Chromatin Maturity and Integrity of Spermatozoa in Male with Infertility

by Achmad Zulfa Juniarto

Submission date: 10-Dec-2021 01:12PM (UTC+0700)
Submission ID: 1726335566
File name: egrity-of-spermatozoa-in-male-with-infertility-615a951ebebbb.pdf (67.24K)
Word count: 2229
Character count: 12986

ISSN: 03875547
Volume 44, Issue 05, October, 2021

Chromatin Maturity and Integrity of Spermatozoa in Male with Infertility

Achmad Zulfa Juniarto[1], Donna Hermawati[2], Rahadian Ramadhan[3], Fransisgor Bai[4], Asmarinah[5*]

Department of medical biology and biochemistry, Faculty of Medicine, Diponegoro University Indonesia[1,2]
Undergraduate medical student of Medical Faculty Universitas Indonesia[3,4]
Biology Department of Medicine Faculty at Indonesia University of Jakarta[5]

Corresponding Author: 5*

Keywords:
Infertility, sperm chromatin, maturity, aniline blue, toluidine blue

ABSTRACT

Infertility is divided into primary, secondary and idiopathic infertility. Idiopathic infertility is infertility that the underlying factors cannot be explained. This study aimed to find alternative support for the examination of undetermined male infertility tests (idiopathic) by analyzing the status of sperm chromatin and the quality of sperm. This study was an observational study with a total sample of 121 consisting of 93 male infertility patients and 28 samples as normal controls. Data collections of patients and sperm samples were conducted at RSIA Gunung Sawo and Hermina Pandanaran Hospital Semarang Indonesia. Sperm analysis was done by macroscopic and microscopic examination. Chromatin spermatozoa examinations were performed by Aniline Blue staining for chromatin maturation and Toluidine Blue staining for chromatin integrity. The ratio of immature chromatin in asthenozoospermia and oligoasthenozoospermia samples were higher than normozoospermia samples. Mann-Whitney test result showed a significant difference between the ratio of immature chromatin asthenozoospermia and oligoasthenozoospermia compared to normozoospermia from fertile men ($p < 0,05$). There was a significant difference in the level of poor sperm chromatin integrity between the infertile samples (asthenozoospermia and oligoasthnenozoospermia categories) compared to the fertile men with normozoospermia category ($p < 0.001$). The level of integrity of sperm chromatin has a positive correlation with infertility in men ($p < 0.001$, $r = 0.454$). Sperm chromatin maturity and integrity in infertile patients with asthenozoospermic and oligoasthenozoospermic condition was lower significant than fertile men with normozoospermic caterogory.

1. INTRODUCTION

Infertility is the absence of pregnancy after marriage for 1 year or more provided that the couple has sexual intercourse regularly without contraceptive use [1]. About 10-15% couples who get married within one year of marriage will experience difficulties for the occurrence of pregnancy. It is estimated that the factors

causing infertility are 40% of female factors, 40% of male factors and 20% combinations of the two. A study mentioned that the cause of infertility is as follow tubal and peritoneal factors 25-35%, male factors 20-33%, ovulation factors 15-25%, unexplained factor 10-20%, cervical factor 3-5% and other factors (uterus, lifestyle, BMI, toxin, activity etc.) around 1-5% [2]. There are several categories of infertility based on sperm analysis. Oligozoospermia is a sperm concentration <20 million/ml. Astenozoospermia is a sperm motility <50%. Normal sperm morphology <30% is called teratozoospermia, and no sperm when ejaculating is called azoospermia. The combination of problem more than one spermatozoa parameter, for example sperm <20 million/ml and sperm motility <50% is called oligoastenozoospermia [3]. There are still many cases of infertility in men that cannot be explained the underlying causes. It is necessary to look for alternative investigations other than examination male fertility that is commonly used. At this time sperm chromatin status has not become one of the clinical parameters in determining a man's fertility, even though several studies have shown that abnormalities in sperm chromatin can cause infertility in men.

This research aims to determine and analyse the sperm chromatin status and sperm quality to look for alternative investigations on male infertility with unknown cause (idiopathic).

To establish the diagnosis and provide management in men who has infertility, health practitioner has been more dependent on the result of semen analysis of motility, morphology, and sperm concentration. In addition, it is common about examinations sperm DNA integrity and sperm immunology that can determine quality sperm. But through these examinations, not all causes infertility can be known. Therefore, examination of the quality of sperm chromatin can be used as an indicator addition in determining quality sperm. Examination of sperm chromatin maturity is with aniline blue and toluidine blue [4].

2. Methods

This study was an observational study with a total number of 121, consisting of 93 patients with infertility and 28 samples as normal controls. Data collection and sperm samples were conducted at RSIA Gunung Sawo Semarang and Hermina Pandanaran Hospital Semarang. Sperm analysis was carried out at the Laboratory of Gunung Sawo Hospital in Semarang and Hermina Pandanaran Hospital in Semarang. Sperm chromatin status, i.e., maturity and integrity, examination was carried out in the Biology laboratory Faculty of Medicine University of Indonesia Jakarta. The study was conducted after getting ethical clearance from the ethics commission of the Faculty of Medicine of Diponegoro University in Semarang and the patient signed an informed consent.

2.1 Sperm analysis

Sperm examination includes macroscopic and microscopic examination. Macroscopic examination consists of checking volume, warmth, odor, pH, liquidity and viscosity. Microscopic examination consists of examination of concentration, motility and morphology of the sperm. Oligozoospermia was defined as sperm concentration of less than 20 million sperm/ml and asthenozoospermia defined as < 40% progressive sperm motility.

2.2 Sperm chromatin examination

2.2.1 Examination of sperm chromatin maturity with Aniline Blue (AB) staining

After experiencing the full liquefaction for about 30-60 minutes, the cement is smeared on an object glass. The smeared sperm is fixed with Glutaraldehyde solution for 30 minutes, then dipped twice in PBS solution for five minutes and dried in the air afterward. After drying, it is stained with a 5% AB solution (pH 35) and waits for seven minutes, rinsed with PBS solution and dried in the air. Cover an object glass with deck-glass and ready for evaluation.

Examination is carried out using a light microscope with 100 times magnification. Observations were made by observing the color of the head of 200 sperm cells. Sperm heads with mature chromatin have a clear color (not colored) while the head of sperm with immature chromatin will be shown blue. The number of mature and immature sperm were then calculated.

2.2.2 Examination of the density of sperm chromatin by Toluidine Blue (TB) staining

Smear is made from the fresh semen after full liquefaction then fixed with ethanol acetone solution (1: 1) at 40° C for 30 minutes. Rinsed the sperm smear three times using distilled water for two minutes each and dried in the air. After drying, the sperm smear is stained with 0.05% TB solution and waits for 10 minutes. Rinsed with distilled water and then dipped in xylol solution twice for three minutes. After drying, covered with a deck-glass for examination under a light microscope with 100 times magnification. Observe the color of the sperm head for 200 cells. Sperm heads with good chromatin density will be bright blue or clear while the poor chromatin will be purple or violet.

3. Results

In this study obtained a total sample of 121, which consisted of 93 samples of male patients with infertility and 28 samples as normal control. From 93 male infertility samples, there were 56 samples (60.2%) with oligoasthenozoospermia and 37 samples (39.8%) with asthenozoospermia (Table 1-5).

Table 1. Average value of the immature sperm chromatin ratio in the normozoospermia sample and asthenozoospermia

	n	**Average value of Immature Chromatin Ratio**	**p ***
Normozoospermia	28	22.79	0,000
Asthenozoospermia	37	40.73	

* Mann-Whitney test

The average value of the Immature Chromatin Ratio in the asthenozoospermia sample is higher than the normozoospermia sample. Mann-Whitney test showed significant differences between normozoospermia samples compared toasthenozoospermia ($p < 0.05$).

Table 2. Average values of Immature Sperm Chromatin Ratio in normozoospermia and oligoasthenozoospermia sample

	N	**Average value of Immature Chromatin Ratio**	**p ***
Normozoospermia	28	22.79	0,000
Oligoasthenozoospermia	56	51.31	

* Mann-Whitney test

The average value of immature chromatin ratio in oligoasthenozoospermia samples is higher thannormozoospermia samples. Mann-Whitney test showed significant differences between normozoospermia samples compared to oligoasthenozoospermia ($p < 0.05$). (Table 2).

Table 3. Sperm chromatin integrity between normozoospermia and asthenozoospermia

	N	Mean ± SD (%)	Median (%)	p-value
Normozoospermia	28	41.73 ± 23.92	32.36	p < 0.001
Asthenozoospermia	38	75.4% ± 10.88	77.5	

Statistical analysis showed the significant difference level of sperm chromatin integrity between normozoospermia samples compared to asthenozoospermia (p<0.001).

Table 4. Sperm chromatin integrity between normozoospermia and oligoasthenozoospermia

	N	Mean ± SD (%)	Median (%)	p-value
Normospermia	28	41.73 ± 23.92	32.36	p < 0.001
Oligoasthenozoospermia	57	75.82 ± 9.97	77	

Mann Whitney analysis showed the significant difference level of sperm chromatin integrity between normozoospermia samples compared to oligoasthenozoospermia (p<0.001).

Table 5. Correlation analysis of sperm chromatin integrity between normozoospermia of fertile men compared to asthenozoospermia and oligoasthenozoospermia of infertile patient

	N	Mean ± SD (%)	Median (%)	p-value
Normozoospermia (fertile men)	28	41.73 ± 23.92%	32.36	p < 0.001
Asthenozoospermia and oligoasthenozoospermia (infertile patients)	95	75.65 ± 10.29%	77	

Spearman test showed positive correlation between sperm chromatin integrity of normozoospermia compared to asthenozoospermia and oligoasthenozoospermia ($p < 0.001$, $r = 0.454$).

4. Discussion

Of the 50% of infertility cases in general, 6-27% are cases of infertility in men whose causes cannot be explained. Various studies are still being conducted from the molecular aspect and which is currently developing is about the mechanism of epigenetics in spermatogenesis. The process of turning histones into protamine at the time of spermatogenesis is an epigenetic mechanism that helps to regulate differentiation of spermatids into spermatozoa [5]. Protamine is a basic protein in the sperm nucleus composed of 100 amino acids are rich in residues of arginine and cysteine [6]. This high arginine causes protamine bonds to DNA become stronger in the phosphate group. Cysteine residues facilitate the formation of disulfide bonds between one protamine to another and will optimally resemble chromatin to support normal sperm function [7]. Sperm chromatin is one of indicators that determine sperm quality base on composition of protamine and histones in the sperm. Mature sperm contain at least 83% protamine and 15% histone [8]. The composition of protamine which is greater than the histone plays an important role in the process of packaging sperm DNA to become more dense or good integrity. This protamine will package sperm DNA optimally and will increase chromatin condensation in order to protect the genetic integrity of the paternal genome from the enzyme nuclease mutagen and other factors that could cause the DNA damage during the transport [9]. In several studies showed that failure of the conversion of histones to protamine in the process of spermatogenesis and lower chromatin integrity in the sperm caused infertility in men [10], [11].

Despite packaging the paternal genome to be denser in the sperm nucleus to protect paternal genome from enzymes nuclease, mutagen, and other factors that can damage DNA, sperm chromatin density also plays an important role in forming the sperm nucleus to become more hydrodynamic so that sperm can move more quickly toward fertilizing the ovum. In general, the quality of sperm chromatin is one of the factors that plays an important role in the process of fertilization [12]. It is known that the level of maturity and sperm chromatin density correlate with zygote development in the Intra Cytoplasmic Sperm Injection program [13].

In this study, we found the quality of chromatin spermatozoa in male infertility patients, i.e., oligozoospermic and astenozoospermic men is lower than normal controls.

5. References

[1] Mascarenhas M, Flaxman S, Boerma T, Vanderpoel S, Stevens G. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. PLoS Medicine. 2012;9(12):1- 12.

[2] ASRM. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril. 2013; Jan 99 (1): 63.

[3] Tjipto, Bambang W. Kajian infertile pria di laboratorium infertile-andrologi puslitbang system kebijakan kesehatan Surabaya tahun 2005-2008. Buletin Penelitian Sistem Kesehatan. 2010;13(2):181-188.

[4] Syauqy, Ahmad. Evaluasi kromatin sperma sebagai indicator kualitas sperma. Jambi Medical Journal. 2014;2(1):87-97.

[5] Rajender Singh, Avery Kelsey, Agarwal Ashok. Epigenetics, spermatogenesis and male infertility. Mutation Research. 2011; 727: 62-71.

[6] Kasinsky HE et al. Protamines: structural complexity, evolution and chromatin patterning. Protein & Peptide Letters. 2011 (18): 755-771.

[7] Iranpour FG. The effects of protamine deficiency on ultrastructure of human sperm nucleus. Adv Biomed Res [Internet]. 2014 Jan 9; 3:24

[8] Zini A, Agarwal A. Sperm chromatin. Springer. New York. 2011: 29.

[9] De Lamirande E, San Gabriel M, Zini A. Human Sperm Chromatin Undergoes Physiological Remodeling During In Vitro Capacitation and Acrosome Reaction. Journal of Andrology. 2012;33(5):1025-1035.

[10] Hekmatdoost Azita et al. Sperm chromatin integrity: etiologies and mechanisms of abnormality, assays, clinical importance, preventing and repairing damage. Avicenna Journal of Medical Biotechnology. 2009; 1 (3): 147-160.

[11] Tsarev I et al. Evaluation of toluidine male fertility potential blue test for sperm chromatin structure assessment. Human Reproduction. 2009; 24 (7): 1569-74.

[12] Ajina T, Ammar O, Haouas Z, Sallem A, Ezzi L, Grissa I et al. Assessment of human sperm DNA integrity using two cytochemical tests: Acridine orange test and toluidine blue assay. Andrologia. 2017;49(10):e12765.

[13] Asmarinah, Syauqy A, Umar L, Lestari S, Mansyur E, Hestiantoro A et al. Sperm chromatin maturity and integrity correlated to zygote development in ICSI program. Systems Biology in Reproductive Medicine. 2016;62(5):309-316.

Chromatin Maturity and Integrity of Spermatozoa in Male with Infertility

ORIGINALITY REPORT

14%	12%	10%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	ejournal.unsri.ac.id Internet Source	2%
2	Submitted to University of Birmingham Student Paper	2%
3	bacandrology.biomedcentral.com Internet Source	1%
4	Submitted to Universitas Sumatera Utara Student Paper	1%
5	Submitted to University of Bristol Student Paper	1%
6	www.ecerm.org Internet Source	1%
7	www.neliti.com Internet Source	1%
8	ossu.ca Internet Source	1%
9	Berlin Pandapotan Pardede, Tulus Maulana, Ekayanti Mulyawati Kaiin, Muhammad Agil et	1%

al. "The potential of sperm bovine protamine as a protein marker of semen production and quality at the National Artificial Insemination Center of Indonesia", Veterinary World, 2021
Publication

10	Submitted to University of Derby Student Paper	1 %
11	www.eurekaselect.com Internet Source	1 %
12	Mikiko Arikawa, Seung Chik Jwa, Akira Kuwahara, Minoru Irahara, Hidekazu Saito. "Effect of semen quality on human sex ratio in in vitro fertilization and intracytoplasmic sperm injection: an analysis of 27,158 singleton infants born after fresh single-embryo transfer", Fertility and Sterility, 2016 Publication	1 %
13	Patricia Peris - Frau, Manuel Álvarez - Rodríguez, Alicia Martín - Maestro, María Iniesta - Cuerda et al. " Unraveling how capacitation alters ram sperm chromatin before and after cryopreservation ", Andrology, 2020 Publication	<1 %
14	www.hindawi.com Internet Source	<1 %
15	jfmpc.com Internet Source	

		<1%
16	mehdijournal.com Internet Source	<1%
17	"Sperm Chromatin", Springer Science and Business Media LLC, 2011 Publication	<1%
18	Abdolhosein Javadneia, Mahin Taheri Moghadam, Armin Alivand, Alireza Kheradmand, Mahvash Zargar, Susan Sabbagh. "Sperm DNA Damage Before and After Kidney Transplantation", Nephro-Urology Monthly, 2019 Publication	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		

Chromatin Maturity and Integrity of Spermatozoa in Male with Infertility

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6