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Article

Effect of the Presence of Pus Cells in Seminal Fluid Samples on Sperm Motility and Morphology in a Group of Males Attended Subfertility Clinics in Galle District, Sri Lanka

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Abstract

Background: The quality of seminal fluid is one of the main determinants of male fertility. The quality of seminal fluid is determined by several parameters such as volume, sperm concentration, percentage of normal morphology, motility, viability, progressivity, etc. The objective of the study was to assess the effects of the presence of pus cells in seminal fluid samples on normal morphology and motility. **Methods:** A cross-sectional study was conducted among a sample of 107 men who attended subfertility clinics in Galle district, Sri Lanka. Consecutive (convenience) sampling method was used. After obtaining the informed consent, semen samples were collected. Pus cell count, sperm motility and normal morphology were evaluated based on the WHO guidelines (2010). Ethical approval was obtained from the Ethics Review Committee, Faculty of Allied Health Sciences, University of Ruhuna. **Results:** The majority of samples had pus cell count less than 5 per high power field (n=75, 70.1%). Only 32 (29.9%) had a pus cell count of 5 or above per high power field (/HPF). 32 samples (42.7%) out of 75 samples with pus cell count <5 (/HPF) had a percentage normal morphology of more than 30%, while only 3 samples (9.4%) with pus cell count ≥5 (/HPF) had a percentage normal morphology of more than 30%. The mean and standard deviation (SD) of normal morphology were 26.88% and 8.69% for samples with pus cell count <5 (/HPF) while 21.39% and 7.00% for samples with pus cell count ≥5 (/HPF). Z value was 3.124 and the level of significance was 0.05. Therefore, the finding was highly significant (p= 0.002). 32 samples (42.7%) out of 75 samples with pus cells <5 (/HPF) had normal motility (≥50%). However, only 6 (19%) of 32 samples with pus cells ≥5 (/HPF) had normal motility (≥50%). The mean and standard deviation (SD) of normal motility were 46.32% and 11.30% for samples with pus cell count <5 (/HPF) while the mean and standard deviation (SD) of normal motility were 41.25% and 10.37% for samples with pus cell count ≥5 (/HPF). Z value was 3.124 and the level of significance was 0.05. Therefore the finding was highly significant (p = 0.032). **Conclusions:** The obtained results demonstrated that the pus cell count can be a factor inversely associated with sperm morphology and motility.

Keywords: Morphology; Motility; Pus cells; Sperm; Seminal fluid

Introduction

Subfertility is defined as the failure to conceive after one year of regular intercourse, without the use of contraceptives. The percentage of subfertile couples is (10-15) (1). The motility and

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morphology of sperms play a significant role in their ability to pass through both the uterus and the fallopian tube, eventually fertilizing an ovulated egg. During intercourse, sperms are deposited in the vagina. It is necessary for the sperm to swim up through the cervix and then travel through the uterus. They must swim from one end of the uterus to the other to ultimately reach the egg that is waiting in the fallopian tube. Although the total distance that the sperm needs to travel is only 10 cm, it is equivalent to running a marathon for the relatively small size of a human sperm. However, the majority of sperm do not reach their destination. During this process, some sperms will lack energy or swim in the wrong direction, while others may swim in circles or travel to the wrong fallopian tube. Less than 1% of the total ejaculated sperms eventually reach the egg. Motility refers to the percentage of moving sperm in the raw ejaculate. Sperm samples are considered normal if they have a motility greater than 40%. Having an adequate percentage of motile sperm with good progression in the ejaculate will help ensure that sperms are capable of arriving at the site of fertilization. Fertility is not only based on the absolute number of sperms but also on the functional capability and one of the common causes of infertility in men with normal sperm parameters is abnormal sperm DNA (2).

Semen analysis is the first step to accurately diagnose male subfertility (3). Sperm count, motility and the percentage of sperm with normal morphology are the main criteria of semen quality (4). Semen quality can vary with various factors like, environmental conditions, smoking, alcohol consumption, infections, prevalence of testicular cancers, genital malformation, presenting of vericocele, radiations, diet, pesticides and lifestyle (5).

A common cause of subfertility is seminal infection (6). High concentrations of white blood cells (WBCs) in semen are an indicator of infection; this condition, marked by pus cells in the semen, is called pyospermia. Although a small number of white blood cells is a normal constituent of sperm, patients are only considered non-pyospermic as long as the concentration of white blood cells remains below 5 per high power field (HPF). However, the white blood cells can have an effect on sperm function. The association of pyospermia with male subfertility has been highlighted by many researchers. Ignorance of the pyospermic factor would have made the patients "pyospermic" as normozoospermic, and the other azoo-, oligo-, astheno- or oligoasthénozoospermic. In such a case, the pyospermic males would not be treated for pyospermia and therefore their state of subfertility would persist. Therefore, it is suggested that the presence of white blood cells in semen should not be ignored by the treating physician and should be considered as a limiting factor in male fertility. It is useful to clinicians, subfertility specialists and andrologists in the treatment and management of patients with pyospermia. Infections can affect male fertility in different ways. The possible consequences are impaired spermatogenesis, induction of an autoimmune mechanism, spematodysfunction and inflammatory obstruction of the ejaculatory duct. Reduced sperm motility was found in semen samples containing high concentrations of bacteria. Likewise, 43% of pyospermic patients showed spontaneous downward variation in the absence of treatment (7, 8). This study aims to evaluate the association between the presence of pus cells and abnormality in the morphology and motility of sperms.

Methods

Study Design

This study was a cross-sectional analysis. One hundred and seven males who attended to subfertility clinics in Galle district, Sri Lanka was selected as the study sample according to consecutive (convenience) sampling method.

Ethical Consideration

Ethical Approval was obtained from the Ethics Review Committee, Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka (Ref no: 01.07.2020:3.4). Institutional permission was

obtained from the head of the institution to carry out the study. Informed written consent was obtained from all the participants. Strict confidentiality of all the data was maintained.

Study Setting

The andrology laboratory of Ruhuna Hospital, Galle, was selected as the study setting since couples from different socio-cultural and economic backgrounds from southern Sri Lanka attend the subfertility clinic in the hospital.

Inclusion Criteria

- The males whose wives could not be able to conceive for at least one year
- The male who gave their consent to participate in the study
- Males with 3 days of abstinence prior to sample collection

Exclusion Criteria

- Males without 3 days of sexual abstinence prior to sample collection
- Incomplete samples (especially if, the first sperm rich fraction was missed. If the sample was incomplete, a second sample was collected, again after 3 days of abstinence)
- The males who were not given their consent

Sample Collection

The consent form was provided in both Sinhala & English, and written consent was obtained prior to collecting samples from participants. Instructions were given to the participants for pre-preparation by contacting them. Samples were collected at the sample collection center on a pre-determined day (from November 2020 to April 2021). The samples were transported from the collection center to the andrology laboratory at ambient temperature (20^0 C- 37^0 C).

Analysis of Semen Samples

The initial analysis of semen parameters, including sperm motility, sperms with normal morphology, and pus cell count, was performed according to WHO standard guidelines for human semen analysis (9). Each sample was examined two times by two examiners (Researcher and andrology technical officer) and mean values were calculated and those were used for statistical analysis of the study. Motility was analyzed as percentages of progressive motility (RP), non-progressive motility (NP), and immotility (IM). Then the total of progressive motility (RP) and non-progressive motility (NP) was taken as the motility of sperms in the specimen.

Statistical analysis

Values of each semen parameter were taken from all study subjects. Variables were analyzed by grouping the samples according to the number of pus cells (pus cell count <5 & ≥5 (/HPF). Then, the mean, median, standard deviation (SD) & variance of semen samples were calculated. Statistical Package for Social Science (SPSS 20) was used for the data analysis. The independent sample t-test was used for the statistical analysis and the level of significance was determined. Values less than 0.05 were considered significant.

Results

The total sample size was 107 and the majority of samples had a pus cell count of less than 5 (/HPF) (n=75, 70.1%). Only 32 (29.9%) had pus cell count 5 or above (/HPF); 32 (42.7%) had a pus cell count <5 (/HPF), and a percentage normal morphology of more than 30%. While only 3 samples (9.4%) with pus cell count ≥5 (/HPF) had a percentage normal morphology of more than 30%. The mean and standard deviation (SD) of normal morphology were 26.88% and 8.69% respectively.

Samples with pus cell count <5 (/HPF) had a mean of 21.39 (SD±7.00) % for samples with pus cell count ≥5 (/HPF) (Table 1).

Based on the results of the independent sample t-test for the morphology of sperms, a statistically significant difference (p = 0.002) was detected between two groups (samples with pus cell count <5 (/HPF) and pus cell count ≥5 (/HPF) (Table 2). Therefore, pus cell count was inversely associated with the normal morphology of sperms.

However, 32 samples (42.7%) out of 75 samples with pus cells <5 had normal motility (≥50%) while only 6 (19%) of 32 samples with pus cells ≥5 (/HPF) had normal motility (≥50%). Samples with pus cell count <5 (/HPF) had a mean and standard deviation (SD) of normal motility of 46.32% and 11.30%, respectively. Samples with pus cell count ≥5 (/HPF) had a mean and standard deviation (SD) of 41.25% and 10.37% (Table 1). According to the independent sample t-test for normal motility of sperms, a statistically significant (p = 0.032) difference between two groups was detected (Table 2). Therefore, normal motility of sperms was inversely associated with the pus cell count.

Table 1. Normal morphology and motility of sperms with pus cell count

Pus cell count (/HPF)	Number of semen samples	Mean ± SD for sperms with normal morphology (%)	Mean ± SD for sperms with normal motility (%)
<5	75	26.88 ± 8.69	46.32 ± 11.30
≥ 5	32	21.39 ± 7.00	41.25 ± 10.37

Table 2. Results of independent sample t-test for normal morphology and normal motility of sperms

	t	p	Mean difference	Std error of the difference
Normal morphology	3.142	0.002	5.49	1.76
Normal motility	2.176	0.032	5.07	2.33

Discussion

Invasion of microorganisms into the male genital tract has been frequently shown to be associated with impaired sperm function and persistent subfertility (10,11). Microorganisms might affect the male reproductive function in different ways. Pathological bacterial strains present in semen may act directly on sperm cells causing agglutination of motile sperm (12), reducing motility and ability for the acrosome reaction and also causing alterations in cell morphology (13). Moreover, the inflammatory process in the genital tract may lead to deterioration of spermatogenesis and obstruction of the seminal tract, worsening characteristics of semen and sperm density (14).

Pyospermia is one of the most important causes of male subfertility, but the distribution, origin and role of pus cells in semen are still controversial. Researchers have reported the negative effect of pyospermia on sperm parameters. Microorganisms trigger a local inflammatory reaction, activating leukocytes and inflammatory mediators such as cytokines and reactive-oxygen species (15, 16, 17, 18, 19, 20). The presence of a significant number of leukocytes, which signifies the presence of infection in semen, correlates with altered semen parameters and decreased fertility. Leukocytes represent the main source of ROS both in seminal plasma and in sperm suspensions and have a negative influence on sperm function and fertilization rates (20).

In this study, a high number of pus cells has been observed in the majority of subfertile patients. The pus cell count showed an inverse relationship with the normal morphology and motility of sperms. High counts of pus cells were seen in the study sample where morphology and motility were compromised. Similarly, reduced pus cell counts were observed in the study sample where the sperm had the fewest morphological defects in the head and neck and the motility.

Aspects of factors affecting male subfertility must be addressed specifically in order to effectively treat the patient. Numerous studies have shown that pyospermia reduces male fertility, and unless the condition of pyospermia is corrected, the fertility of these patients will remain affected (6, 7).

Conclusions

Significantly elevated pus cell counts have been observed in the majority of subfertile males, indicating that infection could be a major cause of male subfertility. The findings of this study revealed that pyospermia is associated with altered normal morphology and reduced motility of sperms. If not properly treated, the underlying cause, subfertility will persist in these patients. Therefore, it is suggested that the presence of pus cells in semen should not be ignored by the attending physician and should be considered as a limiting factor in male fertility.

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