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# Incidence of Genital Mycoplasmas in Women at the Time of Diagnostic Laparoscopy

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The cervicovaginal and endometrial isolation rates of *Ureaplasma urealyticum* and *Mycoplasma hominis* and relevant demographic data were obtained at the time of laparoscopy in 193 women from infertile marriage. For comparative purposes, fertile women undergoing laparoscopy for tubal ligation ($n = 56$) or other purposes ($n = 64$) were also cultured. Blacks were more likely than caucasians to be infected with either organism in all population types ($p \leq .05$); however, no differences were noted in cervicovaginal carriage rates for blacks in different patient populations.

*M. hominis* was isolated more frequently from tubal reanastomosis patients and less often from infertile patients, $p \leq .001$. No differences were noted among the infertile subpopulations. Although the isolation rate of *U. urealyticum* from the different patient populations was similar, one subpopulation within the infertile population (male factor) was identified in which the prevalence of ureaplasmal infection of the female's lower genital tract was over twice as high ($p \leq .005$) as in other infertile women. Yet there were no statistically significant differences in the demographic data of this subpopulation as compared to the population of infertile women as a whole. No other clinical subpopulation with single or multiple diagnoses not including male factor had an increased prevalence of infection. Eighty percent of infected, infertile couples had no clinical evidence of male factor infertility, indicating that only certain individuals are affected. This possibly explains why previous studies involving small numbers of patients without regard to clinical subpopulations have failed to show significant differences between infected and uninfected couples. Neither mycoplasmas, other bacteria, chlamydiae, toxoplasmas, nor viruses were significantly isolated from the endometrium in any population.

Between 1973 and 1976 there was a 45 percent annual increase (i.e., an increase of 122,000 couples per year) in the estimate of non-surgical sterility [1]. The induction of infertility in a variety of animal species by subtle infectious agents [2,3] has given new impetus to the theory that low-grade, clinically silent infections may affect human fertility. Genital mycoplasmas, particularly *Ureaplasma urealyticum,* have become popular infectious agents to incriminate in humans. Over 50 articles purporting to prove or disprove their role in human infertility have been published within the last ten years [4–6], yet investigators still disagree as to whether or not ureaplasmal infection is



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even associated with infertility. Furthermore, the current literature has led to widespread indiscriminate antibiotic treatment of infertile couples. Without resolution of the contradictory findings and proof that such therapy is either effective or necessary, continued administration is unwarranted.

In a recent comparative study [7] of 193 women from infertile marriage and 56 fertile women undergoing tubal ligation, we found no differences in the cervicovaginal or endometrial isolation rate of *U. urealyticum,* even taking into account the major factors known to influence mycoplasmal colonization, including age, race, hormonal status, socioeconomic status, and sexual experience [6]. However, one clinicopathological entity (infertility associated with male factor) within the infertile population was identified in which the prevalence of ureaplasmal infection of the female's lower genital tract was almost twice as high ($p \leq .005$) as in all other infertile women combined and over twice as high as that of fertile tubal ligation controls [7]. A more comprehensive definition of the two patient populations previously reported [7] as well as the inclusion of other patient groups will be discussed in this presentation.

## METHODS

### *Patient Groups*

One hundred ninety-three females with a history of one year or more of involuntary infertility (132 nulliparous and 61 prima-multiparous) and their husbands were examined at the University of Alabama in Birmingham Infertility Clinic. The patients in this study were completely re-evaluated regardless of prior management attempts or diagnostic evaluation prior to referral. The diagnostic regime for both males and females has been described previously [7]. For comparative purposes, women undergoing laparoscopy for tubal ligation ($n = 56$) or other purposes ($n = 64$) were cultured in a similar manner.

Based on the diagnostic routine previously described [7], female infertility patients were classified as being infertile due to: *(a)* ovulatory disorders including inadequate luteal phase, short luteal phase or anovulation, or others; *(b)* endometritis; *(c)* endometriosis; *(d)* tubal obstruction or adhesions; *(e)* uterine anomaly or tumor; *(f)* cervical factor; *(g)* male factor; *(h)* some multiple combination of the above; and *(i)* unexplained causes. All diagnoses were made by the attending gynecologist without prior knowledge of microbial cultural results.

### *Cultural Procedures*

At the time of laparoscopy, endocervical and vaginal swabs, uterine lavage, and endometrium were cultured for mycoplasmas, chlamydiae, toxoplasmas, and viruses. The uterine lavage and endometrial biopsy were collected for culture after surgical preparation (Betadine$^{R}$) of the cervicovaginal area. To further ensure that upper genital tract cultures were not contaminated by endocervical mucus, the sterile Novak$^{R}$ curet (10″ and ¼″ diameter) used to collect the samples was enclosed in a heat-sealed, sterile polyethylene sheath (30 mm thick, 19 mm wide). Cultural procedures have been described in detail previously [7]. In brief, cervical contamination of the uterine lavage and biopsy was monitored on every sample by parallel culture in brain heart infusion broth supplemented with 0.1 percent agar [8]. Shepard's manganese urea agar and broth [9] were inoculated for cultivation of genital mycoplasma (0.1 ml uterine lavage/0.9 ml broth; 0.1 g tissue/0.9 ml broth), and isolates identified as described by Shepard and Lunceford [9]. All cultures were passed blind at least once and incubated for 21 days before designated negative.

Sucrose phosphate transport medium was inoculated for chlamydiae and Earle's 199 transport medium for toxoplasma and viruses. Specimens were immediately processed for *Chlamydia trachomatis* as described by Harrison et al. [10], using McCoy cells treated with cyclohexamide [11]. All negative specimens were passed at least once. A known positive control was included in each cultural assay. Cultures for viruses and toxoplamsa were processed by standard methods in monolayers of African green monkey kidney cells and human foreskin fibroblasts. Cultures were observed for the development of cytopathic effect for a minimum of four weeks before being discarded.

### *Statistical Methods*

Statistical analyses for incidence data were performed by the chi-square test with Yate's correction for continuity employed where appropriate. A $p$ level $\leq 0.05$ was accepted as significant.

## RESULTS

Relevant demographic data for the populations studied are given in Table 1. Educational level was chosen as an indicator of socioeconomic status. The infertile population had a significantly higher number of caucasians, and the number of blacks was increased in the tubal ligation population ($p \leq .001$). Patient ages did not vary significantly among the populations (mean overall age, 30.4 years; standard deviation, 4.1 years). There were no differences among patient groups or between races in the number of women admitting to two or more sexual partners within the last year. Most individuals had only one sexual partner.

There was no difference in the educational level of blacks in the infertile and tubal ligation populations studied; too few blacks were included in other groups for adequate evaluation. Caucasians in the infertile population had higher educational levels, while those in the tubal reanastomosis category had lower educational levels

TABLE 1
Relevant Demographic Data for Different Patient Types Cultured for Genital Mycoplasmas

| Patient Type | Mean Age | Mean Educational Level[a] | Racial Distribution | | Total No. Cultured |
|---|---|---|---|---|---|
| | | | Black | Caucasian | |
| Infertile[b] | 30.2 | 3.6 | 25 | 168 | 193 |
| Habitual aborters[c] | 30.0 | 3.1 | 3 | 23 | 26 |
| Tubal ligation[d] | 31.1 | 2.6 | 32 | 24 | 56 |
| Tubal reanastomosis[e] | 30.6 | 2.1 | 5 | 22 | 27 |
| Chronic pelvic[f] pain | 27.6 | 3.1 | 6 | 5 | 11 |

[a] 1 = Less than high school; 2 = High school graduate; 3 = Some college or technical school; 4 = College graduate; 5 = Post-graduate work

[b] Includes 132 nulliparous and 61 prima-multiparous women; statistical higher percentage of caucasians, $p \leq .001$

[c] Two or more abortions; includes 16 women who were also classified as infertility patients

[d] Includes only those of proven fertility and a statistically higher percentage of blacks, $p \leq .001$

[e] Patients with previously ligated fallopian tubes who were undergoing laparoscopy prior to tubal reanastomosis

[f] Number of patients inadequate for statistical analysis

($p \leq .001$). Within each population there were no statistically significant differences in educational levels between blacks and caucasians. Although not statistically significant, caucasians in the infertile population had a higher percentage of college graduates. Again, the low number of blacks in the other groups precluded statistical evaluation by race. The differences in educational levels for all types and both races were primarily in the completion of college or in the high school education or less categories.

Among the infertile subpopulations, blacks were more likely to have a diagnosis of tubal disease, $p \leq .005$. No differences were observed for caucasians with respect to diagnosis. Educational levels were similar for all infertile diagnoses.

The colonization of the lower genital tract with both *M. hominis* and *U. urealyticum* is affected by race and socioeconomic background [12]. In the present study blacks were more likely to be infected ($p \leq .05$), as were individuals with lower educational levels, regardless of race. The relationship between population type and cultural isolation from the lower and upper genital tract is given in Table 2. *M. hominis* was isolated more frequently from tubal reanastomosis patients and less frequently from infertile patients, $p \leq .001$. No statistically significant differences were observed with respect to ureaplasmal isolation among the populations; however, tubal reanastomosis patients did have a higher overall isolation rate than the other populations.

When the cervicovaginal isolation rates of *U. urealyticum* and *M. hominis* for the subpopulations within the infertile patient group (Table 3) were analyzed, individuals with a diagnosis of male factor infertility had a significantly higher isolation rate of *U. urealyticum* than any other subpopulation ($p \leq .02$). The ureaplasmal isolation rate from this subpopulation was nearly twice that of the isolation rate for the overall infertile population, $p \leq .005$. No significant differences were observed in the isolation rate of *M. hominis* from the various subpopulations.

Both *M. hominis* and *U. urealyticum* were found capable of invading the upper genital tract only in a small number of patients (Table 2). The low number of isolations from the endometrium precluded statistical analysis. However, tubal reanastomosis patients appeared to have the highest isolation rates of any group. Six of 19 (31 percent) infected tubal reanastomosis patients had mycoplasma-positive endometrial cultures. In those patients with positive endometrial cultures, the organisms were also isolated from the cervix, thus indicating a probable ascending route of infection. One of the two infertile patients with *M. hominis* isolated from endometrium had a recent (one month prior to laparoscopy) history of pelvic inflammatory disease of unknown cause (culturally negative for Neisseria, anaerobes, viruses, chlamydia). Her husband had simultaneously undergone treatment for nongonococcal urethritis. The remaining patients with mycoplasma-positive endometria had no evidence of inflammation.

Neither chlamydiae nor toxoplasmas were isolated from the cervix or endometrium of any population. Cytomegalovirus (CMV) was isolated from two infertile patients (endometrium but not cervix of one patient, and cervix only of the other) and from two tubal ligation patients (cervix of one and endometrium of two). Bacterial growth was obtained from endometrial samples in only 14 of 319 (4 percent) patients but never in association with positive viral or mycoplasmal endometrial cultures; thus, vaginal or cervical contamination was not a significant occurrence.

TABLE 2
Incidence of Genital Mycoplasmas in Different Types of Patients Cultured at the Time of Laparoscopy or Hysterectomy

| Patient Type | Cervix and/or Vagina[a] | | Endometrium | | Total Infected[a,b] | Total Cultured |
|---|---|---|---|---|---|---|
| | *M. hominis*[c] | *U. urealyticum* | *M. hominis* | *U. urealyticum* | | |
| Infertile | 33 (17.0) | 77 (39.9) | 2 | 1 | 78 (40.4) | 193 |
| Habitual aborters | 6 (23.1) | 9 (34.6) | 1 | 0 | 9 (34.6) | 26 |
| Tubal ligation | 18 (23.1) | 19 (33.9) | 0 | 0 | 28 (50.0) | 56 |
| Tubal reanastomosis | 13 (48.1) | 16 (59.2) | 3 | 3 | 19 (70.4) | 27 |
| Pelvic pain[d] | 4 (36.4) | 3 (27.3) | 0 | 0 | 5 (45.4) | 11 |

[a] No. in parentheses = no. infected/total no. cultured × 100
[b] Includes individuals positive for *M. hominis, U. urealyticum,* or both
[c] *M. hominis* isolated more frequently from tubal reanastomosis patients and less frequently from infertile patients, $p \leq .001$
[d] No. patients inadequate for statistical analysis

TABLE 3
Incidence of Genital Mycoplasmas in Defined Subpopulations of 193 Infertile Women

| Subpopulation (*N*) | Infected *U. urealyticum* (%)[a] | Infected +*M. hominis* (%)[b] |
|---|---|---|
| Abnormal luteal (42) | 20(47.6) | 9(21.4) |
| Other ovulatory (34) | 10(29.4) | 2(5.9) |
| Endometritis (14) | 8(57.1) | 2(14.3) |
| Endometriosis (55) | 17(30.9) | 10(18.2) |
| Tubal disease (86) | 40(46.5) | 16(18.6) |
| Cervical factor (20) | 8(40) | 3(15) |
| Unexplained (15) | 5(33.3) | 1(6.7) |
| Male factor (20) | 15(75) | 3(15) |

[a] *U. urealyticum* isolated more frequently from individuals with a diagnosis of male factor than all other subpopulations ($p \leq .02$) or the infertile population as a whole ($p \leq .005$)
[b] No significant differences in the isolation rate of *M. hominis* among the infertile subpopulations; *M. hominis* was isolated in the absence of *U. urealyticum* in two individuals.

## DISCUSSION

Race was a predictive factor for cervicovaginal carriage of both *U. urealyticum* and *M. hominis,* with blacks at risk for infection regardless of educational level. Individuals with lower educational levels were more likely to be infected. However, we found no differences in the cervicovaginal isolation rate of *U. urealyticum* or *M. hominis* between the total population of infertile versus fertile tubal ligation patients even when taking into account race, socioeconomic status, sexual experience, and age. All patients were cultured at the time of laparoscopy, which was performed in all cases during the late luteal phase, thus avoiding discrepancies in incidences of mycoplasmas due to cyclical variations.

Individuals with prior tubal ligation (i.e., those women classified in the tubal reanastomosis group) did have an increased prevalence of *M. hominis* in the cervix and/or vagina. Although the number of endometrial isolations was inadequate to achieve statistical reliability, it did not appear that this patient group was also at risk for endometrial colonization by both *M. hominis* and *U. urealyticum.* The low educational level of these individuals might explain these results. However, it is possible that tubal ligation may alter the physiology of the genital tract and create a more conducive environment for colonization.

Our results reflect a much lower endometrial isolation rate from all patient types than reported by other workers [13,14]. The discrepancy might be attributed to differences in patient populations studied or cultural methods but more likely is due to failure of the other two groups to match infertile and control groups for cervical carriage of the organism, failure to quantitate cervical organisms in both patient groups, and subsequent failure to circumvent cervical contamination [13,14]. These differences have been discussed previously [7].

As previously reported [7], isolation of *U. urealyticum* was significantly associated with one infertile subpopulation, those whose infertility was classified as male factor. Eighty-seven percent of women in this study whose infertility was associated with male factor had multiple factor infertility, including tubal disease, endometritis, endometriosis, ovulatory disorders, and/or cervical factors. Importantly, no other subpopulation with single or multiple diagnoses, not including male factor, had an increased prevalence of infection. Had infertility been examined as a single entity rather than a syndrome, the observed association would have been

obscured. It is also important to note that male factor may not necessarily be the primary diagnosis, but only a contributary factor to the overall problem of infertility. Although the observed association of *U. urealyticum* with a single defined infertile subpopulation is epidemiologic support for an etiologic role in infertility, the results are not conclusive. Whether this association is causal or coincidental remains to be determined; however, it is clear that only a limited number of individuals within the infertile population are likely to be at risk.

Other workers [15,16] have noted that ureaplasmal infection is associated with decreased sperm motility and increased aberrant morphology. Recent proof of the etiologic significance of *U. urealyticum* in nongonococcal urethritis [17] and in acute urethroprostatitis [18,19] illustrate this organism's ability to elicit an inflammatory response within the male urogenital tract. The potential pathogenic mechanisms by which *U. urealyticum* could elicit subtle alterations in fertility have been discussed in detail elsewhere [7].

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