MYCOPLASMA, CHLAMYDIA AND VIRUSES IN THE FEMALE GENITAL TRACT AND INFERTILITY: CLINICAL EXPERIENCE

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Summary: In the University of Padua during the last ten years the chlamydia trachomatis, mycoplasma and viruses were studied in human infertility. The results show the influence on chlamydia and ureaplasma urealitycum, in some cases with different pathogenetic mechanisms. The results are discussed.

INTRODUCTION

Among the infections causing female infertility, ascending infections of the higher genital tract (utero tubarica) have particular relevance. Infections by chlamydia and, probably, by mollicules, are among these.

In this respect it must be emphasised that while Mycoplasma hominis and Ureaplasma urealyticum are components of the autoctonous microbiota of the female genital tract, Chlamydia trachomatis are alloctonous pathogenetic agents.

These two different biological situations cause difficulty in determining the true etiological significance of such a micro-organism in human infertility.

In our experience and in literature ([1-9]) endometritis or salpingites by chlamydia trachomatis are frequently the cause of sterility.

METHODS

For the mycoplasma and chlamydia we have adopted the following methods:

Chlamydia

After collection, the samples were put into 2-SP fetal calf serum, Amphotericin B (2.5 mg) and Gentamicin (50 mg), for the transport to the laboratory.

The tissue culture medium was composed of Earle balanced salt solution with aminoacids, vitamins, glucose and fetal bovine serum (5%).

The cultured cells remained in glass botties for 72 hours. Then they were suspended and passed to a flat plastic tube where, after incubation of 24 hours at 37 °C, they started to form cell layers.

After centrifugation, an aliquot of the clinical sample was inoculed onto the cell cultures, together with diluited material from the yolk sac of embryonated eggs.

The count of cellular inclusions was performed after a 3 days incubation at 37 °C.

Mycoplasma

The vaginal swabs were immediately put in test-tube containing 1 ml of "mycoplasma transport broth", BMB (700 Triptic Soy broth, 20% horse serum not warmed up, 10% of yeast extract, 0.002% of red phenol and 500 UI/ml of penicillin) and 50 mg of B-Amphotericin, the pH was corrected with HCl 1N to 6.2+0.1.

In the males, the investigations into mycoplasma were undertaken on seminal fluid obtained by masturbation and collected in sterile containers. Aliquots of 0.1 ml of samples were inoculated into urea broth, arginine broth on corresponding solid media. The positive cultures in liquid media (in which the pH had risen 0.5 units or more) were subcultured in solid media and studied for identification. The remainder for each sample was washed three times in phosphate buffered saline. From the obtained pellets slides were prepared and: a) directly examined by interference phase-contrast microscopy; b) observed after staining with Giemsa solution; c) fixed with cold acetone for immunofluorescence.

Antisera to M. hominis used for this method were prepared in white rabbits according to the immunization method described by Razin and were heated at 56 °C for 30 min. before use. Fluorescein conjugated anti-rabbit IgG produced in goats were used for indirect immunofluores-

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cence. One drop of anti-M. Hominis antiserum was placed on each smear, allowed in a moist chamber at 37 °C for 30 minutes, rinsed with PBS for 15 minutes and covered with fluorescent antibodies for 30 minutes at 37 °C.

RESULTS AND DISCUSSION

In the uterine and salpingis mucosa the pathological effects of chlamydia trachomatis are well known, and can be of different grades in our research. The hysteroscopic microhysteroscopic and histological pictures have shown, in some cases, limited epithelial alteration, in other cases widespread and in others again, massive.

The damage to the epithelian cells and their cilia vibratili is evident in the macroscopic and microscopic examination and the involvement of the underlying stroma is even frequent.

This morphological and functional involvement of the endometrium or tubaric mucosa by chlamydia may be the cause of infertility, even without tubaric occlusion.

In many cases the histological and cultural findings are highly significant in our studies for endometrial damage even without the classic pelvic inflammatory disease which, as we known, is frequently caused by chlamydia trachomatis. On the other hand the chlamydia trachomatis infection does not always involve the higher female genital tract. The chlamydia infections are very frequent in the lower female genital tract, with the characteristic lesions of the mucosa but without an important role in the pathogenesis of infertility. In our case series we found a similar incidence of positive culture for chlamydia in both groups of fertile and of idiopathic infertile couples ([28]) (table 1).

Table 1. — *Chlamydia trachomatis (cultural isolation).*

	Idiopathic infertile couples		*Fertile couples*	
	N	%	N	%
Male	103	0	50	1
Female	126	3	50	2

Chlamydia trachomatis can act in the male on the composition of the seminal fluid and on the motility of the spermatozoa (prostatitis), in the women the presence in the lower genital tract (cervicitis, vaginitis) can act on the capacitation of the spermatozoa.

In our experience however the cultural positivity for chlamydia in the seminal fluid and in the cervical-vaginal mucus in cases of idiopathic infertility is not demonstrative of this etiology, also according to others ([10]).

Regarding the mycoplasmas, not a few doubts exist as to the role of the mollicutes, even if phlogosis and degenerative changes of tubal functions or tubo-ovarian abcesses produced by M. hominis are considered the more probable cause of sterility in regard to endometritis produced by U. urealyticum.

In many cases of pelvic inflammatory disease with consequent infertility due to tubaric occlusion colonies pure of M. hominis have been isolated by us and by others ([11, 12]).

Now, however, there are many doubts about the role of the mollicutes in the etiology of infertility.

We began to study the mollicutes problem in 1975, and we often observed, in agreement with others, the cultural isolation of M. hominis and U. urealyticum in the vagina and in the endometrium in infertile women ([31-16]).

Table 2. — *Epidemiology of mycoplasmic infection in infertile couples.*

	No. cases	Mycoplasmic positive	
		No.	%
Sterile male(*)	116	60	51.7
Sterile female(*)	38	22	57.9

(*) Without other defined causes of sterility.

We also obtained in concordance with other Authors ([20–23]) the cultural isolation of M. hominis and U. urealyticum in the

seminal fluid in many cases of idiopathic infertility. After treatment with Doxicyclin in some cases fertilisation took place.

Table 3. — *Treatment with doxicycline in 56 patients mycoplasmic positive (primary stirility).*

116 Patients with primary sterility (male)
(48.3% positive U. urealyticum)

56 Mycoplasmic positive ⟶ 41 Doxicycline (100 mg/die/20 gg)
↓
19 Mycoplasmic negative
↓
12 Objective improvement of spermatozoa motility (> progress. > % cellular speed motility)
↓
4 pregnancies

We also observed reduction in the motility of the spermatozoa on account of the positive culture of M. hominis and U. urealyticum. Other authors have reported the same results ([22, 24]). Our team observed a close connection of M. hominis with the collar of the sperm ([25]), and consequent alteration of its motility. Other researchers ([25, 27]) of our group demonstrated a reduced capacity of penetration of preincubated spermatozoa with mycoplasmas into hamster zona-free eggs. Even the positive results following treatment with dyoxicyclin ([24]) in infertility are not significant, as for the individuation of the real pathogenic role of the Mollicutes or of the Chlamydias, often associated, inasmuch as both are sensitive to these antibiotics. Our microbiological and clinical experience also confirms this problem.

Virus infection could be responsable for human infertility because of the alteration of the vaginal-cervical fluor.

The Papova virus may be responsable for cervical mucus variation with hostility to the spermatozoa. Endocervical planum condyloma growing in the glandular tissue also causes alterations in the spermatozoa capacitation action on the cervical mucus. Herpes virus even causes local morpho-functional and biochemical alterations.

In our case series the incidence of colposcopical, cytological and positivity for both (herpes and Papova viruses) is significantly high. We are now studying the statistical and clinical significance of vaginal cervical infections in the direct pathogenesis of infertility. Viral infections in the male need to be studied for their role in the direct or indirect – through the female – pathogenesis of infertility.

The role of some viruses in female infertility (Herpes virus, Papova virus) is defined today, not in direct action (as hypothesised in the past) but as indirect iatrogenic consequences in the cervical district by surgical physical and medical therapy.

In conclusion our research cannot allow us to arrive at definite conclusions, but after 10 years of clinical, microbiological histological and pathological study, against all our own doubts, we believe in the role of mycoplasmas in human infertility. We consider right, in every case of idiopathic infertility the culture research of mycoplasmas in the seminal fluid, in the cervical vaginal mucus and in the endometrial specimen.

Exjubantibus, a treatment with dioxicyclin, in these cases can be justified and sometimes useful.

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SPONTANEOUS EXPULSION OF DECIDULIZED PSEUDOPOLYPS IN PREGNANT WOMEN WITH UTERINE MALFORMATION

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Summary: Two cases concerning expulsion of decidualized polyps in early pregnancy associated with uterine malformation are described. The authors discuss the differential diagnosis between the expulsion of cervical polyps during pregnancy and the ectopic pregnancy associated with polyposis. They suggest that a spontaneous expulsion of polyps or pseudopolyps during early pregnancy may be a sign of the presence of uterine malformation.

Decidualization of endometrial mucosa is a well-known phenomenon in pregnancy. When a uterine malformation, such as uterus bicorne, is present, this phenomenon concerns both the endometrial cavities even if only one of them is the seat of placentation.

In this case, in the cavity without placenta, a trophic alteration of the decidualized endometrium occurred, with hormonal insufficiency.

Abortion or threatened abortion may be the result. Sometimes however edges of decidualized mucosa may be lost and