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A new enrichment diagnostic platform for semen culture

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ABSTRACT

Urogenital bacterial infections have been described in literature as a potential cause of infertility. For the consequences that a failure in diagnosis could have on the evolution of male urogenital infectious disease, an accurate microbiological procedure to investigate the bacterial species composition of seminal fluid plays a crucial role to better understand the eventual correlation with infertility. In order to improve the quality of semen culture investigations, we have developed a new enrichment diagnostic platform. Semen samples of 540 infertile men were simultaneously analyzed using the standard microbiological semen culture method and an alternative new experimental technique (Brain Heart Infusion broth, BHI, enrichment). Our results established the possibility to apply BHI enrichment to detect bacteria from semen samples with higher sensitivity (100%) and negative predictive value (100%) than the standard technique.

1. Introduction

Infertility is an emerging health worldwide problem and impacts about 20% of Italian couples (Ruggeri et al., 2016). Many causes may be ascribed for this condition, among which an important role seems to be played by genitourinary infections affecting one or both members of a couple. (Mårdh, 2004; Miri et al., 2016; Pellati et al., 2008).

It is estimated that genital tract infections are associated to about 8–35% of male infertility cases (Askienazy-Elbhar, 2005; Pellati et al., 2008; Ruggeri et al., 2016). Some studies support that spermatogenic process and sperm cell function can be compromised by acute and chronic infections and consequent inflammation in the male reproductive systems (Sanocka-Maciejewska et al., 2005). The relationship between the presence of pathogenic microorganisms in the male reproductive tract and infertility is well documented (Gdoura et al., 2008; La Vignera et al., 2014; Rusz et al., 2012). Several microorganisms, such as *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Mycoplasma hominis*, *Chlamydia trachomatis*, and *Ureaplasma urealyticum* found in the male urogenital tract, may compromise the sperm quality (Kaur and Prabha, 2012; Kaur and Prabha, 2014) with consequent sperm abnormalities, especially aberrant motility, deficient mitochondrial function, and loss of DNA integrity (La Vignera et al., 2011).

Therefore, it is important to investigate the bacterial species composition of seminal fluid to better understand the etiology and pathogenesis of urogenital tract infections and the eventual associations between urogenital infections and infertility.

In particular, microbiology investigation in infertile male could be very useful in asymptomatic infections (Askienazy-Elbhar, 2005) in which the central dilemma is whether to treat these patients or not. Asymptomatic patients often exhibit infertility problems due to prior untreated acute genitourinary infections that become chronic during their life or due to improperly treated acute infections. In chronic infection, the standard microbiological method used to characterize bacteria of seminal fluid are limited and frequently fail to highlight the presence of bacteria, probably because most chronic bacterial infections are nearly universally associated with a biofilm mode of growth (Wolcott and Ehrlich, 2008) that is highly resistant to antibiotic therapy and difficult to culture.

Bacterial biofilms have been identified in culture-negative patients with a past history of chronic bacterial prostatitis which had become refractory to antibiotics (Donlan and Costerton, 2002; Nickel and Costerton, 1993). For this reason, the classical semen microbiological evaluation may be less sensitive and are limited because many species of bacteria are recalcitrant to cultivation.

To overcome this problem and increase the sensitivity of semen

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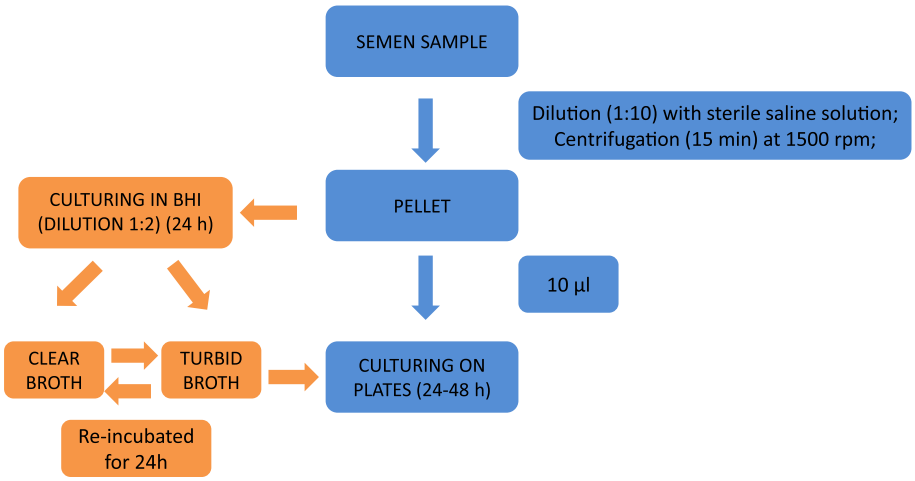

Fig. 1. Scheme graph of standard (blue) and alternative (orange) method for semen culture. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

culture, we have thought to combine the standard microbiological semen culture method (World Health Organization, 2010) with an improvement of BHI enrichment developed by several authors (Alo et al., 2013; Orji et al., 2007).

The purpose of our study was to improve the understanding of the pathophysiology of male infertility caused or complicated by reproductive tract inflammation/infection by the development of a new diagnostic platform for semen culture.

2. Materials and methods

This retrospective study has been conducted at the Laboratory of Clinical Bacteriology of the Virology and Microbiology Unit of the Second University of Naples for a 5-year period between 2009 and 2014.

2.1. Study subjects

During the study period, a total of 610 urine cultures and semen samples of infertile men aged between 30 and 45 years attending the Andrology and Seminology Unit of the Department of Molecular and Clinical Endocrinology and Oncology, University of Naples "Federico II", were collected. Urine cultures and semen samples from a control group of 50 healthy volunteers, of the same age, were also analyzed.

Subjects were eligible for enrollment if they were willing to sign a documented informed consent approved by the Institutional Board of the University of Campania "Luigi Vanvitelli".

The patient's medical history showed that many of them was affected by several episodes of genitourinary infections. The infertility problems were confirmed by abnormal spermiograms (sperm count and/or morphology and/or motility) (data not shown).

2.2. Specimen collection procedure

According to the standard protocol for the examination and processing of human semen (World Health Organization (WHO), 2010), we investigated the patient's history prior to undergoing to semen analysis. The specimen collections were taken from only those males who did not have antibiotic and/or antimycotic treatments for at least a week.

Semen sample were collected by masturbation into a sterile glass receptacle after 3 to 4 days of sexual abstinence. Prior to sample collection, paper guidelines (Woodward and Tomlinson, 2015) were given to the patients on procedures to be followed to prevent sample contamination: first, subjects were asked to urinate, then to wash hands with soap 2–3 times, and, finally to wash penis with warm soapy water swabbing with 75% alcohol 2–3 times before ejaculation.

The freshly collected seminal fluid was used for an accurate microbiological evaluation according to the standard methods and the alternative procedures developed at our laboratory.

2.3. Semen culture

All specimen collected were rapidly transferred within 3 h to the bacteriology laboratory and processed according to the WHO guidelines (World Health Organization (WHO), 2010).

Semen samples were diluted with sterile saline solution at 1:10 (Boitrelle et al., 2012; De Francesco et al., 2011) and centrifuged at 1500 rpm for 15 min. This treatment increased the culture sensitivity and concentrated the bacteria eliminating the seminal plasma that could have an inhibitory effect on bacterial growth (Vicari et al., 1986). The sediment (10 µl) (La Vignera et al., 2014) was seeded on Columbia CNA Agar with 5% Sheep Blood, MacConkey agar, Thayer-Martin agar, Gardnerella Selective Agar with 5% Human Blood, Chocolate agar and Sabouraud agar. Media were incubated for 24 to 48 h in atmosphere supplemented with 5% of CO_2 at 37 °C for detecting aerobic and microaerophilic bacteria. Simultaneously with the standard method above described, we developed an alternative procedure (Fig. 1). Our technique required an extra step in the semen processing to enrich an aliquot of the semen pellet in Brain Heart Infusion broth (BHI-OXOID; Oxoid, Basingstoke, United Kingdom). In particular, a dilution of the specimen (1:2, 250 µl of semen pellet in 500 µl of BHI) was incubated at 36° ± 1 °C for 24 h.

After 24 h, the turbid broths were plated as described above and incubated with the same temperature and aerobic/microaerobic conditions, while if the broth was still clear it was re-incubated for further 24 h and then eventually cultured.

The cultured plates were examined and the bacterial isolates were identified and characterized using Vitek II (Biomerieux, Marcy l'Etoule, France). Isolates from only the solid media plates (standard method) were recorded and compared with isolates obtained using BHI enrichment (alternative method).

2.4. Urine culture

For the isolation of urinary pathogens, a quantitative urine culture was traditionally performed using Mac Conkey Agar, Sabouraud agar, Blood Agar (Biomerieux, Marcy l'Etoule, France). The media plates were incubated aerobically at 37 °C for 24 h, with the exception of Sabouraud agar plates that were incubated at 30 °C for 48 h. The biochemical identification of the isolated bacteria was carried out with the Vitek II (Biomerieux, Marcy l'Etoule, France) (data not shown).