

# **Non infective factors associated with leukocytospermia**

**Submitted as part of fulfilment of the MMED Degree in Obstetrics  
and Gynaecology**

**Researcher: DR RJ KHALEMA**

**Supervisors: DR J du P STRYDOM**

**Clinical technologists:**

**RINEE VAN WYK (M.Tech Reproductive Biology, Central University  
of Technology, 2003)**

**MICHELLE SIEBERT (B.Tech clinical technology, Central University  
of Technology, 2003)**

## **ABSTRACT**

1. *OBJECTIVE*

To investigate non-infective factors possibly associated with leukocytospermia

2. *DESIGN*

Cross sectional study

3. *SETTING*

Unit for Human Reproduction, Universitas Tertiary Hospital in Bloemfontein

4. *PATIENTS*

A total of seventy three, (73) patients were included in the study

5. *OUTCOME MEASURES*

Non infective factors possibly associated with leukocytospermia such as smoking, alcohol use, recreational drug use and HIV infection

6. *RESULTS*

A total of 73 patients were included in the study and leukocytospermia was found in 36% (N=27/73).

In the study the investigated variables were found not to be statistically significantly associated with leukocytospermia (P values>0.05).

7. *CONCLUSION*

From our study, none of the variables were significantly associated with the presence of leukocytospermia

**KEYWORD**

Leukocytospermia, male infertility, white cells, smoking

## 1. INTRODUCTION

Infertility is defined by the World Health Organization as the inability of a sexually active couple to achieve pregnancy in one year or more of adequate sexual exposure.<sup>1</sup>

Infertility can be classified as primary or secondary. Primary infertility is when a couple never conceived before while secondary infertility is the inability to fall pregnant after at least one previous pregnancy or conception. Infertility can be a result of either female or male factors.<sup>1</sup>

Male factor infertility may be due to anatomical abnormalities (congenital or acquired), abnormal male endocrine function or testicular inability to produce sufficient quantity and quality of sperm. To attain a normal pregnancy, a normal sperm has to fertilize a normal metaphase II oocyte and implantation has to occur in a suitable environment primed for sustaining a pregnancy. Spermatogenesis, the process of development of a sperm from a stem cell, takes about 74 days and any insult that occurs during this period can affect semen characteristics.<sup>2</sup>

The sperm cell consists of a head, mid-piece and a tail. The nucleus is situated in the post acrosome area in the head of the sperm. The sperm acrosome is a sac-like structure surrounded by inner and outer acrosomal membranes. Immediately after sperm (receptor)–zona (ligand) binding, the outer acrosomal membrane fuses with the overlying plasma membrane, releasing the acrosomal contents. The mid-piece has a central core with mitochondria for energy production and the tail propels the sperm. These characteristics are vulnerable to changing environmental factors including infections, trauma or surgical intervention to the genitourinary system. All these factors are possibly associated with elevated white cell counts in sperm, also known as leukocytospermia.<sup>2</sup>

Leukocytospermia is defined by the World Health Organization (WHO) as white cell count in sperm of equal or more than a million cells per millilitre of semen.<sup>13</sup> Of these, polymorphonuclear leucocytes account for 50 to 60% while macrophages account for 20 to 30%. At normal levels, leukocytospermia appears to be physiological and is associated with increased sperm fertilization capabilities and pregnancy outcome.<sup>3</sup>

Seminal leukocytes at supra-physiological levels may contribute to male subfertility by damaging sperm through production of reactive oxygen species (ROS) and inducing apoptosis. ROS damage the sperm membrane, reduce sperm motility and decreases the fertilization capacity by compromising sperm DNA.<sup>4, 5</sup>

The correlation between leukocytospermia and infection of the male genitourinary tract seems to be a weak one. Although leukocytospermia is commonly found in patients with genital tract infections, only 20–30 % of patients with leukocytospermia is associated with either bacteriospermia (evidence of bacteria in semen) or clinical evidence of infection<sup>5</sup>. Furthermore, most of the organisms cultured from semen seem to be contaminants from the urethral meatus, skin or gastro-intestinal tract and to confirm the true source of these remains a challenge.<sup>3</sup> This is furthermore supported

by the fact that 40% of patients with increased levels of leucocytes reveal normal levels in semen when re-tested after 3 months, even in the absence of any treatment.<sup>3</sup>

Smoking has been proven to be associated with a decrease in sperm count, motility and morphology as well as an increase in sperm DNA damage, however, evidence linking cigarette smoking to leukocytospermia remains limited.<sup>6</sup>

Male genital trauma or previous surgery cause a possible breach in the blood testis barrier and may therefore be associated with increased white cells.

The objective of the study is to determine if there are non-infective factors associated with leukocytospermia.

## **2. MATERIALS AND METHODS**

### *2.1 STUDY DESIGN:*

Cross-sectional study

### *2.2 PARTICIPANTS*

All males who consented to participate in the study, in the age group 20 to 50 years, who were able to provide a semen sample were included in the study.

### *2.3 EXCLUSION CRITERIA*

All males that did not give consent to participate in the study or were unable to provide a semen sample were excluded from the study.

### *2.4 PROCEDURE:*

Information sheets written in English, Sesotho and Afrikaans were provided and consent forms were signed either physically or telephonically. Questionnaires were completed by the target population under supervision of the primary researcher and the personnel in the Unit for Human Reproduction.

Questionnaires were available in Sotho, English and Afrikaans

The semen analysis was performed by the resident clinical technologists working in the Unit according to the local protocol. Quantification of leucocytes in the semen was determined according to the protocol included in the WHO 2010 handbook.<sup>13</sup>

Semen samples were collected in specimen bottles via masturbation and transferred to the laboratory where analysis were performed within 20 to 60 minutes of collection. No semen samples were stored in the laboratory after analysis was completed.

*2.5 ETHICAL APPROVAL:* The study was approved by the UFS ethics committee, application number ECUFS NR 104/2014.

## 2.6 STATISTICAL ANALYSIS

Data was analysed using a descriptive statistics such as the mean, median as well as frequencies and percentages. The variation in data was described using quantiles and the range. Association of the other variables with leucokytospermia was established using a Chi-square test. Logistic regression was used to assess the association with leukocytospermia with other variables adjusting for all other variables (demographic variables).

## 3. RESULTS

Table 1 presents the demographic information of the participants. The age, weight, height and BMI were analysed. The average age of the participants was  $36.7 \pm 6.1$ , which accounted for 37% of the participants. The lowest age being 23 years and the oldest being 50 years old. The average BMI of the participants was  $26.5 \pm 5.6$  which accounted for 26.5% of the participants in the study.

Table 1: Demographic information N=73

Variable	Min	Mean $\pm$ SD	Max	Low quantile	Median	Upper quantile
Age	23	$36.7 \pm 6.1$	50	32	37	40
Weight	50	$77.7 \pm 18.2$	130	65	75	87
Height	1.6	$1.7 \pm 0.1$	1.8	1.7	1.7	1.8
BMI	16	$26.5 \pm 5.6$	43	22	26.5	30

Table 2 shows the distribution of medical and sexual patterns of the participants. Of the total participants (N=73), 38 (52.1%) had children, 22(31.0%) had a positive history of sexually transmitted infection, 18 (24.7%) had medical conditions including hypertension and diabetes, 32(43.8%) had had operations done on their genitalia. 13 (18.8%) of the study population were HIV positive, and only 2 (2.8%) had a positive RPR, while 5 (6.9%) reported to have used lubricants during sexual intercourse in the past 3 months.

Table 2: The distribution of Medical and Sexual patterns

Questions	Category	Number	Percent (%)
Have you ever had STIs (N=71)	Yes	22	31.0
	No	49	69.0
Do you have any operations to genitalia (N=71)	Yes	32	43.8
	No	41	56.2
Do you have any medical conditions (N=73)	Yes	18	24.7
	No	55	75.3
HIV status (N=69)	Positive	13	18.8
	Negative	56	81.2
Current RPR status(N=72)	Positive	2	2.8
	Negative	70	97.2
Do you use lubricants during sexual intercourse (N=73)	Yes	5	6.9
	No	68	93.1
Do you have children(N=73)	Yes	38	52.1
	No	35	47.9

Table 3 shows the distribution of the social habits, 23/72 (31.9%) were still smoking, 13(18.1%) had previously smoked while 36 (50%) had never smoked before. Fifty two, 52/73 (71.2%) were currently drinking alcohol, 6(8.2%) had previously used it and 15 (20.6%) had never used alcohol. Seven, 7/73 (9.6%) of the participants were using recreational drugs, 10 (13.7%) had previously used them while 56 (76.7%) had never been on drugs before.

Table 3: The distribution of social habits of the participants

Question	Category	Number	Percent (%)
Do you smoke cigarettes? (N=72)	Currently	23	31.9
	Previously	13	18.1
	Never	36	50.0
Do you drink alcohol? (N=73)	Currently	52	71.2
	Previously	6	8.2
	Never	15	20.6
Do you use recreational drugs? (N=73)	Currently	7	9.6
	Previously	10	13.7
	Never	56	76.7

Table 4 presents the findings of any association between the variables and leukocytospermia. None of the measured variables were found to be significantly (all P values >0.05) associated with leukocytospermia.

Table 4: Association between variables and leukocytospermia

Questions	Category	No Leukocytospermia		With Leukocytospermia		P-value
		N	%	N	%	
Ever had STI (N=71)	Yes	15	33.3	7	26.9	0.574
	No	30	66.8	19	73.1	
Having medical condition (N=73)	Yes	14	29.8	4	15.4	0.172
	No	33	70.2	22	84.6	
Having operations to genitalia(N=73)	Yes	23	48.9	9	34.6	0.238
	No	24	51.1	17	65.4	
HIV status (N=69)	Positive	9	20.0	4	16.7	0.736
	Negative	36	80.0	20	83.3	
Current RPR status(N=72)	Positive	1	2.17	1	3.85	0.678
	Negative	45	97.83	25	96.15	
Do you smoke?	Currently	15	32.61	8	30.77	0.976
	Previously	8	17.39	5	19.23	
	Never	23	50.00	13	50.00	
Do you drink alcohol?	Currently	36	76.60	16	61.54	0.273
	Previously	4	8.51	2	7.69	
	Never	7	14.89	8	30.77	
Do you use recreational drugs?	Currently	3	6.38	4	15.38	0.224
	Previously	5	10.64	5	19.23	
	Never	39	82.98	17	65.38	
Do you use lubricants during sexual intercourse? (N=73)	Yes	4	8.51	1	3.85	0.450
	No	43	91.49	25	96.15	
Do you have children?(N=73)	Yes	26	55.3	12	46.1	0.453
	No	21	44.7	14	53.9	

Tables 5 and 6 are summaries of seminal white cell count in age groups above and below the median age group of 37 years. The groups for seminal white cell counts were divided into 3 groups: a control group (WCC < 1 X 10<sup>6</sup>/ml of semen) and 2 study groups; one group with WCC of 1-5 X 10<sup>6</sup>/ml of semen and the other group with WCC > 5 X 10<sup>6</sup>/ml of semen indicative of an acute leukocytospermia.

The age group below 37 years had a larger total number of participants with leukocytospermia compared to the group above 37 years (n=18 vs n=11). The average sperm count in the group with WCC 1-5 X 10<sup>6</sup>/ml was higher in the age group below 37 years (85 vs 74.5 X 10<sup>6</sup>/ml) while the group with a WCC > 5 X 10<sup>6</sup>/ml was higher in the >37 years group (n=50.6 vs 32 X 10<sup>6</sup>/ml). There were similar numbers of participants with a positive history of sexually transmitted infections in the leukocytospermia groups for the two age groups (n=3). The age group below 37 years had a higher HIV infection in total (n=9 vs 5) and in the leukocytospermia groups (n=5 vs 2) compared with the age group above 37 years. There were more participants who had genital surgery, generally (n=19 vs 11) and in the leukocytospermia group (n=6 vs 4); more smokers in total (n=13 vs 9) and with leukocytospermia (n=5 vs 2), more alcohol

users in total (n=30 vs 16) and with leukocytospermia (n=11 vs 3) and more participants using recreational drugs with n=4 being in the leukocyte group vs 0).

*Table 5: Seminal white cell count in age group below median age (37 years)*

	<b>GROUP 1</b> WCC <1 X 10 <sup>6</sup> /ml n=22	<b>GROUP 2</b> WCC 1-5 X 10 <sup>6</sup> /ml n=13	<b>GROUP 3</b> WCC >5 X 10 <sup>6</sup> /ml n=5
Average sperm count	44.6	85.0	32.0
BMI	26.8	25.0	29.4
<b>Measurement</b>	<b>Number of cases</b>		
STI	5	2	1
HIV	4	3	2
Genital surgery	13	5	1
Cigarette smoking	8	2	3
Alcohol use	19	8	3
Recreational drugs	0	3	1

*Table 6: Seminal white cell count in age group above median (37 years)*

	<b>GROUP 1</b> WCC<1 X 10 <sup>6</sup> /ml n=20	<b>GROUP 2</b> WCC 1-5 X 10 <sup>6</sup> /ml n=9	<b>GROUP 3</b> WCC >5 X 10 <sup>6</sup> /ml n=2
Average sperm count	41.5	74.5	50.6
BMI	27.8	27.9	27.5
<b>Measurement</b>	<b>Number of cases</b>		
STI	8	2	1
HIV	3	2	0
Genital surgery	7	4	0
Cigarette smoking	7	1	1
Alcohol use	13	1	2
Recreational drugs	2	0	0



Table 7: Logistic regression of the variables in association to developing leukocytospermia. No variable was found to be statistically significant in association with leukocytospermia.

Table 7: Logistic regression of the variables in association to developing leukocytospermia

Variable	OR	95% CI
Age	0.98	0.85 -1.12
Weight	0.99	0.86 -1.14
BMI	1.08	0.69 -1.72
STIs	0.74	0.25 -2.13
Operation to genitalia	0.55	0.21-1.49
HIV positive status	0.80	0.22-2.93
RPR status	0.56	0.033 -9.27
Smoking	0.94	0.32 -2.82
Alcohol use	0.39	0.12 -1.26
Use of recreational drugs	3.06	0.62 -15.1
Chronic medical conditions	0.43	0.13 -1.47

#### 4. DISCUSSIONS

Out of the study population of 73, 27 participants (37%) had leukocytospermia. Of the parameters measured, none showed any statistical significance to the presence of leukocytospermia.

Genital infection is one of the proposed causes for leukocytospermia although causality has not been proven in a randomised clinical trial.

So far there is lack of data on studies that look at direct factors leading to leukocytospermia. Pasqualotto *et al*, (2008) did a prospective study on 10 fertile donors and 112 infertile patients and evaluated the effect of cigarette smoking on antioxidant levels and the presence of leukocytospermia in infertile men.<sup>5</sup> They concluded that smoking may impair sperm motility and decrease antioxidant activity in seminal plasma.

Surgical procedures are known to cause an inflammatory response or breach in the blood-testis barrier and may also contribute to leukocytospermia, but the duration of the effect nor the data is very limited.

This study aimed to evaluate an association of leukocytospermia to reproductive history; history of STIs, chronic medical illnesses such as hypertension, diabetes, HIV and epilepsy; previous genital surgery, history of cigarette smoking, alcohol use and use of recreational drugs as well as lubricants during sexual intercourse in the past 3 months, and the RPR status.

The age group below 37 years had a higher number of variables that were investigated compared to the age group above 37, clinically putting them at a higher risk of developing leukocytospermia although the numbers were not statistically significant.

## 5. **CONCLUSION**

This study was motivated by the fact that many couples seen at the Unit for Human Reproduction present with leukocytospermia. Even though leukocytospermia has been researched extensively, very little is known about the actual causality and association.

The current study indicates that there is no significant association of non-infective variables with leukocytospermia.

## 6. **LIMITATIONS**

The initial sample size was projected at 250 based on the previous year's statistics at the andrology laboratory, however the sample ended up being small because some of the patients seen in the andrology laboratory for semen analysis refused to participate in the study.

Some participants who were interviewed were unable to provide a semen sample for analysis.

Researchers were unable to include ROS levels and its effect on sperm DNA due to unavailability of reagents.

## 7. REFERENCES

1. World Health Organization.(2009) *The International Committee Monitoring Assisted Reproductive Technologies (ICMART) revised glossary*
2. Hoffman BL,Schorge JO, Schaffer JI, Halvorson LM, Brandshaw KD, Cunningham FG(2008). *Williams Gynaecology*. 2nd ed. Dalas: McGraw Hill Medical. 428
3. Cavagna M, Oliveira JBA, Petersen CG, Mauri AL, Silva LFI, Massaro FC et al.(2012). The influence of leukocytospermia on outcomes of assisted reproductive technology. *Reprod Biol Endocrinol*. 10(1),44
4. Lackner JE, Mark I, Sator K, Huber J, Sator M. (2008). Effect of leukocytospermia on fertilization and pregnancy rates of artificial reproductive technologies. *Fertility and Sterility*. 90(3), 869-871.
5. Pasqualotto, FF, Umezu FM, Salvador M, Borges E, Sobreiro BP, Pasqualotto EB. (2008) Effect of cigarette smoking on antioxidant levels and presence of leukocytospermia in infertile men: a prospective study. *Fertility and Sterility*. 90(2), 278-283
6. Domes T, Lo KC, Grober ED, Mullen JBM, Mazzulli T, Jarvi K. (2012). The incidence and effect of bacteriospermia and elevated seminal leukocytes on semen parameters. *Fertility and Sterility*. 97(5). 1050-1055
7. Lackner JE, Herwig R, Schmidbauer J, Schatzl G, Kratzik C, Marberger M.(2006). Correlation of leukocytospermia with clinical infection and the positive effect of anti-inflammatory treatment on semen quality. *Fertility and Sterility*. 86(3), 601-605
8. Trum JW, Mol BWJ, Pannekoek Y, Spandjaard L, Wertheim P, Bleker OP et al. (1998). Value of detecting leukocytospermia in the diagnosis of genital tract infection in subfertile men. *Fertility and Sterility*. 70(2), 315-319
9. Zini A and Sigman M. (2009). Are tests of sperm DNA damage clinically useful? Pros and cons. *Journal of Andrology* 30(3),219-229
10. Said TM, Agarwal A, Sharma RK, Thomas AJ, Sikka SC. (2005). Impact of sperm morphology of DNA damage caused by oxidative stress induced by  $\beta$ -nicotinamide adenine dinucleotide phosphate. *Fertility and Sterility*. 83(1),95-103
11. Said TM, Aziz N, Sharma RK, Lewis-Jones I, Thomas AJ, Agarwal A. (2005). Novel association between sperm deformity index and oxidative stress-induced DNA damage in infertile male patients. *Asian Journal of Andrology*.7(2), 121-126

12. Cohen-Bacrie P, Belloc S, Menezo YJ, Clement O, Hamidi J, Benkhalifa M.(2009). Correlation between DNA damage and sperm parameters: a prospective study of 1,633 patients. *Fertility and sterility*. 91(5), 1801-1805
13. World Health Organization. ((2010). *WHO laboratory manual for the Examination and processing of human semen*