

Bacterial agents as a cause of infertility in humans

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SUMMARY

Infertility is a problem affecting almost 15% of couples. There are many causes for this condition, among which urogenital bacterial infections seem to play an important role. Many studies have explained the mechanisms by which bacteria cause infertility both in men and women. Therefore we undertook this study to evaluate the presence of genito-urinary infections in infertile couples who sought counselling to investigate their condition. Microbiological analysis was performed on semen and vaginal/cervical samples of both partners of each couple. The percentage of individuals affected by a urogenital bacterial infection was between 14 and 20%. More significantly, most of the species isolated both in men and women have been described in the literature as potential causes of infertility.

Received December 12, 2015

Accepted June 26, 2016

INTRODUCTION

Infertility is a problem affecting almost 15% of couples worldwide (Mascarenhas *et al.*, 2012). In Italy, the estimated percentage rises to 20% (ISS, 2015). There are several causes for this condition, among which an important role seems to be played by urogenital infections (Novy *et al.*, 2008).

Human infertility is defined as the inability for a couple to conceive and produce offspring after at least twelve consecutive months of unprotected sexual intercourse. Infertility is a complex human health situation which particularly alters the quality of life in couples facing the condition. In 2010, around 1.9% and 10.5% of women of reproductive age (20-44 years old) were affected by primary and secondary infertility respectively. A high prevalence of infertility is noted in South Asia, Africa, the Middle East, Central/Eastern Europe and Central Asia (Mascarenhas *et al.*, 2012).

Infertility results from multiple factors that are responsible for impairments of reproductive function in men and/or women. These factors include congenital and hormonal disorders, lifestyle, environmental hazards and psychological state. All these factors can lead to impairments in the function of genital organs, the production of reproductive cells, semen quality, sperm cell transport to the oocyte, fertilization and embryo implantation steps (Abrao *et al.*, 2013; Inoue *et al.*, 2014; Pirkalani *et al.*, 2013; Safarinejad *et al.*, 2010). In the case of many couples, the cause of infertility remains unknown. Sexually transmitted infections (STI) can target different tissues along the genital tract in both men and women and lead to functional alterations. This can result in

reduced fertility or even infertility. It is widely accepted that bacterial infections with *Neisseria gonorrhoeae*, *Treponema pallidum* and *Chlamydia trachomatis* can impair fertility.

Many studies have shown that infections of the reproductive tract both in men and women may impair reproductive function (Novy *et al.*, 2008). The impact on fertility of a few bacteria such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Ureaplasma urealyticum* has been well established. In fact, in women, *C. trachomatis* infection may cause pelvic inflammatory disease (PID) and lead to chronic pelvic pain, ectopic pregnancy and infertility. (Novy *et al.*, 2008, Zanetti *et al.*, 2007; ECDC, 2009). *Neisseria gonorrhoeae* is also known to be involved in damage to the female reproductive tract, and has been recognised as a cause of PID, together with *Gardnerella vaginalis* and *Trichomonas vaginalis* (Mastromarino *et al.*, 2014; Novy *et al.*, 2008; Paavonen, 1996).

Some studies showed that bacteria, yeasts and protozoa may interact directly with sperm. These interactions result in attachment between bacteria and sperm, agglutination phenomena and morphological alterations to sperm (Kaur *et al.*, 2014). Among these species, there are pathogens which cause genito-urinary infections both in men and women, such as *E. coli*, *E. faecalis*, *U. urealyticum* and *Candida spp.* These organisms are present in the human urogenital tract and may be responsible for damage. Most of these phenomena have been observed in *in vitro* experimental studies, but *in vivo* experimental studies confirmed that the presence of spermagglutinant strains of *E. coli* and *S. aureus* in the vagina might be responsible for infertility because their removal from the vagina resulted in the recovery of fertility (Kaur *et al.*, 2014; Kaur *et al.*, 2012). Furthermore, the presence of pathogenic bacteria in the vagina has been associated with the release of proinflammatory cytokines, and a correlation between elevated IL-beta and IL-8 and idiopathic fertility was demonstrated (Mastromarino *et al.*, 2014).

On the basis of these considerations, the aim of this work

Key words:

Infertility, Bacteria, Urogenital infection.

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was to evaluate the presence of pathogenic microorganisms in infertile couples in North Sardinia, a major Italian island, who sought counselling to investigate the cause of their condition.

MATERIALS AND METHODS

Study population

The study population consisted of 246 couples with fertility disorders who contacted the University of Sassari Gynecologic and Obstetric Clinic. The average age of men was 42 years and women 37 years. Microbiological analyses were performed on both members of the couples at the Institute of Microbiology of the University of Sassari.

Samples

Semen samples were collected by masturbation after 3 days of sexual abstinence. Patients were asked to urinate and wash hands and external genitalia before ejaculation to avoid possible contamination.

Vaginal and cervical samples were collected during vaginal examination, by introducing sampling swabs into the vaginal canal and rotating them for at least 10 seconds along the vagina walls and in the cervix before withdrawal.

Microbiological analyses

For the detection of common bacteria and fungi, the samples were seeded on non-selective and selective media, incubated at 37 °C for 48 hours and presumptive pathogens were identified using biochemical test kits (Bio-Mérieux). The presence of mycoplasma was investigated with the Mycoplasma IST 2 kit (Bio-Mérieux). Detection of *C. trachomatis* was performed with the Dx Real-Time System (Biorad). For the detection of *Trichomonas vaginalis*, samples were incubated in Diamond broth at 37°C for 5 days and daily observation of the cultures was performed with a light microscope.

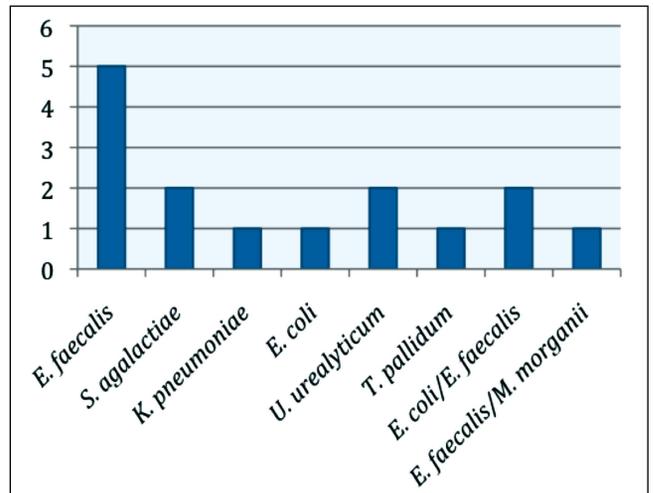


Figure 1 - Microorganisms isolated in women.

RESULTS

In this study we performed microbiological analysis on semen and vaginal/cervical swabs of 246 infertile couples. The results are shown in Figure 1 and 2. 212 semen cultures were negative (86%), 15 (14%) were positive, while 19 showed a mixed flora; 196 vaginal/cervical swabs were negative (79.6%), while 50 (20.4%) were positive. Infections were mostly due to a single agent, and multibacterial infections were more common in women than men (2.9% vs 0.8%).

Enterococcus faecalis was the most isolated pathogen, with similar percentages for both groups (3.6% in women and 2.8% in men).

In most cases it was the only cause of infection, but it was also recovered in multibacterial infections. *Escherichia coli* was the most isolated microorganism in women

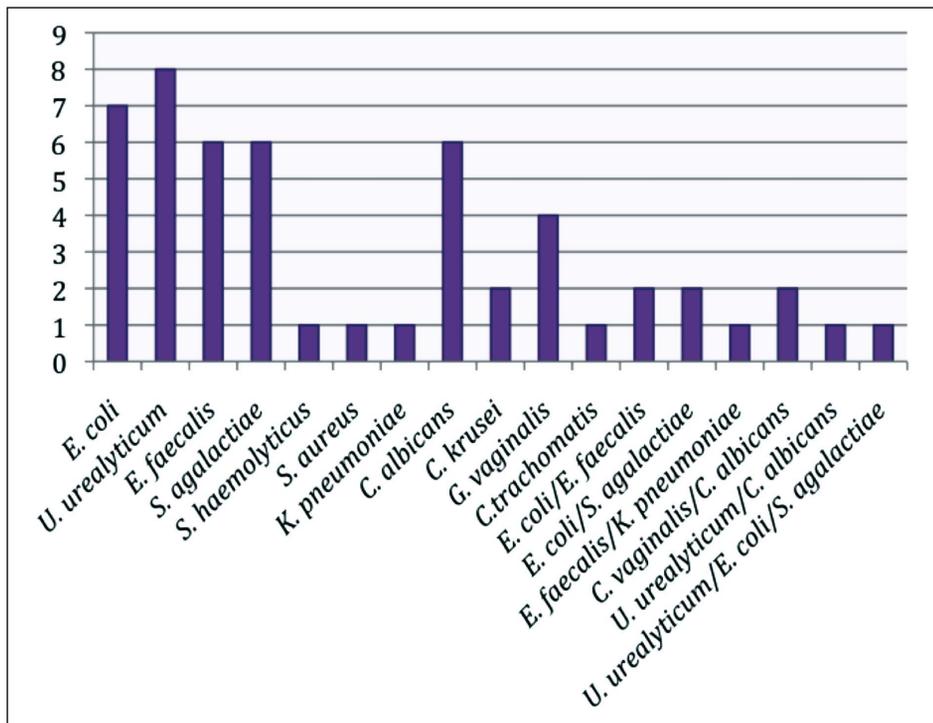


Figure 2 - Microorganisms isolated in men.

(4.8%), whereas in men it accounted for a much lower value (0.8%).

Ureaplasma urealyticum also showed a clearly different percentage of isolation in women and men (3.2% vs 0.8%). *Gardnerella vaginalis* and yeasts belonging to the genus *Candida* were recovered only in women: one patient in this group had a multiple infection with both organisms. A patient of the female group was infected by *Chlamidia trachomatis* and one of the male group was infected by *Treponema pallidum*, the aetiological agent of syphilis.

Regarding the couples, in 191 (77.6%) both partners' microbiological cultures were negative, in 47 (19.2%) only one had an urogenital infection, whereas in 8 both were infected (3.2%). In the latter, the microorganisms causing infection were the same in 5 (2.0%) couples, whereas they were different in the remaining 3 (1.2%).

DISCUSSION

This study was aimed to evaluate the presence of infections in couples with fertility disorders in North Sardinia. Infections of the urogenital tract are directly or indirectly a major cause of infertility in couples since they may impair the reproductive organs both in men and women, and because microorganisms may cause the agglutination of motile sperm, an impairment of acrosome reaction and alteration in cell morphology (Sleha *et al.*, 2013, Novy *et al.*, 2008, Moretti *et al.*, 2009).

The majority of patients were negative and the percentage of positive subjects was higher in women than men. Moreover, in women there was a wider variety of bacterial species responsible for the infections than in men. Both these aspects may be explained considering that women are more susceptible to genitourinary infections because the female urethra is shorter than the male and because the area around the vaginal opening is colonised with potential pathogens.

The most frequently isolated microorganisms in women were *Ureaplasma urealyticum*, *Escherichia coli*, *Enterococcus faecalis* and *Streptococcus agalactiae* among bacteria and *Candida albicans* among yeasts. *Chlamidia trachomatis*, a major bacterium responsible for female infertility, was recovered only in one patient. In men the most frequently isolated microorganism was *Enterococcus faecalis*.

The three most frequently isolated pathogenic bacteria (*Ureaplasma urealyticum*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus faecalis*) have been recognised by several studies as potential causes of infertility. *E. faecalis* is associated with compromised semen quality in terms of sperm concentration and morphology (Mehta *et al.*, 2002). Enterococci do not seem to influence sperm motility, but they seem to have a negative influence on membrane integrity of the human sperm head, neck and mid-piece (Qiang *et al.*, 2007). *U. urealyticum* is one of the most frequent causes of male infertility, because it impairs semen quality and negatively influences sperm motility, density and morphology (Gdoura *et al.*, 2007). *E. coli* showed a spermagglutinating activity *in vivo*. Moreover, studies at ultrastructural level showed its ability to impair the acrosomal function (Kaur *et al.*, 2014, Diemer *et al.*, 2000). In the female group, *Candida* spp and *Gardnerella vaginalis* were also frequently isolated. Species of the genus *Candida* are able to interact with sperms causing reduced motility and profound changes

to their ultrastructure (Tian *et al.*, 2007). *Gardnerella vaginalis*, responsible for bacterial vaginosis, has been associated with the activation of proinflammatory cytokine production in the vagina, that could interfere with sperm viability (Nwaziri *et al.*, 2009). Acute and chronic infections and consequent inflammation in the male reproductive system may compromise sperm cell function and the whole spermatogenic process, resulting in quantitative and qualitative sperm alterations (Urata *et al.*, 2001, Sanocka-Maciejewska *et al.*, 2005). The role of other pathogens, which are rarely encountered as causes of urogenital infections, such as *Klebsiella pneumoniae* or *Morganella morganii*, has not yet been clarified. Most pathogenic bacteria isolated from patients with urogenital infection do not seem to have any impact on sperm motility. However, some works demonstrated that most strains of a pathogenic species do not affect sperm quality, but some strains can cause *in vivo* spermagglutination or morphological alteration of sperm (Kaur *et al.*, 2012; 2014). The existence of different strains, of which only some may interact with sperm may explain why not all subjects with urogenital infections are infertile. Infections occurring in the lower female genital tract represent a complex topic from several different viewpoints. First, there is a clinical consequence associated with these infections. Second, the complexity is increased by the biological diversity of infectious organisms involved including bacteria, fungi, virus, and protozoan life forms. Third, the interaction between the host and infectious organisms through the inflammatory responses to infection and through the elaboration of host factors such as secreted antibodies and defensin molecules inhibitory to intravaginal microorganisms. Finally, the vagina is host to an indigenous microbiota which is credited with contributing to vaginal health, but the flora also interacts with exogenous microorganisms involved in the pathogenesis of vaginal infections. The vaginal infections derive from a biologically diverse collection of microbes. We have to continue to examine both the science behind vulvovaginal infectious pathology and to devise clinically relevant therapeutic approaches to the problems that continue to make women uncomfortable, undermine their quality of life, and in some cases threaten their overall health and the health of their fetuses and infants (De Seta *et al.*, 2012).

In conclusion, the role of urogenital infections in cases of infertility should be taken into account for the negative impact they may have on sperm quality and motility. Appropriate antibiotic treatment should be given before investigating every other possible cause of infertility, because of the brilliant results that may be achieved in a simple way. However, further studies should be carried out for a better understanding of the relationship between bacterial infections and infertility.

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