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RESEARCH ARTICLE

Human Sperm Chromatin Maturation: A Study of Prolactin Supplementation in Sperm Preparation

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Abstract

One of the management of infertile couples is assisted reproduction through the Intra Uterine Insemination (IUI). Paternal factor has been known to influence the success of IUI, such as sperm selection on preparation. Prolactin (PRL) is reported to select sperm with better DNA fragmentation on the preparation process. This study aimed to investigate sperm selection with better chromatin maturation quality, by the supplementation of PRL. Semen samples were obtained from 10 infertile men who experienced sperm preparation for IUI. Prepared sperm were incubated at 1000 ng/ml of PRL, followed by chromatin maturation test. Its index was determined as mature sperms in 100 sperms. The differences in sperm chromatin maturation showed a significant difference ($p < 0.05$), between groups before and after preparation, between groups after preparation without and with PRL administration, as well as between groups before and after preparations given PRL. These differences were supported by the images of sperm chromatin maturation between groups. Prolactin improves sperm quality on sperm preparation, according to sperm chromatin maturation. Nevertheless, the other underlying mechanism that maintain sperm chromatin, are needed to investigate more.

Keywords: *Human sperm, Sperm chromatin maturation, Prolactin, Sperm preparation.*

Introduction

One of the management of infertile couples is assisted reproduction through the Intra Uterine Insemination (IUI) and In Vitro Fertilization (IVF) programs. The success rate for achieving pregnancy through these programs is still low [1].Several factors have been known to influence the success of this assisted reproduction program, namely maternal, paternal and uterine endometrial receptivity. The development of science and the existence of various studies to solve these problems, both clinically and in molecular biology.

Paternal factors include disturbances in concentration, motility, morphology and integrity of DNA, as well as sperm chromatin [2, 3]. Sperm chromatin maturation is also a significant predictive value [4, 5]. One way to select sperm in IUI and IVF programs is sperm preparation.

Sperm preparation is a process of selecting sperm from cement to obtain motile sperm with good morphology and without other contaminants. However, in the preparation of sperm, there are several processes that can damage the sperm themselves, such as repeated centrifugation and storage in a hot incubator. Several studies have shown that the addition of certain substrates can reduce the detrimental effects of sperm preparation processes. One of the substrates that have been reported to play a role is prolactin (PRL), which has an important role in the male reproductive tract.

Other studies have also proven that administering prolactin to sperm preparations can improve the quality of sperm in terms of the number of motile sperm and sperm DNA fragmentation [6, 7].

In this study, we selected higher quality sperm using the Density Gradient Centrifugation (DGC) method for sperm preparation, in terms of chromatin maturation, through the supplementation of PRL. This research has never been conducted in Indonesia and is expected to increase the success of assisted reproduction both IUI and IVF in the management of infertile couples.

Material and Methods

Human Sperm Collection

Semen samples were obtained from 17 infertile men who experienced sperm preparation for IUI. Agreement of all patients was obtained by signing informed consent forms. Approval of Faculty of Medicine of Universitas Indonesia (FMUI) research ethical committee was obtained with the ethical number 86/UN2.F1/ETIK/PPM.00.02/2020. Semen samples were collected by masturbation after 3 - 5 days of abstinence [7].

Sperm Preparation

Sperm preparation was performed by Density Gradient Centrifugation (DGC) method using Sperm Grade reagent. (Vitrolife, Gothenburg, Sweden) Two point five ml of gradient 45%, 90% and semen were put in 15 ml tube. Then, the centrifugation (Thermo Scientific Centrifuge, New York, USA) was demonstrated at 300xg for 20 minutes.

The supernatant was removed and the pellet was supplemented with 2 ml of Sperm rinse reagent (Vitrolife, Gothenburg, Sweden). Another centrifugation was demonstrated at 300xg for 10 minutes after homogenization. The supernatant was removed again, and then the pellet was supplemented with 2 ml of Sperm Rinse reagent. Again, another centrifugation at 300xg for 8 minutes was demonstrated after homogenization. Finally, the obtained pellet was investigated further.

Prolactin Incubation

Two µl of sperm samples in 5 million/ml concentration were supplemented with prolactin (Human recombinant prolactin, Merck, USA) at concentration at 1000 ng / ml. Consequently, the sample was incubated at 37°C for 30-45 minutes.

Chromatin Maturation Assay

Sperm chromatin maturation was assessed applying Sperm Nuclear Chromatin Kit. The guided protocol was in accordance with the prior research [2].The blue staining was marked for immature chromatin, while the red-purple was marked for mature chromatin. The chromatin maturation index was determined as mature sperms in 100 sperms.

Statistical Analysis

22nd version of SPSS was implanted to calculate the data analysis. The t-test was demonstrated to compare the chromatin maturation index in two groups between before preparation, after preparation without PRL supplementation and after preparation with PRL supplementation. The level of significance was set at $p<0.05$.

Result

In this study, sperm chromatin maturation test was performed, as shown in Figure 1 between groups of sperm after preparation, with and without the supplementation of PRL. (Figure 1) In the three groups, it was seen that sperms which were in pink or purple color showed mature chromatin and in bluish color showed immature chromatin. The PRL group showed more mature sperms, compared to the group without PRL.

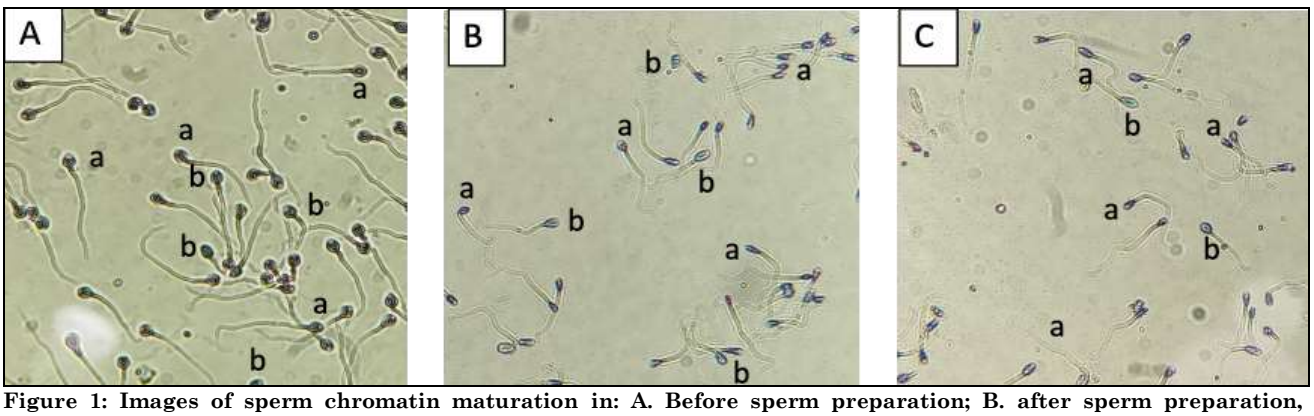

Figure 1: Images of sperm chromatin maturation in: A. Before sperm preparation; B. after sperm preparation, without PRL supplementation and C. After sperm preparation with PRL supplementation group

In addition to the images of sperm with chromatin maturation, a comparative test conducted to observe differences in sperm chromatin maturation between these three groups was the paired t test, which showed a significant difference ($p < 0.05$), both before and after preparation, between groups after preparation without and with PRL administration, as well as between groups before and after preparations given PRL (Table 1).

Table 1: Sperm chromatin maturation

	Group			p
Sperm chromatin maturation	Before preparation	After preparation, without PRL supplementation	After preparation, with PRL supplementation	<0,05[a,b,c]
	82,29 ± 6,01	87,47 ± 3,61	92,35 ± 3,55	

a = p value between before preparation vs after preparation without PRL supplementation; b = p value between after preparation with and without PRL supplementation; c = p value between before and after preparation with PRL supplementation

Discussion

Sperm Chromatin Maturation

Sperm chromatin is tightly packed to protect DNA during the transit that occurs before fertilization. In sperm chromatin, DNA packaging by protamine is one of the mechanisms that regulate the differentiation of spermatids into sperm in the spermatogenesis process. The quality of this chromatin is one of the indicators for determining the quality of sperm. Mature sperm contains at least 85% protamine and 15% histone [8].Failure to convert histone to protamine and suboptimal chromatin quality are associated with failure of fertilization.

At present, in several studies and applications in the field of andrology, the quality of sperm both from semen analysis (sperm concentration, motility and morphology) and examination of sperm DNA fragmentation can act as biomarkers or screening of male fertility, but it is not known yet for this chromatin maturation. It is hoped that knowing the chromatin maturation of infertile men and its role in sperm preparation can contribute to the development of progress in infertility management.

Prolactin Supplementation and Sperm Chromatin Maturation

In this study, preparation was performed so that the produced sperm had better quality with a higher chromatin maturation value. The results of this study are in line with other studies stated that in preparation process, most of the abnormal sperms are removed, hence the result of preparation contain more motile sperms with normal morphology [9, 10]. However, it cannot be negated that the preparation process can also increase ROS which results in oxidative stress itself, such as rapid and repeated centrifugation and storage at temperatures and periods that can damage sperm (ex. 37°C for more than 1 hour).

These conditions may lead to DNA fragmentation and damage to the sperm chromatin. PRL has been shown to act as a sperm pro-survival factor that affords to suppress apoptosis [11, 13]. Furthermore, this study is also in line with previous studies which have proven that giving PRL to the sperm preparation process can reduce sperm DNA fragmentation [6].

In addition to PRL, which acts as a sperm pro-survival factor and suppresses apoptosis, there is also a study on the chromatin maturation of sperm by administering ALA as an antioxidant. It has been known from previous studies that free radicals or ROS can induce oxidative stress if the amount exceeds the antioxidant capacity [14].This causes damage to DNA or chromatin fragmentation through lipid peroxidation in the sperm membrane [15].

Since high polyunsaturated fatty acids (PUFA) are contained in the sperm membrane, the interaction between ROS and the membrane will produce lipid electrophiles associated with oxidative stress that causes lipid peroxidation [16].

ALA as an antioxidant seeks to suppress the activity of these free radicals so that ALA administration in the preparation process can improve the quality of sperm, one of which is in terms of chromatin maturation [17].This study is the first study on the effect of PRL on sperm preparation, in terms of chromatin maturation.

Apart from having good motility and morphology, sperm if they are completed with intact DNA integrity and packed with mature chromatin, are certainly an ideal paternal factor for fertilization with oocytes to produce a perfect embryo. PRL has been proven in vitro to improve the quality of sperm after preparation, both in terms of DNA fragmentation and chromatin maturation, but it is not yet known whether PRL application to sperm preparation can improve the quality of embryos produced from IVF-ICSI.

Conclusion

Prolactin improves sperm quality in sperm preparation, according to sperm chromatin maturation. Nevertheless, the other underlying mechanism that maintain sperm chromatin, are needed to investigate more. The results of this study are expected to increase the success rate of assisted reproduction technology, particularly in IUI.

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References

1. Abdelkader AM, Yeh J (2009) The potential use of intrauterine insemination as a basic option for infertility: a review for technology- limited medical settings. Obstet Gynecol. Int., 1-11.
2. Agarwal A, Gupta S, Sharma R (2016) Sperm preparation for intrauterine insemination using density gradient separation. Andrological Evaluation of Male Infertility: Springer, 101-7.
3. Lestari SW, Sari T, Pujianto DA (2016) Sperm DNA fragmentation and apoptosis levels: A comparison of the swim up and the density gradient centrifugation methods for sperm preparation. On Line Journal of Biological Sciences, 16(4):152-8.
4. Bungum M (2012) Role of Sperm DNA Integrity in Fertility. In: Pereira PLV, editor. Embryology-Updates and Highlights on Classic Topics: In Tech., 23-40.
5. Murray MT, Hechtman L (2015) Infertility, Male. Textbook of Natural Medicine: Amazon Customer, 1515-34.
6. Lestari SW, Lestari SH, Pujianto DA (2018a) Sperm quality after swim up and density gradient centrifugation sperm preparation with supplementation of alpha lipoic acid (ALA): A preliminary study. AIP conference proceedings, 1933/1.
7. Lestari SH, Lestari SW, Pujianto DA, Selene NB, Izza EF (2018b) Prolactin supplementation in sperm preparation: A study of sperm DNA fragmentation. Journal of Global Pharma Technology, 10(6):224-230.
8. Asmarinah, Syauqy A, Umar LA, Lestari SW, Mansyur E, Hestiantoro A, et al (2016) Sperm chromatin maturity and integrity correlated to zygote development in ICSI program. Systems biology in reproductive medicine, 62(5):309-16.
9. Hammadeh M, Zeginiadov T, Rosenbaum P, Georg T (2001) Predictive value of sperm chromatin condensation (aniline blue staining) in the assessment of male fertility. Archives of andrology, 46(2):99-104.
10. Arny M, Quagliarello J (1987) Semen quality before and after processing by a swim-up method: relationship to outcome of intrauterine insemination. Fertility and sterility, 48(4):643-48.
11. Pujianto DA, Curry BJ, Aitken RJ (2010) Prolactin Exerts a Prosurvival Effect on Human Spermatozoatozoa via Mechanisms that Involve the Stimulation of Akt Phosphorylation and Suppression of Caspase Activation and Capacitation. Endocrinology, 3: 1269-79.
12. Koppers AJ, De Iuliis GN, Finnie JM, McLaughlin EA, Aitken RJ (2008) Significance of mitochondrial reactive oxygen species in the generation of

oxidative stress in spermatozoatozoa. The Journal of clinical endocrinology and metabolism, 93(8):3199-207

13. Aitken RJ, De Iuliis GN (2010) On the possible origins of DNA damage in human spermatozoatozoa. Mol. Hum Reprod., 16(1):3-13.
14. Menezo YJR, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie, et al (2007) Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. Reprod Biomed Online, 14(4):418-21.
15. Schulte RT, Ohl LE, Sigman M, Smith GD (2009) Sperm DNA damage in male infertility: etiologies, assays and outcomes. Journal of assisted reproduction and genetics, 27(1):3-12.
16. Rochette L, Ghibu S, Richard C, Zeller M, Cottin Y, Vergely C (2013) Direct and indirect antioxidant properties of α-lipoic acid and therapeutical potential. Mol. Nutr. Food Res., 57(1):114-25.
17. Lestari SW, Hinting A, Supardi, Bowolaksono A, Asmarinah (2019) Sperm DNA fragmentation and chromatin maturation of prepared sperm with ALA supplementation: The effect of embryo development quality. Journal of global pharma technology, 11(9):196-202.

