## Clomiphene Citrate in PCOS

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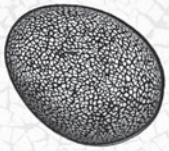
# Does the postcoital test predict pregnancy in WHO II anovulatory women? A prospective cohort study



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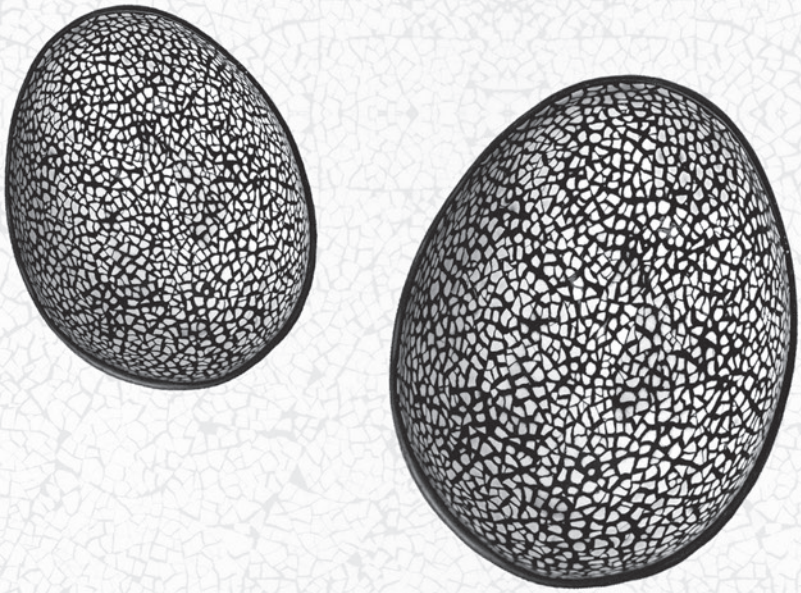

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## ABSTRACT

**Objective:** To assess the capacity of the postcoital test (PCT) to predict pregnancy in WHO II anovulatory women who are ovulatory on clomiphene citrate (CC). In these women, an abnormal PCT result could be associated with lower pregnancy chances, but this has never been proven or refuted.

**Study design**: Prospective cohort study was performed between December 2009 and September 2012 for all women who started ovulation induction with CC in one university clinic and two teaching hospitals in the Netherlands. A PCT was performed in one of the first three ovulatory cycles. Ovulation induction with CC was continued for at least six cycles. The PCT was judged to be positive if at least one progressive motile spermatozo was seen in one of five high power fields at 400× magnification. The primary outcome was time to ongoing pregnancy, within six ovulatory cycles.

**Results:** In 152 women the PCT was performed. 135 women had a reliable, well-timed PCT. The ongoing pregnancy rate was 44/107 (41%) for a positive and 10/28 (36%) for a negative PCT. The hazard rate for ongoing pregnancy was 1.3 (95% CI 0.64–2.5) for a positive versus a negative PCT.
Thirty five of 77 (46%) women with clear mucus had an ongoing pregnancy versus 12 of 45 (27%) women in whom the mucus was not clear (HR 2.0; 95% CI 1.02–3.84, $p = 0.04$).

**Conclusion:** The present study suggests that the outcome of the postcoital test in women with WHO-II anovulation that undergo ovulation induction with CC does not have a large effect on ongoing pregnancy chances over time.

## INTRODUCTION

Anovulation and oligo-ovulation are important causes of subfertility and are estimated to contribute to 20% of all cases of female subfertility.[1,2] The recommended first-line treatment for ovulation induction is the anti-oestrogen clomiphene citrate (CC) according to the ESHRE/ASRM-Sponsored PCOS Consensus Work- shop Group. [3,4] CC will restore ovulation in almost 80% and will result in pregnancy in 50% of all women.

Though CC results in high pregnancy rates, several studies have shown that CC has a negative effect on the cervical mucus.[5-7] The Dutch guidelines and the National Institute for Health and Clinical Excellence guidelines are not specific on the necessity to perform a PCT to exclude or demonstrate a cervical factor in women ovulating after induction as evidence on the relation between cervical factor and pregnancy chances does not exist.

The studies on the negative effect of CC on the cervical mucus did not evaluate whether abnormal cervical mucus was associated with lower pregnancy chances and the only prospective follow-up study on the relation between the result of the PCT and pregnancy rates describes the PCT outcome in some women, while the pregnancy rates are described in other women.[5-8] Therefore the association between outcome of the PCT and pregnancy chances cannot be determined from this study.

In view of this lack of knowledge we initiated a prospective cohort study to evaluate the relationship between the result of the postcoital test and time to ongoing pregnancy after ovulation induction with CC in women with WHO II anovulation.



## MATERIAL AND METHODS

Between December 2009 and September 2012, we performed a multicenter prospective cohort study in one university clinic and two teaching hospitals in the Netherlands. All women with WHO II anovulation attending these clinics were asked to participate in this study. The study protocol was approved by the Institutional Review Board of the Medical Spectrum Twente of Enschede (registration number: P08-37), and had local approval from the board of the other participating hospitals.

### Participants

We studied women with WHO class II anovulation who started ovulation induction with CC. Women needed to have oligo- or anovulation, combined with signs of hyperandrogenism or polycystic ovaries on ultrasound. Women younger than 18 years and women with other causes of anovulation, like thyroid disease or hyperprolactinaemia were not eligible for the study. The total motile sperm count had to be above 1 million in at least one semen analysis before starting ovulation induction. The cut-off point of 1 million was chosen to exclude severe male factor. Tubal patency tests before start of treatment were not mandatory, as the

incidence of bilateral tubal obstruction is low within this group of women and tubal patency testing is not without risks,[9] but women with already known bilateral tubal obstruction were excluded. Women could enter the study only once.

**Study design and treatment regimen**

Ovulation induction with CC was started after a spontaneous or progesterone induced menstrual bleed. From the third or fifth day until the seventh or ninth day after menstruation women took CC with a minimum of 50 mg to a maximum of 150 mg a day. If ovulation did not occur with the lowest dose of 50 mg/day, it was increased with steps of 50 mg to a maximum of 150 mg a day in the next cycles. Ovulation was established according to local protocol. Centres used a biphasic temperature curve, a follicle with a diameter ≤18 mm on transvaginal ultrasonography, progesterone ≤16 nmol/l in the second half of the cycle or a cycle length ≤35 days to define ovulation. The menstrual cycle was considered regular if the duration of the cycle was between 23 and 35 days. A PCT was performed during the basic fertility work-up in one of the first three ovulatory cycles. The test was planned based on cycle history or the basal body temperature (BBT) curve in the preceding cycle or ultrasound findings in the present or preceding cycle. In couples in whom the timing depended on the BBT and cycle length, the PCT was scheduled the day before the expected ovulation. In couples where the timing depended on ultrasound findings, the PCT was performed once the dominant follicle was ≤18 mm. The couple was asked to have intercourse four to sixteen hours before the appointment. The PCT was carried out by cleaning the cervix, followed by aspiration of endocervical mucus using a 1 ml disposable syringe or forceps. Clarity (clear or not clear) and spinnbarkeit were assessed and recorded. Mean number of progressive motile spermatozoa in a high power field at 400× magnification were determined. The PCT was judged to be positive if at least one progressive motile spermatozo was seen in one of five high power fields at 400× magnification. All other PCT results were considered to be negative. In case of a normal, positive test, only one PCT had to be performed. If progressive motile spermatozoa were absent, the test was scheduled again two days later or following the confirmation of a dominant follicle on ultrasound. In case the timing of the PCT was not optimal the test was planned again next month, based on ultrasound measuring of the follicle or LH tests. In case the test was negative again, and timing was appropriate the PCT test was considered to be negative. Follow-up started immediately after starting ovulation induction and ended at six ovulatory cycles. CC was continued for at least six cycles for both a positive and negative result of the PCT within a time horizon of 12 months.

**Outcome measures**

The primary endpoint of this study was time to an ongoing, viable intrauterine pregnancy, confirmed by ultrasonography, defined as a fetal heart beat seen by vaginal ultrasonography

at 12 weeks' gestation. The first day of the last menstrual period was considered to mark the end of time until natural conception. Time to pregnancy was censored at the day of start of any other treatment within six months after the start of ovulation induction with CC or at the day of the last contact during follow up, if the couple had no ongoing pregnancy.
Secondary outcomes were ovulation, clinical pregnancy, defined as any registered heart beat at sonography, ectopic pregnancy and miscarriage, defined as loss of an intra uterine pregnancy (confirmed by ultrasound or histological examination) before the 12th week of pregnancy and multiple gestation, defined as a registered heartbeat of at least two fetuses at 12 weeks of gestation.



**Power calculation and statistical analysis**

We planned a comparison between women with a positive and women with a negative result of the PCT. We anticipated that 50% of women would have an ongoing pregnancy within six ovulatory cycles, and that the ratio of a positive versus a negative PCT was 1.5:1. This ratio was based on data reported in the literature and a retrospective search in the clinics where women would be included. To prove that a negative PCT indicates a decrease of 20% chance for an ongoing pregnancy within six months compared to a standard of 50%, with a power of 80% and an alpha of 5%, 234 women needed to be included. To account for drop out, which we estimated not to be substantial, we aimed to include 250 women.
We compared time to ongoing pregnancy by constructing Kaplan Meier curves for women with a positive and negative result of the PCT. We performed Cox proportional hazard analyses to assess the association between the outcome of the PCT with time to ongoing pregnancy as a dependent variable adjusted for female age, total motile sperm count and duration of subfertility. Associations were expressed as hazard rate ratios (HR). We performed two sensitivity analyses. In the first we assumed that all the unrepeated negative PCTs would have been negative, in the second we assumed that all the unrepeated negative PCTs would have been positive.
We performed a separate analysis based on number of progressive motile spermatozoa per high power field and mucus quality. We classified the findings at PCT into four groups and compared pregnancy rates for women without progressive motile spermatozoa, for those with 1 progressive motile spermatozo, for those with 1–5 progressive motile spermatozoa and for women with more than five progressive motile spermatozoa per high power field at 400× magnification. Log rank test was used to test whether time to ongoing pregnancy differed significantly between groups. We classified the cervical mucus as clear or not clear. The effect of CC dose was not be taken into account since no evidence exists that the dose has any influence on pregnancy rates as long as women are ovulatory with CC.

# RESULTS

## PCT

The study profile is shown in Fig. 1. A total of 251 women with WHO class II anovulation starting ovulation induction with CC in three hospitals were included in this follow up study. There were 152 women (61%) with at least one PCT during one of the first three ovulatory cycles. The PCT was not performed in the remaining 99 women for various reasons, of which the main reason was pregnancy before the PCT could be planned.
Of the 152 remaining women, the PCT could be adequately performed in 135 women (89%) starting ovulation induction with CC, of whom 107 had a positive and 45 women had a negative PCT result. Baseline characteristics are shown in Table I. For all women included, 63% of the women had a BMI below 25 (159/251), 18% had a BMI between 25 and 30 (45/251), 11% had a BMI above 30 (28/251) and for another 8% the BMI was unknown. No differences between women with the three different test results were observed.

**Table I.** Baseline characeteristics of women with a PCT (n = 152)

| | **Results of the PCT** | | | |
|---|---|---|---|---|
| | **Positive n = 107** | **Negative n = 28** | **Negative, not well timed n = 17** | **Significance (p < 0.05)** |
| **Clinical parameters** | | | | |
| Female age in years (mean) | 29.15 | 29.59 | 29.30 | 0.62 |
| Duration of subfertility in months (mean) | 16.1 | 18.2 | 14.7 | 0.46 |
| Primary subfertility n (%) | 77 (72) | 22 (79) | 13 (77) | 0.51 |
| Smoking n (%) | 21 (20) | 3 (11) | 3 (18) | 0.86 |
| Amenorrhea n (%) | 29 (27) | 7 (25) | 1 (6) | 0.17 |
| BMI (mean) | 23.7 | 22.7 | 22.22 | 0.34 |
| **Male partner** | | | | |
| Total motile sperm count (median) | 67 | 64 | 79 | |

A total of 200 PCTs were performed in the 152 women. In 131 women one ovulatory cycle was needed to perform an adequate PCT, while in 21 women PCTs in two or more ovulatory cycles were necessary. In 107 women who finally had a positive PCT more than one PCT was performed in 20% of the women, whereas in 28 women with a negative PCT more than one PCT was performed in 50% of women.

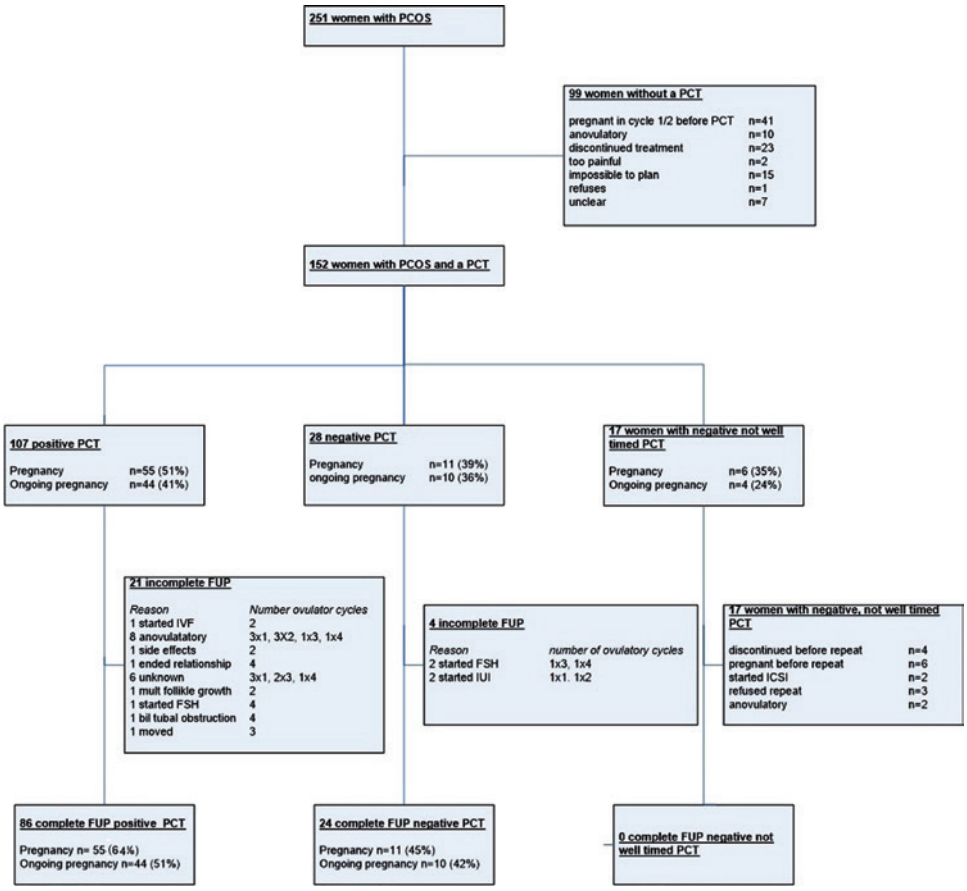


**Figure 1.** Study profile

## Pregnancy

Overall, 72 of the 152 women (47%) had a clinical pregnancy (Fig. 1). Clinical pregnancy rate per cycle is shown in Table II.

Unsuccessful pregnancies occurred in 13 women (18%) of whom 12 miscarried and 1 had an ectopic pregnancy.

From the 152 women, 59 (39%) had an ongoing pregnancy within 6 ovulatory cycles after start with CC. From the 135 women with a well-timed, adequate performed PCT, 54 (40%) had an ongoing pregnancy within 6 ovulatory cycles after start with CC. Of the 107 women with a positive PCT, 44 women (41%) had an ongoing pregnancy. Of the 28 women with a negative PCT, 10 women (36%) had an ongoing pregnancy. Of the 17 women in whom the PCT was not well-timed and negative, but not repeated, 4 women (24%) had an ongoing pregnancy. All ongoing pregnancies were singleton pregnancies (Fig. 1). There

were 24 women who discontinued treatment before the sixth ovulatory cycle, without a pregnancy. Kaplan–Meier analysis of the cumulative probability of ongoing pregnancy up to six ovulatory cycles are shown in Fig. 2.

**Table II.** Pregnancy rate per ovulatory cycle

| Ovulatory cycle | Women started | Pregnancy n (total) | | | Pregnancy rate per cycle (%) |
|---|---|---|---|---|---|
| | | Positive | Negative | Negative, not well timed | |
| 1 | 152 | 10 | 0 | 1 | 7 |
| 2 | 133 | 11 (21) | 2 (2) | 3 (4) | 12 |
| 3 | 110 | 10 (31) | 1 (3) | 0 (4) | 10 |
| 4 | 92 | 17 (48) | 4 (7) | 1 (5) | 24 |
| 5 | 59 | 3 (51) | 2 (9) | 0 (5) | 8 |
| 6 | 52 | 3 (54) | 2 (11) | 1 (6) | 13 |
| Total pregnancies | | 54/107 (50%) | 11/28 (39%) | 6/17 (35%) | 72/152 (47%) |

**Pregnancy over time and associations**

There was no evidence for a difference in ongoing pregnancy chance over time between women with a positive and women with a negative PCT result (HR 1.3; 95% CI 0.64–2.5). Under the assumption that all the unrepeated negative PCT would have been true negative PCT outcomes, the negative PCT group would entail 45 women of whom 14 women had an ongoing pregnancy. The ongoing pregnancy chance over time remained almost the same (HR 1.3; 95% CI 0.74–2.5). Under the assumption that the unrepeated negative PCT tests would actually have been positive PCT outcomes, the positive PCT group would entail 124 women of whom 48 women had an ongoing pregnancies. The ongoing pregnancy chance over time would also remain comparable (HR 1.2; 95% CI 0.61–2.4). For all these analyses there was no association of female age, total motile sperm count and duration of subfertility with ongoing pregnancy rates. Furthermore there was no interaction with between these variables and PCT.

**Ranking motile spermatozoa and clarity of mucus**

When ranking women, with a well-timed PCT, based on number of progressively motile spermatozoa per high power field, no differences in pregnancy rate were reported for women with none, one, one to five or more than five progressively motile spermatozoa per high power field (Table III). No statistically significant differences were found between those four groups in time to pregnancy (log rank test $p = 0.31$). When ranking women, with a well-timed PCT, based on clarity of the mucus, women with clear mucus had a significantly higher chance of an ongoing pregnancy. Thirty five of 77 (46%) women with clear mucus had an ongoing pregnancy versus 12 of 45 (27%) women in whom the mucus was not

clear (HR 2.0; 95% CI 1.02–3.84, p = 0.04). For all these analyses there was no association of female age, total motile sperm count and duration of subfertility with ongoing pregnancy rates. Furthermore none of these factors had any interaction with mucus clarity. There was no absence of mucus for any PCT (Table III).

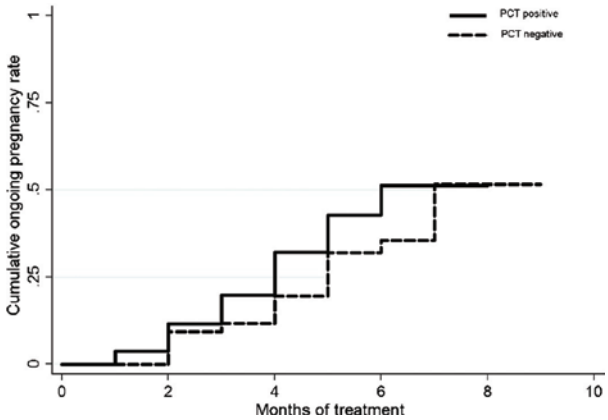


**Figure 2.** Cumulative proportion of women with a positive and negative result of the PCT (time in months from first ovulatory cycle).

**Table III.** Ongoing pregnancy rate per woman included in subgroup analysis based on 1) Number of progressive motile spermatozoa per high power field and 2) Mucus quality.

| Progressive motile spermatozoa/ high power field | | Positive | Negative | Negative, not well timed |
|---|---|---|---|---|
| 0 | | | 10/28 (36) | 4/17 (24) |
| 1 | | 4/11 (36) | | |
| 2-5 | | 13/34 (38) | | |
| >5 | | 22/51 (47) | | |
| Unknown | | 7/11 (63) | | |
| **Mucus** | **Positive** | **Negative** | **Negative, not well timed** | **Total** |
| Clear | 29/67 (43) | 6/10 (60) | 1/7 (14) | 36/84 (42) |
| Not clear | 8/27 (30) | 4/18 (22) | 2/7 (29) | 14/52 (27) |
| Unknown | 8/13 (62) | 0/0 | 1/3 (33) | 9/16 (56) |

## DISCUSSION

In this prospective cohort study we included 251 women with WHO II anovulation who started ovulation induction with CC. A PCT could be performed in 152 women who became ovulatory with CC. Women with an abnormal PCT had an ongoing pregnancy rate of 36%

compared to an ongoing pregnancy rate of 41% in women with a normal PCT. There was no evidence of a difference in time to ongoing pregnancy as expressed by a hazard rate of 1.3 (95% CI 0.64–2.5). In women with clear mucus the ongoing pregnancy rate was significantly higher (HR 2.0; 95% CI 1.02–3.84, p = 0.04).

The strength of our study is that we were able to assess the value of the PCT and cervical mucus in a prospective multicentre cohort. All women attending the fertility clinics with subfertility and WHO II anovulation were included. When ovulatory with CC a PCT was performed, or the reason for not performing was registered, which was usually due to pregnancy. The PCT was performed by various clinicians in different hospitals, but this is an adequate reflection of the true performance of the PCT in daily practice.

A limitation of our study is the sample size. The subset of women with a negative PCT was smaller than expected and from the 251 included women in only 152 women a PCT could be performed as a substantial number of women either got pregnant very fast, remained anovulatory and or just refused to do the test. Nevertheless, the differences in pregnancy rates between women with a positive and a negative PCT were small, and, more importantly, the absolute pregnancy rate was with 36%–41% rather high.

Women with clear mucus had a higher chance of pregnancy than women in whom the mucus was judged as not clear. Thirty five of 77 (46%) women with clear mucus had an ongoing pregnancy versus 12 of 45 (27%) women in whom the mucus was not clear. This difference could not be explained by a difference in female age, total motile sperm count and/or duration of subfertility. We could only speculate that spermatozoa migrate more easily through clear mucus than unclear mucus since evidence for this theory is lacking.

Compared to all other groups, the subgroup of women with a negative PCT and cervical mucus of poor quality had the lowest chance on an ongoing pregnancy (22%). This could in theory be the group that benefits from doing a PCT and a mucus determination. However, this group accounted for only 7% of the 250 women included in this study and therefore probably not worth of doing, especially since the PCT may result in emotional stress.[10] We noticed that several women refused the PCT, some thought it to be painful or the PCT was impossible to plan.

Performing PCTs may also have unnecessary side effects like switching treatment. In this study 4 out of 28 women (14%) with a negative test immediately switched treatment after the negative result of the PCT; two women started IUI and two women started ovulation induction with gonadotrophins. Also 6 out of 17 women (35%) with a negative and not well timed PCT stopped treatment or switched treatment before the PCT could be repeated as indicated. In daily practice this number may be higher. Finally, 17 out of 45 women (38%) with a negative PCT became pregnant.

In summary, the findings of this prospective cohort study demonstrated that the postcoital test has only limited value in women with WHO II anovulation, ovulatory with CC. Women

with clear mucus may have a higher chance of pregnancy than women with unclear mucus but an explanation for this is not available. We advocate that women who start ovulation induction with CC can safely do so without performing a PCT.

## DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## FUNDING

None

## REFERENCES

1. Hull MG, Glazener CM, Kelly NJ, et al. Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)* 1985; 291(6510): 1693-7.
2. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod* 2010; 25(2): 544-51.
3. Thessaloniki EA-SPCWG. Consensus on infertility treatment related to polycystic ovary syndrome. *Hum Reprod* 2008; 23(3): 462-77.
4. Thessaloniki EA-SPCWG. Consensus on infertility treatment related to polycystic ovary syndrome. *Fertil Steril* 2008; 89(3): 505-22.
5. Gelety TJ, Buyalos RP. The effect of clomiphene citrate and menopausal gonadotropins on cervical mucus in ovulatory cycles. *Fertil Steril* 1993; 60(3): 471-6.
6. Roumen FJ. [Decreased quality of cervix mucus under the influence of clomiphene: a meta-analysis]. *Ned Tijdschr Geneeskd* 1997; 141(49): 2401-5.
7. Thompson LA, Barratt CL, Thornton SJ, Bolton AE, Cooke ID. The effects of clomiphene citrate and cyclofenil on cervical mucus volume and receptivity over the periovulatory period. *Fertil Steril* 1993; 59(1): 125-9.
8. Hammond MG, Halme JK, Talbert LM. Factors affecting the pregnancy rate in clomiphene citrate induction of ovulation. *Obstet Gynecol* 1983; 62(2): 196-202.
9. Nahuis MJ, Oosterhuis GJ, Hompes PG, van Wely M, Mol BW, van der Veen F. The basic fertility workup in women with polycystic ovary syndrome: a systematic review. *Fertil Steril* 2013; 100(1): 219-25.
10. Eimers JM, Omtzigt AM, Vogelzang ET, van Ommen R, Habbema JD, te Velde ER. Physical complaints and emotional stress related to routine diagnostic procedures of the fertility investigation. *J Psychosom Obstet Gynaecol* 1997; 18(1): 31-5.