Original Article

To study the effect of microbial contamination in semen and its effect on male infertility

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Abstract

According to the 3rd WHO methods, normal sperm morphology has been considered to be directly related with the fertilization process and pregnancy. The quality of semen such as sperm morphology, count, and motility has been reduced due to abnormalities in male thus, causing infertility. A considerable factor responsible for infertility among male partners may be as a result of microbial contamination. Semen may contain contamination while collecting sample. However, there are some sperm preparation techniques that can be used for the removal of contamination in semen such as; swim up, density gradient, simple wash, discontinuous density gradient. The main objective of this study is to find out the presence of infections and microbial contamination. This study will compare sperm count and sperm motility of patient's semen sample with numbers of debris/ present infectious agent. Microbiological assay of semen sample will be performed. Also, various infectious agents causing infertility will be evaluated and studied. Semen analysis, semen preparation by density gradient, semen culture were carried out to look into the effect of microbial contamination on semen sample of infertile men.

Keywords: Infertility; Microorganisms; spermatogenesis; Polyvinylpyrrolidone; Microbial contamination; Semen culture; Microbiota.

INTRODUCTION

Nowadays, Infertility is becoming major problem among most of the couples. Worldwide most of the infertility cases are caused due to abnormalities in the male partner. Contamination in semen mostly arises due to infectious agents surrounding men. (1) Many studies have shown that presence of bacteria in semen is deteriorating the sperm quality. These bacteria present in the semen may appear through intercourse or by infection in urinary tract.(2)

Over 10 years, the most important infectious agents found in semen are HIV, Hepatitis B and C viruses. Therefore, appropriate caution should be use to handle and dispose it with special care. It also contains microbial contaminations such as bacteria, yeast, fungi, and other toxins.

Many other male factors also responsible for infertility such as descended testes, erectile and ejaculatory dysfunction, endocrine disorders, testicular tumours, etc. The effect of these conditions may be sperm damage, poor motility, and immotile form. Other factors such as hormonal disorders, lifestyle modifications, environmental hazards, improper use of antibodies, new ways in drug intake are the reason for male infertility. Bacteria affects the fertility in a male by several mechanisms such as decreased sperm motility, altered sperm morphology, altered acrosomal reaction, deterioration of spermatogenesis.(3)

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For removal of these contamination from semen sample various sperm preparation techniques are used such as simple wash, swim up, density gradient (4).For ICSI in case of having low sperm count, it is necessary to have high quality of sperm count and for IVF and IUI sperm preparation with good quality of sperm is necessary. Sperm preparation of liquefied semen sample is usually done by a density gradient method through polyvinyl pyrrolidone coated silica particles.(5)

The main clinical aim of a procedure is the expulsion of presence of infectious and microbial contamination. Some of the genitourinary tract infections are mainly asymptomatic which create confusion whether to treat them or not. In this study we arranged this study to determine the contamination and their impact on semen quality in infertile men.(3, 5)

Mainly, the bacteria species which is responsible for urogenital infections causing contamination in semen is E.coli. Though some studies have suggested that bacteria like Neisseria gonorrhoeae and Staphylococcus have adverse effects on some organs like testis, epididymis. Further affecting quality of sperm their rate of motility, count and concentration.(6)

Semen is one of the main source of contamination during ART. In many of the studies, bacteria has been found out the reason behind the contamination in the semen. The common contamination of semen is Chlamydia. One study has shown that we cannot remove Chlamydia from semen by density gradient centrifugation because it can bind and form inclusion bodies in sperm.

In semen, viruses are also found which enhances and alter the sperm quality, implantation and pregnancy.

SEMEN COLLECTION-

- Semen sample should be collected by masturbation and ejaculated into a wide mouth sterile container.
- For the collection of sample, abstinence period should be 3-7 days.
- The sample should be collected in private room near the laboratory so that the exposure of the semen to the temperature fluctuation can be limited.(7)(6)

Semen analysis should be done after liquefaction of sample. Minimum 10-15 minutes take to liquefy the sample.

SPERM PREPARATION TECHNIQUE- (Density Gradient)

- Take 15 ml conical test tube. Prepare density gradient medium by layering 1ml of 80% solution.
- Add 1ml of 40% solution.
- Add 2 ml of semen sample.
- Centrifugate the sample for 10 minutes at 600 g.
- Discard the supernatant leaving the sperm pellet.
- Transfer the pellet into a new tube resuspending in 3 ml of media.
- Centrifuged the tube again for 5-7 minutes at 250 g so that the silica particles found in gradient solutions gets removed.
- Remove all supernatant leaving the pellet and resuspended it 1 ml of media.

(5)

SEMEN CULTURE-

- Blood agar and MacConkey agar plate were used as a semen culture media.
- In this plate semen sample were inoculated with 1 hours of sample collection and aerobically incubated at 37 0 C for 24-48 hours.
- Whereas chocolate agar cultures were incubated at 5% Co2 candle jar.
- The bacterial concentration of greater than 103 CFU/ml for certain pathogens and greater than 104 for occasional pathogens was considered as significant and isolated colonies were identified by colony morphology, gram staining and biochemical tests.(3)(8).

Rationale:

At the time of initial consultation of infertile couple, the diagnostic information that can be obtained by a single semen analysis. For semen analysis, Semen is obtained by masturbation in a sterile wide mouth container with 2-5 Day of abstinence period. Appropriate care should be taken by patient during collection to avoid microbial contamination. There may be several etiological factors including bacteria, fungus, virus which led to deteriorate the quality of semen which having adverse effect on the sperm count and motility. Sperm preparation techniques are used for removal of debris and microorganisms and immotile sperm from motile sperm. The main aim of sperm preparation for ART is to maximize the chance for fertilization and reducing contamination from semen.

Many investigations indicated in IVF that oocytes fertilization was decreased in presence of microorganism in semen and that bacterial contamination reduces the quality of sperm. In January 2012- A study was conducted by Kimball O Pomeroy on Contamination of Human IVF Cultures by Micro-organisms- a review.

In this prospective study, it has been mentioned about the microbes present in 140 semen sample. 63% of contaminations were found with bacteria. The most common microbes found were Staphylococcus epidermidis which was 29%, Streptococci which was 30%, diptheroids which was 14%, b-streptococci which was 12.1%, Streptococcus viridanswhich was 10% and E. Coil which was 6.4%. Anaerobes were also identified with presence of 17 % of Mycoplasma were also observed in semen sample. Contamination in semen sample was found to be reduce by

usage of antibiotics during semen processing. Embryologists suggest that most of the contamination was occur due to poor sterile methods and therefore there was need to develop different methods to decrease the effect of microbial contamination on semen sample. There was need to know the source of microbial contamination. (9-21)

In June 2018, A study was conducted by Yasser Nasralla et all from Shaqra University on Microbiological profiles of semen culture in male infertility. This study was conducted to determine the effect of genitourinary infections on semen parameters of infertile men. They perform semen analysis, bacteriological analysis, and sensitivity analyses. This study includes 200 semen sample from men with primary and secondary infertility. In which 47% of patients were of 26-30 years old, 71% had primary infertility and 29 % had secondary infertility. Overall, 50% had infertility from 1 to 2 years. Among 200 semen cultures, after 24-48 hours 108 samples were observed with positive sample for bacterial growth.(21)

On 6 January 2016, A study was conducted on Bacteriospermia and Its Impact on Basic Semen Parameters among Infertile Men. The main aim of this study to examine the semen cultures and its impact on semen parameters among infertile men. The samples were collected from 85 male partners attending to infertility clinic during the period of June 2014 to June 2015. According to WHO guidelines Samples were collected and analysed.Also samples were cultured in blood agar and MacConkey agar in microbiological laboratory and incubated at 370C. The microbial organisms were isolated in a concentrate of >103 CFU/ml were considered as significant. Sensitivity testing by Kirby-Bauer disc diffusion method was also conducted to identified microbes. Among total 85 sample, 47 sample had normal sperm count, 37 sample had oligozoospermia and only 1 sample had azoospermia. The most common abnormality was teratozoospermia which was 81.17% followed by asthenozoospermia which was 28.23 %. The spreading of bacteriospermia was nearly 35.3%. The most common micro-organism Enterococcus faecala(30%) was the most common isolated followed by coagulase negative Staphylococcus(23.33%), Staphylococcus aureus (20%) and E.coil(10%) Klebsiella pneumoniae (6.66%) Proteus sp (6.66%) and Citrobacter sp (3.33%)(3)

S. R.Steyaert et all conducted a study on Infections In IVF. In this study, identification of infections in male and female partner was observed. Also the infection with HBV, HCV, HIV, etc was identified. In IVF there is need to maintain sterility to prevent microbial contamination. The main aim of this study is to feature the issue of infections. The purpose is to maintain sterile nature in laboratory. Patient whose sample was collected to cryopreserved first of all there was need to screening the presence of infectants such as HBV, HCV, HIV and other. Different containers must be used for infected and non-infected sample. In most of the cases infections occurs from the patients. Therefore special care should be taken to decrease the risk of contaminations. (10)

Aim: To study the effect of microbial contamination in semen and its effect on male infertility.

Objectives:

- To compare sperm count and sperm motility of patient's semen sample with numbers of debris/ present infectious agent.
- To perform microbiological assay of semen sample.
- To correlate infection present in semen sample with sperm count and motility.
- To evaluate and study various infectious agents causing infertility.

Hypothesis:

In this study, we hypothesized that the microbial composition may have influence on the quality of sperm. Presence of micro-organisms deteriorated the quality of semen and thus it is responsible for the infertility in men. The most frequently used density gradient techniques for sperm preparation may remove micro-organism."

Methods:

Study design:

Observational Study

Methodology:

This study will be done in Wardha Test Tube Taby Centre AVBRH (SAWANGI) WARDHA. Regarding data on the treatment history as well as the indications will be recorded. Counselling to the patient for research work will be done. Patients gave written as well as informed consent before recruit. There is routine protocol in our set up as follows: History of patient will be taken.

Screening of the patients will be done which are suitable for research work by considering the parameters such as previous treatment, indications, BMI, age etc. Appropriate verbal as well as written consent for research work will be taken from the patients that are enrolled for research work.

Prior to Semen analysis, according to standard, patient have to go through by clinical and physical examination conducted by Urologist. Patient with transformed hormonal level, late or current ongoing any treatment, varicocele, cryptorchidism were excluded from this study.

Semen sample collection:

Abstinence period of 2-7 days, must noticed for particular patient who was already counselled to wash the hands properly to avoid bacterial contamination. Sample should be collected by masturbation.

Semen processing:

According to WHO standard, semen sample were examine after liquefying for 15-20 minutes and evaluate pH, volume, count, motility, at 370 C.

Culture Procedure:

Priory, Semen sample were allowed to liquefy completely and then inoculated as undiluted 1:10 diluted and 1:100 undiluted sample as standard loop on agar plates. Semen sample were culture: MacConkey, Chocolate and Blood agar. Now sample are ready to incubate at 370 C in a microareophilic (5% CO2) and aerobic condition.

Setting:

Location: Wardha Test Tube Baby Centre, AVBRH

Relevant dates, including periods of recruitment: August 2020 – August 2022

Participants:

Inclusion criteria

• Male causing infertility factors and healthy male visiting WARDHA TEST TUBE BABY CENTRE, AVBRH.

Exclusion criteria

• Patient having infections like HIV, HBV, HCV, etc

• Patient having asymptomatic disease, endocrine disorders, testicular disorders will be excluded from this study.

• Patient not ready to give consent for research.

Study size:

Sample size:

50 infertile couple

$$N = \frac{\chi^2 * N * p(1-p)}{C^2(N-1)+\chi^2 p(1-p)}$$

Total population = N=100during 15 months

χ2=Chi-square value for 1 degrees at some desired probability level. This is 3.84 at 5% level of significance

P=50% proportion

Q= 100 – p

=50

C= Confidence interval of the one choice (95% CI)

=0.05

$$N = \frac{3.84*120*0.5*0.5}{(0.05)2 *24+3.84*(0.5*0.5)}$$

=50

Expected Outcomes:

50 men will be included. Expected outcome will be in the form of infertility present in semen of male partner and its effects on his fertility. According to WHO, normal range is 20-120*106/ml for fertile men (normozoospermia). The count and motility of sperm may be decrease by the presence of microbial contamination.

Discussion:

Semen consists of seminal fluid and spermatozoa. Various separation techniques such as density gradient, mini gradient and discontinuous density gradient have been introduced. The advantages of these procedures are very rapid, by requiring 20 minutes of centrifugation which is performed under sterile condition. It is considered as the abnormal sperm and microorganisms/ debris are eliminated and free from contamination is possible. Density gradient with combination of swim up method has been decided to reducing the viral infections from sample and minimize the risk of infection and improve the rate of fertilization. There are some studies on the uriogenital tract and bacterial contamination among infertile men but the consequence of these agents on the quality of semen is still under discussion.

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