Gynecology & Obstetrics
ISSN: 2161-0932

Cens et al., Gynecol Obstet (Sunnyvale) 2015, 5:7
DOI; 10.4172/2161-0932.1000305

Research Article Open Access

The Diagnostic and Therapeutic Values of the Huhner Test: A Retrospective Study of 718 Subfertile Couples

Cens Steven[1,2]*, Biopyrénées's Team of Biologists and Faure Celine[3]

[1]Centre d'Assistance Médicale à la Procréation de PAU, Polyclinique de Navarre, Pau, France
[2]Directeur du laboratoire de spermiologie, laboratoire Biopyrénées, Pau, France
[3]Service de Biologie de la Reproduction, CECOS, Hôpital Jean Verdier, Assistance Publique-Hôpitaux de Paris, Bondy, France

Abstract

Background: This retrospective study aims to investigate the value of Huhner Test (HT) or Post-coïtal Test (PCT) in the routine evaluation of infertile couples and to compare 4 different cut off levels to consider the positivity of the test.

Methods: Pregnancy outcomes of 718 couples, who underwent a Huhner Test from January 2004 to December 2012, were assessed. The follow up was stopped in 04/30/2013. The results were calculated according to different criteria of positivity for HT.

Results: After exclusion of women's FSH > 15 IU/ml and altered sperm, total pregnancy rate was higher in the positive HT group than in the negative one (70.5% vs 57.8% p<0.05). A negative HT was significantly associated with half chance of natural conception (38.8% vs 20.3% P<0.05). For simple stimulation, pregnancy rate was three times higher in the positive HT group than in the negative HT group (17.8% vs 6.8% p<0.05). For IUI, there were twice as many pregnancies in the negative HT group compared with the positive HT group (30.5% vs 13.2% p<0.01) and three times more in ICSI (27.1%vs 9.3% p<0.01). For IVF, there was no significant difference.

The best cut off level, for a positive HT, appeared to be 1 motile spermatozoon seen in all the cervix according to WHO 2010.

Conclusion: This study showed that the HT was still useful in predictive and therapeutic diagnosis: If a woman is young and the Huhner Test is positive, we should simply give a chance to a natural conception and therefore give more time to the couple before moving on to simple stimulation. With a negative HT, no clear conclusions can be drawn, but it seems logical to proceed directly to IUI and shift more rapidly towards ICSI.

Keywords: Assisted reproductive technology, infertility, Huhner test, post coital test, pregnancy, intrauterine insemination, IVF, ICSI

Introduction

The subfertility diagnosis tries to understand the type of subfertility in order to be more efficient and less expensive. The HT is simple, non-invasive, cheap and commonly used in standard fertility investigations but its clinical utility is subject to debate.

The purposes for the HT are numerous: confirm a complete intercourse, identify women with abnormalities in quality or quantity of cervical mucus, determine whether the sperm-mucus interaction is favourable or not to the vitality of the spermatozoa [1], and is the only test to diagnose a cervical factor in idiopathic subfertility [2].

Several studies demonstrated a strong association between the HT and pregnancy rates [3-9]. In a prospective study, Glazener et al., conclude that HT is an effective predictor of conception if duration of infertility is less than 3 years [10]. The use of HT improved the discrimination of two models for the prediction of the chance of treatment-independent pregnancy leading to live birth among subfertile couples [11,12]. Similarly, Van der Steeg et al., got an improved predictive model by adding the HT [13]. The HT has a diagnostic importance to discriminate cervical and unexplained infertility [14]. More recently, Leushuis et al., showed that subfertile couples with an abnormal HT have a lower probability of a spontaneous pregnancy [15]. And Hessel and al., observed, after a follow-up of three years, that a positive HT was still associated with a higher spontaneous and a higher overall ongoing pregnancy rate [16].

On the other hand, Oei et al., concluded, with a substantial number of participating women, that the use of HT would only lead to more interventions without an increase in pregnancy rates [17]. But the study was criticized because it did not assess the HT only [18,19]. Similar findings were published by the team 3 years later [20]. Similarly to Oei, Helmerhost et al. explained that the HT has poor diagnostic and prognostic properties and stated that has no benefit on pregnancy rates [21].

The HT identifies a cervical factor in subfertiles couples which has important implications for treatment. Three studies clearly indicated a beneficial effect of IUI in couples with an isolated cervical factor [22-24] whereas four others did not report such an effect [25-28]. But Helmerhost et al., in a literature review, revealed numerous biases in these studies [29].

Another limit in HT is the criterion for the positivity of the test. For WHO 1992, more than ten progressive motile spermatozoa per high power microscope field were necessary for positivity [30]. Seven years later, only one progressive motile spermatozoa was needed in six fields [31].

***Corresponding author:** Dr Steven Cens, Service de Spermiologie, Laboratoire Biopyrenees, 3 rue bayard 64000 PAU, France, Tel: +33559275131; E-mail: centrefivpau@gmail.com

Received June 15, 2015; **Accepted** July 20, 2015; **Published** July 27, 2015

Citation: Cens S, Biopyrénées's Team of Biologists, Celine F (2015) The Diagnostic and Therapeutic Values of the Huhner Test: A Retrospective Study of 718 Subfertile Couples. Gynecol Obstet (Sunnyvale) 5: 305. doi:10.4172/2161-0932.1000305

In the meta-analysis of Griffith, the 6 studies used different criteria from 0 (<1 per field) to 5 progressive motile spermatozoa per field. In the retrospective study, Oei et al. concluded that the best definition for a negative HT is <1 progressive motile spermatozoa per field (and specificity and sensitivity compared with different cut off: <1, <5, <10 and <20 progressive motile spermatozoa per field). For Van der Steeg et al., a HT was considered negative if no progressive motile spermatozoa was observed throughout the mucus. In France, most teams consider the HT as positive if more than 5 progressive motile spermatozoa were observed by field.

But even if the positivity criteria varied according to the teams, Glatstein et al. in a study with blinded observers has shown that the test is reproducible on the number and mobility of sperm [32].

The present retrospective study aims at comparing couples with positive HT and couples with negative HT regarding the occurrence of pregnancy, mode of pregnancy obtained and the period of infertility from the realization of HT. Furthermore, the results are expressed according to different criteria of positivity.

Materials and Methods

Patients

Outcomes of all couples who underwent a Huhner test as part of their routine fertility work-up in 2 sites of Pau's laboratories from 1st January 2004 to 31st December 2012, were evaluated. All of the couples had a duration of infertility>12 months. To avoid bias, couples were excluded if the woman had a FSH>15 IU/l. Similarly, men with an altered sperm (<20 M/ml, motile spermatozoa<30%) were excluded from the study. We have not taken into account tubal disease because hysterography has not been performed for every women. Anovulation wasn't either a criterion of exclusion in our study.

About 854 HT were realized in the laboratory. Time of conception was based on the date of the first HT.

Huhner test (HT) procedure

Patients were given standardized instructions. The Huhner test was planned 8-12 hours after intercourse. The HT was not performed if a couple failed to have intercourse as recommended, and was reprogrammed.

The HT and slide preparation for each subject were performed by 6 biologists in an identical standardized fashion according to the HAS guidelines.

The patient was placed in the lithotomy and a sterile non-lubrificated speculum was inserted into the vagina and the cervix was exposed. After assessment of the opening of the cervix, endocervical mucus was collected by suction in a standard narrow 1ml disposable syringe. 2 collections were performed, the first from the endocervix and the second into the pouch of Douglas to ensure an effective intercourse.

Evaluation of the cervical factor

The cervical mucus quantity and quality were determined using the Insler score [33], which is based on evaluation of spinnbarkeit, ferning, cervical mucus volume, and appearance of the external cervical os (condition of the cervix) and ranges from 0–12 (cervical index). Samples with an Insler score of ≥9 were considered ovulatory.

The pH of the cervical mucus was measured by using paper strips (pH Indikatorpapier; Merck, Darmstadt, Germany)

Cellularity

After collection of the test specimens, the biologist examined vaginal pool specimens microscopically for the presence of spermatozoa. A sample of cervical mucus was covered with a glass slip (22x22mm) and examined at low (x100) and high (x400) power magnification. The number of sperms moving forward per high field was recorded.

4 different cut-off levels were considered varying from one to 10 motile spermatozoa per high power field for positivity.

Cervical mucus inflammation was evaluated with number of leucocytes.

Pregnancy diagnosis

Diagnosis of clinical pregnancy was defined with the presence of embryonic sac visible at sonography. The research regarding women's potential pregnancies were stopped on 04.30.13.

Semen analysis

Men produced semen samples by masturbation into a specimen cup at the laboratory. Lubricants were not used for masturbation. The men were asked to abstain from ejaculation for 3–5 days before the clinic visit and to report the time of their previous ejaculation.

Samples were processed within 30 minutes of collection. After semen liquefaction, semen analysis was performed according to World Health Organization guidelines [34] for assessing semen volume and sperm concentration. Total sperm count was calculated as concentration x volume. Motility was assessed, classifying the spermatozoa as progressive motile (a+b), total motile (a+b+c), or immotile (d).

Statistical methods

Descriptive statistics were used to assess the similarity of the groups. Cumulative pregnancy rates were calculated as for life table analysis and compared with the Wilcoxon test. Data were assessed by the student test. $P<0.05$ was considered significant.

Results

Out of the 854 HT performed in the laboratory, 20 were excluded because of FSH>15 IU/ml and 116 were not taken into account (one HT per couple, if more than one was done for the same couple, we only kept the positive one). Infertility was primary in 95.4% of the women and secondary in 4.6%.

The remaining 718 tests were divided into 2 groups: positive HT or negative HT.

Long term follow up was known for over 80% of women who underwent the test.

After exclusion of couples with bad semen analysis, 408 couples remained and follow up was known for 89.6% of them (figure 1).

In table 1, HT was considered positive if at least one motile spermatozoon was observed in cervical mucus. The characteristics of the participants did not differ significantly between the 2 groups.

The cumulative pregnancy rate was 13.5% higher ($P<0.01$) and occurred quickly (0.80 year vs 0.99 year) in the positive HT group compared with the negative HT group.

For spontaneous pregnancy: In the positive HT group, there were more spontaneous pregnancies (+19.8%) than in the negative HT

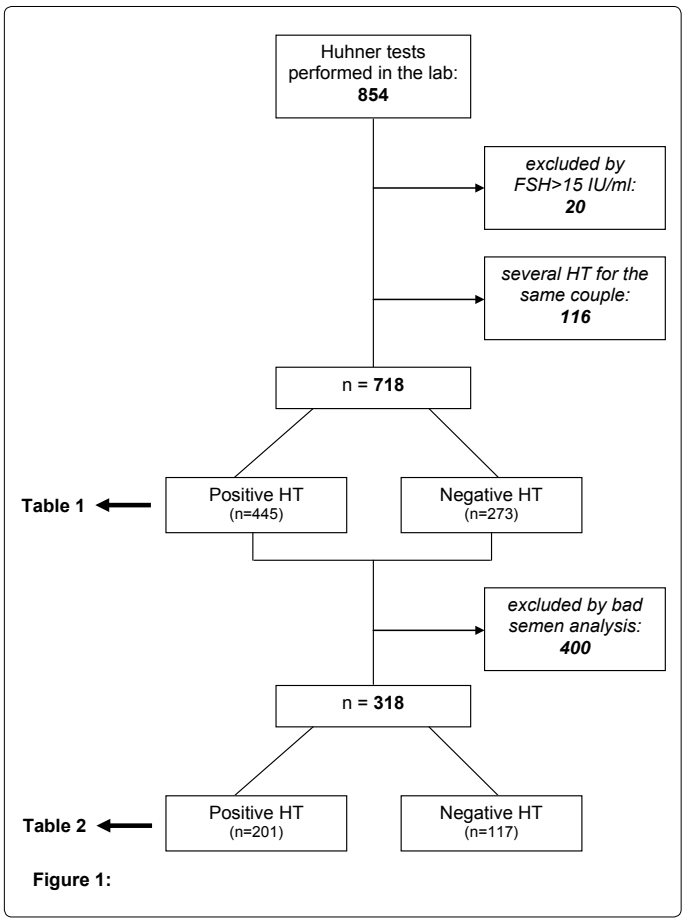

Figure 1:

group. The lapse of time to pregnancy was the same (6 months) in the two groups.

For simple stimulation: there were twice as many pregnancies in the positive HT group as in the negative HT group (22.1% vs 12.2%). But the time to pregnancy was 3 times longer in positive HT group than in negative HT group (0.9 year vs 0.3 year).

For intrauterine insemination (IUI): there were 50% less pregnancies (P<0.001) in the positive HT group than in the negative HT group (9.9% vs 18.7%). But time to pregnancy was longer in the negative HT group than in positive HT group (1.2 year vs 0.8 year).

For in vitro fertilization (IVF), no significantly difference was found between the positive and the negative HT groups (10.5% vs 10.1%).

For intracytoplasmic sperm injection (ICSI): there were three times fewer pregnancies (P<0.001) in the positive HT group than in the negative HT group (7.5% vs 28.8%). And time to pregnancy was shorter in the negative HT group than in the positive HT group (1.3 year vs 1.8 year).

In table 2, after exclusion of altered sperm, similar results were obtained: total pregnancy rate was higher in the positive HT group than in the negative HT group (70.5% vs 57.8%) (P<0.05).

A negative HT was significantly associated with half chance of natural conception compared with a positive HT (38.8% vs 20%) (P<0.05).

For simple stimulation, pregnancy rate was three times higher in the positive HT group than in the negative HT group (17.8% vs 6.8%) (P<0.05).

For IUI, there were twice as many pregnancies in the negative HT group compared with the positive HT group (30.5% vs 13.2%) (p<0.01) and three times more in ICSI (27.1%vs 9.3%) (p<0.01). For IVF, there was always no significant difference between groups (20.9% vs 15.2% for the positive and the negative HT groups respectively).

In table 3, we compared 4 different cut-off levels to consider a HT positive or negative, varying from one to 10 motile spermatozoa. We considered a positive result of a HT to be more than 1) 10 progressively motile spermatozoa per field or 2) 5 progressively motile spermatozoa per field or 3) one progressively motile spermatozoon per field or 4) one progressively motile spermatozoon in the whole cervical mucus sample.

With the third criterion (1 spermatozoon per field), we could see two significant differences between positive and negative HT with pregnancies by stimulation (19.6% vs 8.1%) and ICSI (8.8% vs 22.1%).

Differences were more obvious with the last criterion: at least one spermatozoon seen in the cervix appears to be the best cut off probably because of its simplicity and better reproducibility according to WHO 2010 [27]. We observed significant differences between positive and negative HT with spontaneous pregnancy (38.5% vs 20.3%), pregnancy by treatment (61.2% vs 79.7%), pregnancy by stimulation (17.8% vs 6.8%), pregnancy by insemination (13.2% vs 30.5%) and ICSI (9.3% vs 27.1%).

Discussion

HT was still recommended in WHO and continues to do so in the 2010 edition. In France, for ANAES (Agence Nationale d'Accréditation et d'Evaluation en Santé), HT is still indicated in the first place contrary to the NICE (National Institute for Clinical Excellence) guideline that

Cut-off point :1 or more motile spermatozoa in the whole cervix	HT +			HT -			
Number of tests	445			273			
Women average age	31,6			31,7			
Follow up known	381	85.6%		218	79.9%		
Lost	64	14.4%		55	20.1%		
Total Pregnancy	294/381	77.2%	0.8 year	139/218	63.7%	1 year	P<0.001
Spontaneous pregnancy	147/294	50%	0.5 year	42/139	30.2%	0.5 year	P<0.001
Pregnancy with treatment	147/294	50%	1 year	97/139	69.8%	1.1 year	P<0.001
Ovulation induction	65/294	22.1%	0.9 year	17/139	12.2%	0.3 year	P<0.05
Intrauterine insemination	29/294	9.9%	0.8 year	26/139	18.7%	1.2 year	P<0.01
IVF	31/294	10.5%	1.7 year	14/139	10.1%	1.7 year	NS
ICSI	22/294	7.5%	1.8 year	40/139	28.8%	1.3 year	P<0.001

Table 1: Occurrence of pregnancy by result of the Huhner Test (HT). Positive HT criterion: 1 or more motile spermatozoa in the whole cervix.

Cut-off point :1 or more motile spermatozoa in the whole cervix	HT +			HT -			
Number of tests	201			117			
Women average age	31,6			32,1			
Follow up known	183	91%		102	87.2%		NS
Lost	18	9%		15	12.8%		NS
Total Pregnancy	129/183	70.5%	1 year	59/102	57.8%	1.3 year	P<0.05
Spontaneous pregnancy	50/129	38.8%	0.6 year	12/59	20.3%	0.8 year	P<0.05
Pregnancy with treatment	79/129	61.2%	1.3 year	47/59	79.7%	1.4 year	P<0.05
Ovulation induction	23/129	17.8%	1 year	4/59	6.8%	0.3 year	P<0.05
Intrauterine insemination	17/129	13.2%	0.8 year	18/59	30.5%	1.2 year	P<0.01
IVF	27/129	20.9%	1.6 year	9/59	15.2%	1.5 year	NS
ICSI	12/129	9.3%	2.1 year	16/59	27.1%	1.9 year	P<0.01

Table 2: Occurrence of pregnancy by result of the Huhner Test (HT) after exclusion of altered sperm (< 20 M/ml, motile spermatozoa < 30%). Positive HT criterion : 1 or more motile spermatozoa in the whole cervix.

Cut-off point (motile spermatozoa per field)	>9		>4		1 or more		1 or more in the whole cervix	
HT Result	+	-	+	-	+	-	+	-
Pregnancy	70%	65.1%	71.3%	63.6%	70.8%	61%	70.5%*	57.8%*
Spontaneous pregnancy	37.1%	32%	37.1%	30.9%	35.3%	30.2%	38.8%*	20.3%*
Pregnancy with treatment	62.9%	68%	62.9%	69.1%	64.7%	69.8%	61.2%*	79.7%*
Pregnancy with stimulation	17.1%	13.7%	21%	11.1%	19.6%*	8.1%*	17.8%*	6.8%*
Pregnancy with insemination	8.6%	20.9%	9.7%	23%	13.7%	24.4%	13.2%**	30.5%**
Pregnancy with IVF	27.7%	17.6%	22.6%	17.5%	22.5%	15.1%	20.9%	15.2%
Pregnancy with ICSI	11.4%	15.7%	9.7%	17.5%	8.8%*	22.1%*	9.3%**	27.1%**

Table 3: Occurrence of pregnancy by result of the Huhner Test (HT) after exclusion of altered sperm (< 20 M/ml, motile spermatozoa < 30%) *: p<0.05 ; **: p<0.01.

decides not to recommend the HT because of "its low predictive value on pregnancy rates", based on 2 peer-reviewed publications [35,36].

We conducted this retrospective study to assess the usefulness of the controversial HT in investigating subfertility. We aimed to show the interest of the non-invasive and cheap HT regarding etiological, predictive and therapeutic diagnosis.

The diagnosis's interest is to identify a cervical factor in the subfertility and avoid classifying these couples in idiopathic subfertility. And it's essential to understand the type of subfertility in order to be more efficient

The therapeutic interest is to adapt the strategy according to the positivity or negativity of the test.

Although this is not a propective study, our team followed the same guideline for each group. Positive HT induced no ART or IUI in 75% of the study and appeared to be less cost-effective. We showed predictive interest of the HT for spontaneous pregnancy. As in 5 other studies [15,16,35,37,38], we have demonstrated that spontaneous pregnancy rate was higher with a positive HT than with a negative HT. One thinks that the result of the HT may have induced a quicker treatment in case of negative HT, but our medians delay for spontaneous pregnancy are the same (6 months). When the HT is positive and the woman young, we must let the chance of natural conception and therefore give more time to the couple before moving on to simple stimulation.

With a negative HT, no clear conclusion can be drawn, but it seems logical to proceed directly to IUI (if all other criteria are met: patent tubes, good ovulation...). Similar to our results, Van der Steeg et al., believe that IUI is an effective treatment for cervical factor subfertility [39]. And, if IUI failure, it might be worth considering ICSI rapidly.

Hypothesis that could explain a higher rate of pregnancies with ICSI in case of a negative HT is the presence of anti-sperm antibodies (ASA). Marshburn et al., has demonstrated, that a negative HT could be due to an immune subfertility [40]. Effectively, a poor sperm penetration of cervical mucus (HT negative) could be due to the presence of ASA either in the woman or in the man [41]. These ASA are directed against sperm surface antigens, interfering with sperm motility and their migration through the female reproductive tract. ASA also can inhibite capacitation or acrosome reaction [42-44]. These antibodies in the female can cause sperm agglutinate or become immobilised in the cervical mucus. ASA in the husband prevented the sperm from penetrating the cervical mucus, even though the sperm appear normal on seminal analysis. ASA have been considered as subfertility cause in 10-30 % of subfertile couples [45,46] and prevalence rates of ASA in subfertile men range between 6 and 11% [47].

In conclusion, for a negative HT and IUI failure, it would be judicious to detect ASA in both men and women, and shift rapidly towards ICSI.

Finally, because of the lack of a uniform definition of positive test, we have analyzed several cut offs. To standardized the HT methodology, we have shown that the best criterion to consider a HT positive is, at least, one motile spermatozoon seen in the cervix, according to WHO 2010 [48].

Although some authors may feel uncertain about the HT, we are convinced of the usefulness of this test in etiological diagnosis as well as its predictive and therapeutic interests.

Competing Financial Interests

All authors declare that they have no financial interests in this study.

Biopyrénées's Team of Biologists

Cens S, Chauveau H, Couture JF, Domercq P, Fargheon D and Uthurriague C.

Acknowledgements

The authors thank Pau region's Gynaecologists, the sperm lab technicians Guicharnaud N, Pereira C, Bordinat S.

References

1. Sims JM (1868) Illustrations of the value of the microscope in the treatment of the sterile condition. BMJ 2: 465-466.
2. Barnea ER, McInnes DR, Arronett GH (1982) Infertility due only to the cervical factor: management and long-term prognosis. Int J Fertil 27: 85-89.
3. Hull MG, Savage PE, Bromham DR (1982) Prognostic value of the postcoital test: prospective study based on time-specific conception rates. Br J Obstet Gynaecol 89: 299-305.
4. Griffith CS, Grimes DA (1990) The validity of the postcoital test. Am J Obstet Gynecol 162: 615-620.
5. Eimers JM, te Velde ER, Gerritse R, van Kooy RJ, Kremer J, et al. (1994) The validity of the postcoital test for estimating the probability of conceiving. Am J Obstet Gynecol 171: 65-70.
6. Hall LL, Montgomery Rice V (1995) Ovulation predictors as postcoital testing. Inf Reprod Med Clin North Am. 6: 179-197.
7. Snick HK, Snick TS, Evers JL, Collins JA (1997) The spontaneous pregnancy prognosis in untreated subfertile couples: the Walcheren primary care study. Hum Reprod 12: 1582-1588.
8. Cohlen BJ, te Velde ER, Habbema JD (1999) Postcoital testing. Postcoital test should be performed as routine infertility test. BMJ 318: 1008-1009.
9. Hull MGR, Evers JLH (1998) Postcoital testing: criterion for positive test was not given. Br Med J 317: 1007
10. Glazener CM, Ford WC, Hull MG (2000) The prognostic power of the post-coital test for natural conception depends on duration of infertility. Hum Reprod 15: 1953-1957.
11. Hunault CC, Habbema JD, Eijkemans MJ, Collins JA, Evers JL, et al. (2004) Two new prediction rules for spontaneous pregnancy leading to live birth among subfertile couples, based on the synthesis of three previous models. Hum Reprod 19: 2019-2026.
12. Hunault CC, Laven JS, van Rooij IA, Eijkemans MJ, te Velde ER, et al. (2005) Prospective validation of two models predicting pregnancy leading to live birth among untreated subfertile couples. Hum Reprod 20: 1636-1641.
13. van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, Hompes PG, et al. (2007) Pregnancy is predictable: a large-scale prospective external validation of the prediction of spontaneous pregnancy in subfertile couples. Hum Reprod 22: 536-542.
14. Brandes M, Hamilton CJCM, de Bruin JP, Nelen WLDM, Kremer JAM (2010) The relative contribution of IVF to the total ongoing pregnancy rate in a subfertile cohort. Hum Reprod 25: 118-126.
15. Leushuis E, van der Steeg JW, Steures P, Koks C, Oosterhuis J, et al. (2011) Prognostic value of the postcoital test for spontaneous pregnancy. Fertil Steril 95: 2050-2055.
16. Hessel M, Brandes M, de Bruin JP, Bots RS, Kremer JA, et al. (2014) Long-term ongoing pregnancy rate and mode of conception after a positive and negative post-coital test. Acta Obstet Gynecol Scand 93: 913-920.
17. Oei SG, Helmerhorst FM, Bloemenkamp KW, Hollants FA, Meerpoel DE, et al. (1998) Effectiveness of the postcoital test: randomised controlled trial. BMJ 317: 502-505.
18. Bossuyt PM, Lijmer JG, Mol BW (2000) Randomised comparisons of medical tests: sometimes invalid, not always efficient. Lancet 356: 1844-1847.
19. Zorn JR (2006) Current place of the Hühner test in the work-up of couple infertility. Gynecol Obstet Fertil 34: 142-146.
20. Oei SG, Helmerhorst FM, Keirse MJ (2001) Routine postcoital. Testing is unnecessary. Hum Reprod 16: 1051-1053.
21. Helmerhorst FM, van Vliet HA, Gornas T, Finken MJ, Grimes DA (2006) Intrauterine insemination versus timed intercourse for cervical hostility in subfertile couples. Obstet Gynecol Surv 61: 402-414.
22. Te Velde ER, Van Kooy RJ, Waterreus JJ (1989) Intrauterine insemination of washed husband's spermatozoa: a controlled study. Fertil Steril 51: 182-185.
23. Check JH, Bollendorf A, Zaccardo M, Lurie D, Vetter B (1995) Intrauterine insemination for cervical and male factor without superovulation. Arch Androl 35: 135-141.
24. Steures P, van der Steeg JW, Hompes PG, Bossuyt PM, Habbema JD, et al. (2007) Effectiveness of intrauterine insemination in subfertile couples with an isolated cervical factor: a randomized clinical trial. Fertil Steril 88: 1692-1696.
25. Glazener CM, Coulson C, Lambert PA, Watt EM, Hinton RA, et al. (1987) The value of artificial insemination with husband's semen in infertility due to failure of postcoital sperm-mucus penetration—controlled trial of treatment. Br J Obstet Gynaecol. 94: 774-778.
26. Friedman A, Haas S, Kredentser J, Stewart E, Schiff I (1989) A controlled trial of intrauterine insemination for cervical factor and male factor: a preliminary report. Int J Fertil 34: 199-203.
27. Chaffkin LM, Nulsen JC, Luciano AA, Metzger DA (1991) A comparative analysis of the cycle fecundity rates associated with combined human menopausal gonadotropin (hMG) and intrauterine insemination (IUI) versus either hMG or IUI alone. Fertil Steril 55: 252-257.
28. Kirby CA, Flaherty SP, Godfrey BM, Warnes GM, Matthews CD (1991) A prospective trial of intrauterine insemination of motile spermatozoa versus timed intercourse. Fertil Steril 56: 102-107.
29. Helmerhorst FM, van Vliet HA, Gornas T, Finken MJ, Grimes DA (2006) Intrauterine insemination versus timed intercourse for cervical hostility in subfertile couples. Obstet Gynecol Surv 61: 402-414.
30. World Health Organization (1992) WHO laboratory manual for the examination of human semen and sperm-cervical mucus interactions. 3rd ed. Cambridge University Press, Cambridge, UK.
31. World Health Organization (1999) WHO laboratory manual for the examination of human semen and sperm-cervical mucus interactions. 4th ed. Cambridge University Press, Cambridge, UK.
32. Glatstein IZ, Best CL, Palumbo A, Sleeper LA, Friedman AJ, et al. (1995) The reproducibility of the postcoital test: a prospective study. Obstet Gynecol 85: 396-400.
33. Insler et al., (1972)
34. World Health Organization (1999) WHO laboratory manual for the examination of human semen and sperm - cervical mucus interactions. 4th ed. Cambridge University Press, Cambridge, UK.
35. Oei SG, Helmerhorst FM, Bloemenkamp KW, Hollants FA, Meerpoel DE, et al. (1998) Effectiveness of the postcoital test: randomised controlled trial. BMJ 317: 502-505.
36. Helmerhorst FM, Van Vliet HA, Gornas T, Finken MJ, Grimes DA (2006) Intrauterine insemination versus timed intercourse for cervical hostility in subfertile couples. Obstet Gynecol Surv 61: 402-414.
37. Eimers JM, te Velde ER, Gerritse R, van Kooy RJ, Kremer J, et al. (1994) The validity of the postcoital test for estimating the probability of conceiving. Am J Obstet Gynecol 171: 65-70.
38. Snick HK, Snick TS, Evers JL, Collins JA (1997) The spontaneous pregnancy prognosis in untreated subfertile couples: the Walcheren primary care study. Hum Reprod 12: 1582-1588.
39. van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, van der Veen F, et al. (2004) Should the post-coital test (PCT) be part of the routine fertility work-up? Hum Reprod 19: 1373-1379.
40. Marshburn PB, Kutteh WH (1994) The role of antisperm antibodies in infertility. Fertil Steril 61: 799-811.
41. Eggert-Kruse W, Buhlinger-Gopfarth N, Rohr G, Probst S, Aufenanger J, et al. (1996) Antibodies to chlamydia trachomatis in semen and relationship with parameters of male fertility. Hum Reprod 11: 1408-1417.
42. Alexander NJ (1988) The effects of antibody sperm-egg interaction. Ann N Y Acad Sci 541: 317-323.
43. Menge AC, Christman GM, Ohl DA, Naz RK (1999) Fertilization antigen-1 removes antisperm autoantibodies from spermatozoa of infertile men and results in increased rates of acrosome reaction. Fertil Steril 71: 256-260.
44. Restrepo B, Cardona-Maya W (2013) Antisperm antibodies and fertility association. Actas Urol Esp 37: 571-578.
45. Mahdi BM, Salih WH, Caitano AE, Kadhum BM, Ibrahim DS (2011) Frequency of antisperm antibodies in infertile women. J Reprod Infertil 12: 261-265.

46. Naz RK (2011) Antisperm contraceptive vaccines: where we are and where we are going? Am J Reprod Immunol 66: 5-12.

47. Pattinson HA, Mortimer D (1987) Prevalence of sperm surface antibodies in the male partners of infertile couples as determined by immunobead screening. Fertil Steril 48: 466-469.

48. World Health Organization (2010) Laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 5th ed. Cambridge.