that these lesions have a different genetic profile from ovaries from women with a genetic risk for ovarian cancer. Therefore it might be that this dysplasia will not develop into cancer (Dauplat *et al.*, 2009).

The ESHRE/ASRM PCOS Consensus Workshop Group proposes to combine CC or FSH with IUI when PCOS is associated with male subfertility or when women fail to conceive despite successful induction of ovulation (The Thessaloniki ESHRE/ASRM PCOS Workshop Group, 2008). Evidence for the value of combined treatment of ovulation induction and IUI in women with anovulation is, however, not available. So far, there has been one RCT conducted in women with anovulation comparing the effectiveness of IUI versus timed intercourse during ovulation induction (Abu Hashim *et al.*, 2011). This trial randomized 188 women with PCOS

for either CC and IUI or CC and timed intercourse. Clinical pregnancy rates in both groups were comparable (23.6 versus 22.1%, $P = 0.33$). A retrospective cohort study of women with PCOS receiving ovulation induction (with CC, gonadotrophins or letrozole) with IUI ($n = 86$) or with timed intercourse ($n = 70$) also showed no significant difference in clinical pregnancy rates; 16.6 and 17.5%, respectively (Wiser *et al.*, 2012). Therefore, more research is needed on what treatment regimen is most successful in women with anovulation WHO type II.

There have been speculations that CC, due to its anti-estrogenic effect, might negatively influence the thickness of the endometrium (Gonen and Casper, 1990; Dickey *et al.*, 1993; Haritha and Rajagopalan, 2003; Casper 2011). In view of this possible effect of CC, various agents such as tamoxifen and aromatase inhibiters have been examined within trials but clear cut evidence to replace CC as first line therapy by these drugs has not been generated (Steiner *et al.*, 2005; Brown *et al.*, 2009; Badawy and Gibreal, 2011; Franik *et al.*, 2014).

Given the equipoise between continuing ovulation induction with CC after six failed cycles or starting gonadotrophins as second line treatment with or without IUI, a multicenter randomized controlled trial is now conducted, in which women are included after six ovulatory cycles with CC and randomized for continued treatment with CC, either with and without IUI or for six cycles with gonadotrophins with or without IUI (Nahuis *et al.*, 2013).

Conclusion

For women with WHO type II anovulation who are ovulatory with CC, pregnancy rates continue to rise until at least 12 treatment cycles with CC. This outcome, although unexpected, may be explained if we bear in mind that healthy ovulatory women also have high chances of conceiving within 12 cycles (te Velde, 2000). Whether treatment regimens like gonadotrophins and IUI give better outcomes should be investigated in a randomized setting. In the meantime, we suggest to only install the less convenient and more expensive treatment with gonadotrophins for women who do not conceive after 12 ovulatory cycles with CC.

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Authors' roles

N.S.W. collected parts of the data, performed the analyses and wrote the article. S.B., T.E.K., M.L.H., C.J.H., J.M.J.S. and C.A.M.K. helped providing the data. C.B.L., F.v.d.V., B.W.J.M. and P.G.A.H. contributed to the design of the cohort, helped with interpreting the outcomes of data and drafting the manuscript. M.v.W. assisted with the analyses, interpreting the outcomes of data and drafting the manuscript.

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Conflict of interest

None declared.

References

Abu Hashim H, Ombar O, AbdElaal I. Intrauterine insemination versus timed intercourse with clomiphene citrate in polycystic ovary syndrome: a randomized controlled trial. *Acta Obstet Gynecol Scand* 2011;**90**:344–350.

Badawy A, Gibreal A. Clomiphene citrate versus tamoxifen for ovulation induction in women with PCOS: a prospective randomized trial. *Eur J Obstet Gynecol Reprod Biol* 2011;**159**:151–154.

Balen AH. Ovulation induction in the management of anovulatory polycystic ovary syndrome. *Mol Cell Endocrinol* 2013;**373**:77–82.

Brown J, Farquhar C, Beck J, Boothroyd C, Hughes E. Clomiphene and anti-oestrogens for ovulation induction in PCOS. *Cochrane Database of Syst Rev* 2009;**4**:CD002249.

Casper RF. It's time to pay attention to the endometrium. *Fertil Steril* 2011;**96**:519–521.

Chene G, Penault-Llorca F, Le Bouëdec G, Mishellany F, Dauplat MM, Jaffeux P, Aublet-Cuvelier B, Pouly JL, Dechelotte P, Dauplat J. Ovarian epithelial dysplasia after ovulation induction: time and dose effects. *Hum Reprod* 2009;**24**:132–138.

Dauplat J, Chene G, Pomel C, Dauplat MM, Le Bouëdec G, Mishellany F, Lagarde N, Bignon YJ, Jaffeux P, Aublet-Cuvelier B *et al.* Comparison of dysplasia profiles in stimulated ovaries and in those with a genetic risk for ovarian cancer. *Eur J Cancer* 2009;**45**:2977–2983.

Dickey RP, Olar TT, Taylor SN, Curole DN, Matulich EM. Relationship of endometrial thickness and pattern to fecundity in ovulation induction cycles: effect of clomiphene citrate alone and with human menopausal gonadotropin. *Fertil Steril* 1993;**59**:756–760.

Eijkemans MJ, Imani B, Mulders AG, Habbema JD, Fauser BC. High singleton live birth rate following classical ovulation induction in normogonadotropic anovulatory infertility (WHO 2). *Hum Reprod* 2003;**18**:2357–2362.

Franik S, Kremer JA, Nelen WL, Farquhar C. Aromatase inhibitors for subfertile women with polycystic ovary syndrome. *Cochrane Database Syst Rev* 2014;**24**:CD010287.

Gadducci A, Guerrieri ME, Genazzani AR. Fertility drug use and risk of ovarian tumors: a debated clinical challenge. *Gynecol Endocrinol* 2013;**29**:30–35.

Gonen Y, Casper RF. Sonographic determination of a possible adverse effect of clomiphene citrate on endometrial growth. *Hum Reprod* 1990;**5**:670–674.

Hammond MG, Halme JK, Talbert LM. Factors affecting the pregnancy rate in clomiphene citrate induction of ovulation. *Obstet Gynaecol* 1983;**62**:196–202.

Haritha S, Rajagopalan G. Follicular growth, endometrial thickness, and serum estradiol levels in spontaneous and clomiphene citrate-induced cycles. *Int J Gynaecol Obstet* 2003;**81**:287–292.

Homburg R. Clomiphene citrate—end of an era? A mini review. *Hum Reprod* 2005;**20**:2043–2051.

Homburg R, Hendriks ML, König TE, Anderson RA, Balen AH, Brincat M, Child T, Davies M, D'Hooghe T, Martinez A *et al.* 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. *Hum Reprod* 2011;**27**:468–473.

Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA, Coulson C, Lambert PA, Watt EM, Desai KM. Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)* 1985;**14**:1693–1697.

Kousta E, White DM, Franks S. Modern use of clomiphene citrate in induction of ovulation. *Hum Reprod Update* 1997;**3**:359–365.

López E, Gunby J, Daya S, Parrilla JJ, Abad L, Balasch J. Ovulation induction in women with polycystic ovary syndrome: randomized trial of clomiphene citrate versus low-dose recombinant FSH as first line therapy. *Reprod Biomed Online* 2004;**9**:382–390.

Nahuis MJ, Weiss NS, van der Veen F, Mol BWJ, Hompes PG, JurOosterhuis J, Lambalk CB, Smeenk JMJ, Koks CAM, van Golde RJT *et al.* The M-OVIN study: does switching treatment to FSH and/or IUI lead to higher pregnancy rates in a subset of women with world health organization type II anovulation not conceiving after six ovulatory cycles with clomiphene citrate—a randomised controlled trial. *BMC Womens Health* 2013;**13**:42.

NICE. Fertility: Assessment and Treatment for People with Fertility Problems. Clinical Guideline 156, February 2013, www.nice.org.uk.

Rossing MA, Daling JR, Weiss NS, Moore DE, Self SG. Ovarian tumors in a cohort of infertile women. *N Engl J Med* 1994;**331**:771–776.

Steiner AZ, Terplan M, Paulson RJ. Comparison of tamoxifen and clomiphene citrate for ovulation induction: a meta-analysis. *Hum Reprod* 2005;**20**:1511–1515.

The ESHRE Capri Workshop Group. Health and fertility in World Health Organization type 2 anovulatory women. *Hum Reprod Update* 2012; **18**:586–599.

The Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to PCOS. *Hum Reprod* 2004;**19**:41–47.

The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. *Hum Reprod* 2008;**23**:462–477.

te Velde ER, Eijkemans R, Habbema HD. Variation in couple fecundity and time to pregnancy, an essential concept in human reproduction. *Lancet* 2000;**355**:1928–1929.

Veltman-Verhulst SM, Fauser BC, Eijkemans MJ. High singleton live birth rate confirmed after ovulation induction in women with anovulatory polycystic ovary syndrome: validation of a prediction model for clinical practice. *Fertil Steril* 2012;**98**:761–768.

Wiser A, Shalom-Paz E, Reinblatt SL, Holzer H, Tulandi T. Controlled ovarian hyperstimulation in women with polycystic ovarian syndrome with or without intrauterine insemination. *Gynecol Endocrinol* 2012;**28**:502–504.

Wolf LJ. Ovulation induction. *Clin Obstet Gynecol* 2000;**43**:902–915.