Abnormal cervical cytology in women eligible for IVF

D.van Hamont¹,²,⁵, L.H.C.Nissen¹, A.G.Siebers³, J.C.M.Hendriks⁴, W.J.G.Melchers², J.A.M.Kremer¹ and L.F.A.G.Massuger¹

¹Department of Obstetrics and Gynaecology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, ²Department of Medical Microbiology, Nijmegen University Centre for Infectious Diseases, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, ³Department of Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, and ⁴Department of Epidemiology and Biostatistics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

⁵To whom correspondence should be addressed at: Department of Obstetrics and Gynaecology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands. E-mail: d.vanhamont@obgyn.umcn.nl

BACKGROUND: *Chlamydia trachomatis* is more prevalent in subfertile women than in the general population and is the leading cause of tubal factor subfertility. As *C. trachomatis* infections are sexually transmitted, it can be expected that infections with human papillomavirus (HPV) are also more prevalent in this group of women. HPV is a necessary cause for the development of cervical (pre-)malignancies. We therefore hypothesized that subfertile women are more likely to have HPV-induced cervical abnormalities compared to the general population. METHODS: In this retrospective case–control study, all cervical smears of women visiting the fertility clinic for IVF (cases) and of women attending the population-based screening programme for cervical cancer (controls) were retrieved from an electronic database and assessed. RESULTS: The cases (*n* = 669) showed significantly more abnormal cervical smears compared to the controls (77 055) (6.1 and 3.9%, respectively, *P* < 0.02). CONCLUSIONS: The probability that subfertile women eligible for IVF are diagnosed with a high-grade cervical lesion is almost twice as high compared to women in the general population. We therefore suggest to take a cervical smear from all women referred for fertility problems.

Key words: case–control study/cervical cytology/screening/sexually transmitted infections/subfertility

Introduction

Sexually transmitted infections (STIs) in adolescents and young adults (e.g. 10–19 and 20–24 years of age, respectively) are a significant burden for health care worldwide. In the United States, this age group represents approximately 25% of the sexually active population; however, they account for more than 65% of all registered STIs (Centers for Disease Control and Prevention, 1996; Brown et al., 2005). *Chlamydia trachomatis* and human papillomavirus (HPV) infections are STIs of specific interest because of the high prevalence rates and the association with tubal factor subfertility and cervical cancer, respectively. Both can be considered a marker reflecting promiscuous, precarious sexual behaviour noticeably increasing in the general population (van Valkengoed et al., 2001).

Prevalence of *C. trachomatis* infections varies from 5% in asymptomatic women in the general population (Stergachis et al., 1993) to 24% in women assessed for fertility problems (Bjercke and Purvis, 1993). Adolescence, nulligravidity, promiscuity and unmarried state are independently associated with an increased risk for *Chlamydia* infection (Stergachis et al., 1993). In a small percentage of infected women, an ascending infection will lead to pelvic inflammatory disease and ultimately to tubal factor subfertility, the third cause of subfertility in industrialized countries (Hull et al., 1985).

The estimated lifetime risk of contracting a genital HPV infection is 80%, whereas 50% of the sexually active women will be infected within 2 years following the sexarche (Koutsky, 1997). Molecular and epidemiologic studies have shown that a persistent infection with high-risk HPV is the most important risk factor for the development of both cervical cancer and its precursors (Remmink et al., 1995; Wallin et al., 1999; Cuschieri et al., 2005). Close to all of the cervical cancers (Walboomers et al., 1999), 84% of high-grade cervical intraepithelial neoplastic lesions (CIN) and 74% of low-grade CIN lesions harboured the high-risk virus (Melchers et al., 1999). Recent studies have shown that HPV persistence is associated with concurrent *Chlamydia* infection (Samoff et al., 2005) and that *C. trachomatis* antibodies were associated with a significant increase of squamous cell cervical cancer (Smith et al., 2004).

The presumed increase in promiscuous, precarious sexual behaviour results in sexually active adolescents who are at risk of acquiring HPV and *C. trachomatis* infections unaware of the fact that (tubal factor) subfertility and (the treatment of) severe cervical pathology could negatively affect family planning even many years following an infection. Because women with fertility problems have the highest *Chlamydia* prevalence, suggesting promiscuous, precarious sexual behaviour and thus possible HPV infections, we hypothesize that women visiting...
fertility clinics are more likely to harbour high-grade cervical lesions than women in the general population. This study was undertaken to study this hypothesis.

Materials and methods
In this retrospective case–control study, the cases were women who consulted the fertility clinic of the Department of Obstetrics and Gynaecology of the Radboud University Nijmegen Medical Centre in the period from 1 January 2000 to 31 December 2003 and who had had either IVF or ICSI treatment. According to the policy of the department, cervical smears were taken before starting IVF or ICSI. The controls comprised women who had had a cervical smear taken within the population-based screening programme for cervical cancer in the same period. These smears were taken by a general practitioner. Because both fertility clinic and screening programme operated in the same region, demographic features of both cases and controls were considered comparable.

Women aged 29–42 years were included. The upper limit was applied because IVF is not performed in women older than 42 years, whereas the lower limit is based on the age of the first screening within the population-based screening programme. Although this programme starts at age 30, some women have their first smear taken at 29 years of age, explained by the fact that women are generally invited in the year they are turning 30. Applying these criteria led to the inclusion of 669 cases and 77,055 controls.

The cytological results were retrieved from the local pathology database and were classified using the Dutch CISOE-A classification (Hanselaar, 2002). This classification system discriminates between normal cytology, borderline nuclear changes (BNC), mild dyskaryosis, moderate dyskaryosis, severe dyskaryosis, carcinoma in situ and carcinoma. Smears diagnosed equal to or more severe than BNC were considered abnormal. Furthermore, it is the hospitals’ policy to perform colposcopy-guided histology in all smears indicating equal to or more than moderate dyskaryosis. Smears showing BNC or mild dyskaryosis are repeated after 6 months and are referred for colposcopic evaluation in case of persistence. On the basis of classification system guidelines, all cervical smears classified as ‘insufficient quality for reliable diagnosis’ were repeated within 6 weeks. Presuming that all ‘insufficient’ smears were reiterated, all cervical smears classified accordingly were excluded from the controls. With regard to the cases, ‘insufficient’ smears were identified and replaced by the first consecutive cervical smear of sufficient quality. In case the only available smear was ‘insufficient’, the woman was excluded from the study.

To study the relation between the incidence of abnormal smears and the primary cause of subfertility, additional data were collected from the electronic database of the fertility clinic. Only the most important subfertility diagnosis was listed in the database and thus taken into consideration. All women of subfertile couples, irrespective of age and fertility treatment modality, who had had a cervical smear and who were diagnosed with subfertility were taken into account. For this part of the study, 1,629 women were included.

Statistical analysis
The chi-square test was used to test for significant differences between cases and controls in categorical nominal variables. Fischer’s exact test was used in case of two by two tables. Univariate logistic regression analysis was used to test differences in the incidence of severe abnormalities between groups. The (crude) odds ratio (OR) with 95% confidence interval (95% CI) is presented. Multivariate logistic regression was used to test differences in the incidence of severe abnormalities between groups adjusted for age. The adjusted odds ratio (adj OR) with 95% CI is presented. All test results with a probability (P) of <0.05 were considered to be statistically significant. The statistical analyses were performed with SAS version 8.2 software.

Results
Meeting the inclusion criteria, that is age between 29 and 42 years and cervical smear taken of sufficient quality, the cases consisted of 669 women aged 34±3 years, whereas the mean age of the 77,055 controls was 35±4 years. Age distribution for cases and controls is shown in Figure 1.

The distribution of cervical cytology in the two groups is summarized in Table I. In comparison to the cases, the control group showed more normal smears. Smears indicating BNC and the smears showing moderate dyskaryosis or more severe were more frequently observed in the cases. The percentage of abnormal cytology (BNC or more severe) as observed in the cases was significantly different from the abnormal cytology as expected based on the findings in the control group (P = 0.014; chi-square test).

From moderate dyskaryosis onwards, it is compulsory to evaluate and verify the cytological abnormalities using (colposcopy-guided) histology. Table I summarizes that significantly more cases (1.95%) than controls (1.01%) had a cytological diagnosis equal to or more severe than moderate dyskaryosis [P = 0.017; OR 1.94 (95% CI = 1.12–3.37)]. Although the age distribution in the cases differed from the distribution in the controls (Figure 1), this did not affect the significant difference [adj OR 1.77 (95% CI = 1.19–2.65)].

Table I. Percentage of the distribution of cervical cytology in the cases (n = 669) and the controls (n = 77,055)

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Cases (n = 669) (%)</th>
<th>Controls (n = 77,055) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>93.87</td>
<td>96.06</td>
</tr>
<tr>
<td>BNC</td>
<td>3.74</td>
<td>2.36</td>
</tr>
<tr>
<td>Mild dyskaryosis</td>
<td>0.45</td>
<td>0.56</td>
</tr>
<tr>
<td>Moderate dyskaryosis</td>
<td>0.60</td>
<td>0.34</td>
</tr>
<tr>
<td>Severe dyskaryosis</td>
<td>0.75</td>
<td>0.38</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>0.45</td>
<td>0.27</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>0.15</td>
<td>0.02</td>
</tr>
</tbody>
</table>

BNC, borderline nuclear changes.
Abnormal cytology in women eligible for IVF

### Discussion

In this study, abnormal cytology (i.e. ≥BNC) was observed significantly more often in women eligible for IVF treatment (e.g. the cases) as compared with the women in the population-based screening programme for cervical cancer (e.g. the controls) (6.1 and 3.9%, respectively) (chi-square test, \( P < 0.02 \)). These results are in contrast to the observations by Lundqvist et al. In a similar study, they found abnormal cytology in 2.3% of women admitted for IVF and in a corresponding 4.1% of the healthy control women from a screening programme (Lundqvist et al., 2002). However, in the latter study, both case and control groups were substantially smaller, encompassing just 214 and 197 women, respectively. In contrast to this study, Lundqvist and colleagues did not apply age limits—the cases were aged 20–40 years, whereas the controls were aged 25–59 years. Moreover, the results could neither be interpreted scientifically nor be compared to our results because statistical analyses were not performed.

The most important factor known in the development of CIN and cervical cancer is a persistent type-specific high-risk HPV infection (Remmink et al., 1995; Wallin et al., 1999; Cuschieri et al., 2005). Because this study is entirely based on electronic data, HPV status was not and could not be included. Hormonal stimulation promotes HPV replication in vitro (Gloss et al., 1987) and in vivo (Arbeit et al., 1996). Long-term exogenous estrogen exposure in humans has shown an increased detection rate of HPV (Hildesheim et al., 1990) and appeared to be a risk factor for high-grade cervical lesions (Negrini et al., 1990). However, because the cases in our study consisted of non-pregnant women eligible for IVF, who neither used oral contraceptives nor received hormonal treatment at intake, higher hormone levels were considered unlikely to explain the observed difference. Moreover, Strehler and colleagues (1999) did not find a significant difference in cervical HPV DNA prevalence between infertile women undergoing ovarian hormonal stimulation and healthy control women. In addition, the study of Lundqvist and co-workers did not report significant differences in hr-HPV genotype prevalence in IVF women compared to healthy controls (7 and 9.1%, respectively) (Lundqvist et al., 2002). Even cumulative HPV prevalence rates in infertile and healthy women do not seem to be significantly different. Van Ham and colleagues (2002) found a cumulative HPV prevalence of 75% in infertile women with normal cytology, whereas Brown and colleagues (2005) found 82% in healthy adolescents.

The pathogenesis of cervical intraepithelial neoplasia is however not solely dependent on HPV. Smoking, promiscuity, early sexarche, long-term contraceptive use and immunosuppression have been proposed as co-factors. Moreover, an independent role for the most prevalent STI i.e. *C. trachomatis* was suggested by several epidemiologic and case–control studies (Lehtinen et al., 1996; Anttila et al., 2001; Smith et al., 2004; Friedek et al., 2005). Although endocervical glandular cells are

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### Table II. The number (percentages) and totals of the distribution of cervical cytology in all subfertile women for the various subfertility diagnoses

<table>
<thead>
<tr>
<th>Subfertility diagnosis</th>
<th>Normal [n (%)]</th>
<th>BNC [n (%)]</th>
<th>Mild dyskaryosis [n (%)]</th>
<th>Moderate dyskaryosis [n (%)]</th>
<th>Severe dyskaryosis [n (%)]</th>
<th>Carcinoma in situ [n (%)]</th>
<th>Carcinoma [n (%)]</th>
<th>Total [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male subfertility</td>
<td>644 (93.6)</td>
<td>25 (3.6)</td>
<td>5 (0.7)</td>
<td>4 (0.6)</td>
<td>5 (0.7)</td>
<td>5 (0.7)</td>
<td>0 (0)</td>
<td>688 (100)</td>
</tr>
<tr>
<td>Menstrual cycle disorder</td>
<td>239 (93.5)</td>
<td>11 (4.3)</td>
<td>1 (0.4)</td>
<td>2 (0.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>253 (100)</td>
</tr>
<tr>
<td>Cervical factor</td>
<td>44 (93.6)</td>
<td>2 (4.3)</td>
<td>0 (0)</td>
<td>1 (2.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>47 (100)</td>
</tr>
<tr>
<td>Tubal/uterine factor</td>
<td>167 (88.8)</td>
<td>14 (7.4)</td>
<td>2 (1.1)</td>
<td>1 (0.5)</td>
<td>3 (1.6)</td>
<td>1 (0.5)</td>
<td>0 (0)</td>
<td>188 (100)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>67 (91.8)</td>
<td>6 (8.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>73 (100)</td>
</tr>
<tr>
<td>Sexual problems</td>
<td>12 (92.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (7.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>13 (100)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>342 (93.2)</td>
<td>15 (4.1)</td>
<td>2 (0.5)</td>
<td>1 (0.3)</td>
<td>5 (1.4)</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
<td>367 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>1515 (93.0)</td>
<td>73 (4.5)</td>
<td>10 (0.6)</td>
<td>10 (0.6)</td>
<td>13 (0.8)</td>
<td>7 (0.4)</td>
<td>1 (0.06)</td>
<td>1629 (100)</td>
</tr>
</tbody>
</table>

BNC, borderline nuclear changes.

### Table III. The number (percentages) and totals of the distribution of cervical cytology in all subfertile women for the various subfertility diagnoses

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Tubal/uterine factor [n (%)]</th>
<th>Other factors [n (%)]</th>
<th>Total [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>167 (88.8)</td>
<td>704 (93.5)</td>
<td>871 (92.6)</td>
</tr>
<tr>
<td>BNC</td>
<td>14 (7.4)</td>
<td>34 (4.5)</td>
<td>48 (5.1)</td>
</tr>
<tr>
<td>Mild dyskaryosis</td>
<td>2 (1.1)</td>
<td>3 (0.4)</td>
<td>5 (0.5)</td>
</tr>
<tr>
<td>Moderate dyskaryosis</td>
<td>1 (0.5)</td>
<td>5 (0.7)</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>Severe dyskaryosis</td>
<td>3 (1.6)</td>
<td>5 (0.7)</td>
<td>8 (0.9)</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>1 (0.5)</td>
<td>1 (0.1)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>0</td>
<td>1 (0.1)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Total</td>
<td>188 (100)</td>
<td>753 (100)</td>
<td>941 (100)</td>
</tr>
</tbody>
</table>

Male factor subfertility was not included in the groups. BNC, borderline nuclear changes.
the targets for *C. trachomatis*, the association between *Chlamydia* and cervical carcinoma was only applicable to squamous cell carcinomas (Smith et al., 2004). However, the association is modest in comparison with the strong effect of HPV infections. Possibly, a *C. trachomatis* infection might increase the host susceptibility to HPV or enhance the effects of HPV (Felley-Bosco, 1998; Castle et al., 2001). Because *C. trachomatis*-seropositive women appear to elicit a humoral- and cell-mediated response to particular antigens, they may have an impaired ability to clear HPV (Eckert et al., 1997). This hypothesis is supported by the finding that a self-reported history of previous *Chlamydia* infection was the most significant risk factor for the persistence of a HPV infection (Silins et al., 2005). Therefore, it remains unlikely that *C. trachomatis* individually acts as a carcinogen of the cervix, but it may act as a trigger or an enhancer for HPV-mediated carcinogenesis of the uterine cervix. Both microorganisms are however sexually transmittable and evidently related to promiscuous, precarious sexual behaviour.

Another consequence of persistent *C. trachomatis* infections is a significant increase in the likelihood of developing tubal pathology and, subsequently, tubal factor subfertility (den Hartog et al., 2005). In addition to the association between *Chlamydia* infection and cervical pre-malignancies, we expect a higher frequency of abnormal smears in subfertile women suffering from tubal pathology. However, despite one case of moderate dyskaryosis, three cases of severe dyskaryosis and one case of carcinoma *in situ* in the ‘tubal pathology’ group, the data of this study do not support this hypothesis, because the majority of the abnormal cytological results were found in the ‘unexplained subfertility’ and the ‘male subfertility’ groups. This latter group is of specific interest because HPV has been found in substantial proportions of the sperm cells of patients who attended fertility clinics (Lai et al., 1996; Pao et al., 1996) and in the vas deferens of vasectomized middle-aged men (Rintala et al., 2002). Moreover, HPV negatively affects sperm cell motility, and the incidence of asthenozoospermia appears associated with sperm harbouring HPV (Lai et al., 1997). Although insufficiently studied, this work emphasizes the interesting possibility of sperm cells as a carrier for HPV transmission in unprotected sexual intercourse, eventually leading to cervical (pre-)malignancies.

Two other or alternative confounding factors could however explain the observed differences in the present study: (i) an urbanization trend and (ii) intra- and inter-observer variability. Squamous and glandular cell abnormalities were observed more frequently in cities exceeding 250,000 inhabitants in comparison with towns of 20,000–250,000 inhabitants and villages of less than 20,000 inhabitants (Boon et al., 2003). This positive urbanization trend has also been described in association with HPV infection, bacterial vaginosis and Gardnerella infection (Boon et al., 2002, 2003). However, both the case and the control groups of women assessed in this study populate the same geographical area that encompasses only towns and villages. The town/village ratio was assumed to be equally distributed over the cases and controls. Several studies have described a significant intra- and inter-observer variability in the subclassification of squamous and glandular pre-malignancies based on cytology (Yobs et al., 1987; Klinkhamer et al., 1988; Young et al., 1994; Woodhouse et al., 1999) and histology (Ismail et al., 1990). Although all patients in this study lived in the same area, the control group smears were taken by a different group of health-care workers (i.e. general practitioners), and the smears were assessed by various laboratories in the district, whereas all smears from the cases were taken by gynaecologists and subsequently evaluated by the pathology department of the Radboud University Nijmegen Medical Centre.

The higher prevalence of severe abnormal cytology in subfertile women visiting the outpatient clinic because of IVF treatment compared to the general population in the same geographical area suggests that sexually transmitted diseases are more present in subfertile women. Because pathologists might be influenced by the clinical origin of a cervical smear, thereby judging smears from cases in a more enquired perspective, a possible bias could be considered. Provided that the results of this study are validated in a prospective randomized investigation with standardized sampling and assessment methods, we suggest to structurally perform cytological examination of the uterine cervix in all women assessed for fertility problems. Especially since cytological abnormalities are induced or enhanced by highly prevalent STIs that are related to promiscuous, precarious sexual behaviour and appear to be more common in patients visiting subfertility clinics.

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