Journal of Fertilization: In vitro - IVF-Worldwide, Reproductive Medicine, Genetics & Stem Cell Biology

Lucena et al., JFIV Reprod Med Genet 2014, 3:1
DOI: 10.4172/2375-4508.1000139

Case Report

Unexplained Infertility Caused by a Latent but Serious Intruder: *Trichomonas vaginalis*?

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Abstract

Trichomona vaginalis infection is a sexual transmitted disease that affects human fertility. In men, trichomoniasis has been related to infertility by deficit of sperm cell quality and function due to physical damage. In women, trichomoniasis has been related to infertility due to pelvic inflammatory disease that compromises tubal patency. In this article, a case of unexplained infertility in a couple that was undergoing IVF treatment is discussed. Semen sample analysis demonstrated the presence of *Trichomona vaginalis,* polymorphonuclear cells, and asthenozoospermia. A protocol for separation and capacitation of optimal motile sperm needed for IVF procedure was utilized and a capacitated sperm sample with complete removal of trichomonas and polymorphonuclear cells was obtained. Capacitated motile sperm were used to achieve IVF fertilization and embryo development and the embryo obtained was transferred into the uterus. However, embryo implantation failed and pregnancy was not achieved, probably as a consequence of trichomoniasis in the asymptomatic female partner. The result indicates that *Trichomona vaginalis* pathogenicity, adverse reproductive health outcomes, in time diagnosis, and treatment may improve implantation rate in patients with unexplained infertility undergoing ART.

Keywords: Unexplained infertility; Implantation failure; Sexual transmitted disease; Pelvic inflammatory disease

Background

Trichomonas vaginalis is a flagellated protozoan. Sexually transmitted disease by this parasite has a high prevalence worldwide [1]. In the male reproductive tract, this microorganism is found in the urethra and the sub-preputial sac, and the infection can cause lesions in the penis. In the female, this microorganism is found in the vagina, urethra, and paraurethral glands [2-4]. Urethral infection by *T. vaginalis* is present in at least 80% of infected women and over 73% of male partners of women diagnosed with vaginal trichomoniasis[3]. No symptoms are shown in 70-100% of male population and in 35-85% of female population with confirmed *T. vaginalis* infection and *T. vaginalis* can persist in the reproductive tract from 3-12 months. Patients with no symptoms of trichomoniasis are classified as a chronic asymptomatic carrier [2,3,5,6]. In addition, *T. vaginalis* infection has high rates of recurrence and high resistance rates to the metronidazole treatment [6-7]. .

Transmission of *T. vaginalis* infection is exclusively through sexual intercourse, however some vertical transmission cases have been reported at the time of delivery. Symptoms in males with *T. vaginalis* are present only in 15-50% of the cases and they are defined as urethral discharge, urethral irritation and/or dysuria [3,5,6]. Several studies report that patients with *T. vaginalis* infection display deleterious outcomes in reproduction. In men, this has been associated with urethritis, prostatitis, epididymitis, and infertility through inflammatory damage or interference with the sperm function [3,5,6,8-11]. In women, *T. vaginalis* has been linked to vaginosis, vaginitis, endometritis, adnexitis, and can trigger inflammatory responses in the mucosal genital tract, increasing the risk of pelvic inflammatory disease by microhemorrhages [1,10,12-15]. Also, *T. vaginalis* has been associated with up to 30% of acute salpingitis and 16% of postpartum endometritis cases [16]. During pregnancy, *T. vaginalis* infection is associated with a 30% increase of preterm delivery, 30% of the low birth weight infants, and a predisposition to postpartum maternal sepsis [17]. The medical community considers *T. vaginalis* microorganism as a harmless inhabitant of the human reproductive tract [18]. Additionally, the *T. vaginalis* synergism with vaginal microflora and the host responses provide the key to severe reproductive complications. The interaction of vaginal pathogens with epithelium and mucosa of the reproductive tract affects the immunological harmony needed for the success of embryo implantation. Nowadays, *T. vaginalis* is a latent pathogen in the reproductive tract, in this way is responsible of the adverse reproductive health outcomes in humans. Trichomoniasis proves the way for several bacterial intruders of the inflammation processes, thereby increasing the risk of failure in reproductive capacity and increasing the risk by 1.5-3 times of HIV and VPH acquisition [19,20]. *T. vaginalis* evades host immunity by the presence of adhesion proteins, cysteine proteases, and lipophosphoglycan molecules, all of which increase the pathogenicity of this intruder. The parasite adheres to the vaginal and cervical epithelial cells and triggers an immunosuppressive response from monocytes, macrophages, and dendritic cells. Also, *T. vaginalis* carries viruses and other parasites, such as mycoplasma and gardenella, causing chronic mucosal damage and an inflammatory reaction which gives rise to severe consequences in reproductive outcomes [21-24]. Goodman et al. [24] reported the presence of a *Totiviridae* viral family, which is a virus with a doubled stranded RNA that concurrently, is infecting the *T. vaginalis* parasite [24-26]. Infection of *T. vaginalis* by such a virus increases severely the immunological genesis of trichomona virulent factors by changes in its genome organization, protein coding, and replication signals. *Trichomona virus* increases the recurrence of the parasite infection and resistance to the metronidazole treatment [7,24,27-29].

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Received December 12, 2014; **Accepted** January 05, 2015; **Published** January 12, 2015

Citation: Lucena E, Moreno-Ortiz H, Coral L, Lombana O, Moran A, et al. (2014) Unexplained Infertility Caused by a Latent but Serious Intruder: *Trichomonas vaginalis*? JFIV Reprod Med Genet 3: 139. doi:10.4172/2375-4508.1000139

Several reports punctuate the serious and detrimental outcome of trichomoniasis in pregnancy but exists a gap of knowledge in the correlation of T. vaginalis infection with unexplained infertility in humans. In addition, it is well understood today that embryo implantation depends on both the quality of the embryo and the receptivity of endometrium, the latter being dependent on an appropriate immune environment needed to achieve implantation and pregnancy. The presence of pathogens in the reproductive tract could easily contribute to the unexplained reproductive failure in an infertile couple. The *T. vaginalis* microorganism present in semen samples affects the quality of sperm cells, impeding the ability to achieve oocyte fertilization in a natural intercourse in humans. Patients showing unexplained infertility underwent assisted reproductive techniques and benefited from *in vitro* novel procedures to improve oocyte fertilization, embryo development, and pregnancy rate. In this case, the preparation of ejaculated semen sample by using density gradients protocol and ProInsert™ device allowed a complete separation of motile sperm and complete removal of pathogens and polymorphonuclear cells. The semen sample preparations resulted in optimal motile sperm cells used to improve fertilization rates in the IVF procedure. The aim was to achieve clean and capacitated sperm cells for *in vitro* fertilization purposes. As a consequence of this, normal development of embryos was obtained for transfer into the uterus of the patient´s partner. Successful protozoan isolation from semen sample with a novel procedure combining the density gradient protocol with the ProInsert™ device was achieved. Furthermore, improving selection and capacitation of pathogen free motile sperm without cell contaminants for the IVF procedure has not previously been reported in this setting, making this a very interesting case. Additionally, considering *T. vaginalis* as a latent pathogen in the reproductive tract is neccesary the diagnosis and treatment a priori as a protocol to improve implantation achievement in patients with unexplained infertility.

Case Presentation

In March 2014, a 39 year-old Caucasian male came to the clinic with a medical history of unexplained infertility. As a relevant personal history, he had unilateral varicocelectomy in 2012. Seminal analysis in 2013 reported moderated teratozoospermia due to abnormalities of the sperm head and flagellum. The patient and his partner decided to undertake assisted reproduction techniques to achieve pregnancy. From his female partner, oocyte retrieval was made on the 13th day of the controlled ovarian stimulated cycle obtaining two mature oocytes. The same day, semen was collected from the patient through masturbation. The sample was incubated at 37ºC in a hot plate until liquefaction was complete. A sample from the liquefied semen was analyzed directly with microscope for motility and total concentration of spermatozoa. Motile trichomonas were observed when scanning the semen for sample preparation. The presence of *T. vaginalis* was determined by morphology and motility: oval, spherical, motile, and flagellated microorganisms with barbed tails. Additionally, several polymorphonuclear cells were also observed. The sample had a total count of 115.250.000 spermatozoa per ml, 23% progressive, 51% non-progressive, and 26% immotile spermatozoa prior to capacitation process. At the same time, the sperm capacitation protocol by density gradients separation with PureSperm™ 40-80 (Nidacon-Sweden) was initiated. First, a layer of 80% gradient was added, then a layer of 40% gradient, and finally adding a liquefied semen layer via outer chamber of the Proinsert™ (Nidacon-Sweden) device. ProInsert was centrifuged at 300 x g during 20 minutes. The pipette provided with the device was passing slowly into the ProInsert central channel, down to the sperm pellet, avoiding disturbing the pellet or contaminating it with the gradients. The sperm pellet was aspirated and transferred to a new conical tube that contained PureSperm™ Wash (Nidacon-Sweden). A new centrifugation was done at 400 x g for 10 minutes, the supernatant was aspirated and the pellet was resuspended in 1ml of PureSperm™ Wash to be incubated at 37ºC until IVF was done. An aliquot of the capacitated sample was analyzed under microscope and neither microorganisms nor polymorphonuclear cells were identified. The capacitated sperm sample had a total count of 71.125.000 spermatozoa per ml, 49% progressive, 32% non-progressive, and 19% immotile spermatozoa. One hour later, 0.7ul of capacitated semen sample was added to each drop in the IVF dish to achieve 20.000 motile sperm per drop. IVF dishes were prepared the day before with IVF-Plus Media (Vitrolife- Sweden) in microdrops under mineral oil. Plates were equilibrated at 37ºC, 6% CO_2, and 5% O_2 during 18 hours. After that mature oocytes were added one by one to each drop containing capacitated sperm. The IVF dish was incubated for 24 hours at 37ºC, 6% CO_2 and 5% O_2. Dishes are kept in incubation during three consecutive days to allow embryo fertilization and cleavage. After 72h of *in vitro* culture, two embryos were obtained. One embryo was selected to be transferred into the uterine cavity to achieve implantation. The second one embryo was vitrified to future embryo transfer. Fourteen days later B-hCG levels were not detectable.

Outcome and Follow-up

ProInsert and the method of density gradient centrifugation were effective in removing both the *T. vaginalis* protozoan and polymorphonuclear cells from the semen sample. Two quality embryos were obtained after 72h of *in vitro* culture. One embryo was transferred and pregnancy was not achieved. The second embryo was vitrified to future embryo transfer, once the female partner has finished the treatment for trichomoniasis.

Discussion

T. vaginalis is not routinely screened in asymptomatic patients and the infection can persist from 3-12 months in the genital tract. Older patients with asymptomatic trichomoniasis are classified as a long-standing asymptomatic carrier [2,3,5,6]. Patients with *T. vaginalis* infection are asymptomatic in 70-100% of male cases *vs* 35-85% of female cases [2]. Additionally, previous studies have reported that *T. vaginalis* infection has high rates of recurrence due to resistance of the protozoan to metronidazole treatment [6,7] making this microorganism a serious reproductive tract enemy. Higher infection rates are reported in minority populations and disadvantaged communities worldwide [30]. Serious adverse reproductive health outcomes, including pregnancy complications, pelvic inflammatory disease, and an increased risk of HIV acquisition, have been linked to *T. vaginalis* infection.

The *T. vaginalis* protozoan attaches to vaginal epithelial cells through its barbed tail, membrane expression of surface protein p270, secretion of proteases and a cell-detaching factor, leading to an intense host inflammatory response, inducing local cytotoxic effects, genital tract damage, and reproductive effects [17,31]. Recently, trichomonas have been isolated from fallopian tubes, peritoneal fluid, and the pouch Douglas, suggesting that motile trichomonas may be able to invade the whole genital tract [32,33]. There are several studies that conclude that *T. vaginalis* causes urogenital damage to different types of cells and tissues, such as connective and muscular tissues, due to an excessive cytotoxic local effect suggesting high risk of reproductive failure [9,33-39]. Trichomonas bind to the cells inducing membrane retraction, cell blebbing, and apoptosis. These changes of cell architecture can be evidenced under microscopy and characterized by condensed chromatin and intense cytoplasm vacuolization [39].

In this case, the female partner did not report any infectious symptom and, therefore, she was not tested for *T. vaginalis*, but she might have been affected with latent trichomoniasis. She is an example of asymptomatic patient who obtained a negative outcome of implantation failure after ART. More than 20% of women with trichomoniasis have a chronic inflammatory process in the reproductive tract and that may explain the embryo implantation failure after ART procedure. Consequently, the clinical implication of embryo implantation failure depends on both the quality of the embryo and the receptivity of endometrium mainly marked by the correct and exact immune environment needed for a successful pregnancy.

On the other hand, men infected with *T. vaginalis* display abnormal motility of the spermatozoa and high semen agglutination. A relevant tropism from *T. vaginalis* microorganism to the head or flagella of the human spermatozoa has been reported. The adhesion of *T vaginalis* and sperm affects sperm motility followed by phagocytosis, lysis, and digestion of sperm cell [40,41]. In 2008, Benchimol et al. [9] report that, after one hour of interaction between *T. vaginalis* and sperm cells in an *in-vitro* environment, 75% of the sperm cells were immotile or dead. Under scanning electron microscopy, the interaction of *T. vaginalis* and sperm cells results in high agglutination, membrane protrusions, or channels between the sperm plasma membrane and parasite surface as a mechanism of phagocytosis of *T. vaginalis* [9,42]. Keeping this in mind, cyto-adhesion and phagocytic activity of trichomonas to ingest and digest spermatozoa in an *in-vitro* environment suggest a similar behavior in an *in-vivo* environment as a cause for decreasing numbers of motile sperm directly affecting reproductive success. Latent trichomoniasis infection could be the cause of the unexplained infertility in men, since this case showed sperm damage evidenced by severe asthenozoospermia.

This report showed that density gradient separation protocol combined with the ProInsert™ device for selection, isolation, and capacitation of motile sperm should be used as an alternative tool on couples that will undergo ART techniques. Use of optimal motile sperm samples separated with this novel method might be a beneficial procedure in patients with sexually transmitted diseases, such as trichomoniasis, gonorrhea, chlamydia, mycoplasma, and HIV. Couples with unexplained infertility must be widely screened and tested for sexually transmitted pathogens to ensure adequate conditions of the female reproductive tract needed to achieve pregnancy. Also, semen samples become easy to clean and prepare using the discussed protocol, with the aim of improving the fertilization rate and embryo development *in-vitro*. This case study presents a novel management of semen samples contaminated with *T. vaginalis* or other pathogens for couples undergoing ART. Further studies are required to accurately fill the gap of knowledge between trichomoniasis, unexplained infertility, and implantation failure.

Learning Points/Take Home Messages

T. vaginalis pathogenicity, adverse reproductive health outcomes, in-time diagnosis, and treatment may improve the implantation rate in patients with unexplained infertility undergoing ART. Managing semen sample showing *T. vaginalis* by density gradient separation utilizing a ProInsert™ device is a successful protocol for patients undergoing ART.

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