Available online at www.sciencerepository.org

Science Repository

Research Article

ATP Content: An Indicator of Sperm Metabolic Fitness

Rajasingam S. Jeyendran[1*], Aleksandra Lazarević[1], Laurence Fishel[1], Milica Ivanovic[1] and Seth Levrant[2]

[1]*Androlab Inc, Chicago, Illinois, USA*

[2]*Institute for Human Reproduction, Chicago, Illinois, USA*

ARTICLE INFO

Article history:

Received: 10 June, 2022

Accepted: 29 June, 2022

Published: 13 July,2022

Keywords:

ATP

sperm

metabolic fitness

ABSTRACT

The association of sperm adenosine triphosphate (ATP) content with the standard sperm parameters and among different populations of sperm within an ejaculate was determined. ATP was determined in sperm using Promega CellTiter-Glo^{R} 2.0 and the chemiluminescence read on a Molecular Devices SpectraMax L plate reader. ATP was calculated per 10^6 sperm and all results were presented as mean ± standard deviation. Pearson correlation coefficients between ATP content per 10^6 sperm and all other variables analysed had no significant association ($p < 0.05$). Student t-test however yielded significant differences among motile and progressively motile sperm in the filtrate and retentate respectively, following silica wool filtration ($p < 0.0005$). This rapid, quantitative ATP assay provides new information regarding overall metabolic function of the sperm independent of other sperm variables, especially sperm motility. Future studies of pregnancy outcome will corroborate whether this assay helps to identify the minimum number of metabolically active sperm needed to classify an ejaculate as fertile, sub-fertile or infertile.

Introduction

The diagnostic value of routine semen analysis results to identify fertile or infertile males is not reliable as there is considerable overlap between these two groups of males [1-3]. This may be due to the inability to simultaneously identify a progressively motile sperm as the one which also has absolutely normal sperm morphology. A test that can simultaneously assess the normalcy of most of the known sperm characteristics of a population of sperm is an ideal that has not yet been achieved.

After ejaculation, sperm acquire energy from nutrients found in the seminal plasma and in the female reproductive tract environment, and this energy is transformed into adenosine triphosphate (ATP) by anaerobic glycolysis and oxidative phosphorylation [4, 5]. Glycolysis takes place mainly in the fibrous sheath of the main piece of the tail, whereas oxidative phosphorylation occurs in mitochondria, which are localized exclusively in the mid-piece of the sperm tail. ATP is needed for many of the sperm functions such as motility, to transport ions and other molecules through membranes against concentration gradients, and membrane changes during the fertilization process such as the sperm capacitation and acrosome reaction, including hyper activated motility [4, 6, 7].

Therefore, we postulate that the determination of ATP content of an ejaculate may be closely correlated with the overall quantity of functional sperm in that ejaculate. The objective was to determine the association of ATP content of the ejaculate with the standard sperm parameters and among different populations of sperm within an ejaculate.

Materials & Methods

Ejaculates from apparently healthy men who were referred for semen analysis were obtained by self-masturbation. Institutional review board approval was obtained from Wayne State University and was exempted under IRB-19-11-1489. Twenty-five ejaculates were analysed for ATP content following routine semen analysis, including sperm vitality by dye exclusion and sperm viability by the hypo-osmotic swelling test, as previously described in Study 1 [8-10].

Correspondence to: Rajasingam S. Jeyendran, Androlab Inc, 680 North Lake Shore Drive, Suite 807, Chicago, Illinois, 60611, USA; E-mail: jeyendran@sbcglobal.net

http://dx.doi.org/10.31487/j.CROGR.2022.01.02

In study 2, ten ejaculates were filtered through silica (glass) wool as described previously [11]. ATP content of the sperm in the filtrate and retentate following silica wool column were determined following routine analysis.

ATP Determination

ATP content of the ejaculate was determined using the CellTiter-Glo^R 2.0 Assay 2.0 Assay (Promega, Madison, WI; Link). Briefly the procedure is as follows: 5 µL of semen was diluted with 95 µL of Tyrode salt solutions (Sigma Aldrich, St. Louis, MO). 50 µL of diluted semen was mixed with 50 µL of ATP reagent (CellTiter-Glo^R 2.0) by pipetting up and down several times, transferred to a 96-well dark plate and the chemiluminescence read at 470 nm on a Molecular Devices SpectraMax L plate reader. The chemiluminescence (ATP) obtained was expressed (or output) in Relative Light Units (RLU) per 10^6 sperm.

All data are expressed as the mean ± standard deviation (SD) and the chemiluminescence (ATP) obtained was calculated per 10^6 sperm. Pearson correlations were calculated for all the sperm variables determined against the calculated ATP chemiluminescence per million sperm. Comparison between filtrate and retentate was performed by Student's two-tailed t-test and P-values of <0.05 were considered to be statistically significant.

Results

The mean ± S.D obtained from 25 different ejaculates for ATP content per million sperm was 77483 ± 42868 RLU. The results of sperm variables analysed are detailed in (Table 1). The Pearson correlation coefficient between the ATP content per million sperm and all other variables analysed revealed no significant ($p < 0.05$) association. The mean ± S.D. of the ATP content per million sperm obtained from 10 different filtrates and their respective retentates for overall sperm motility and for progressively motile sperm were significantly different from each other ($p=0.0005$, Table 2).

Table 1: Sperm variables and their correlation with ATP content.

Semen Parameters*	**Mean**	**SD**	**r^2**
Sperm 10^6/ml	80.4	40.5	-0.30
Sperm 10^6/ejaculate	267.6	301.5	-0.24
Overall Sperm Motility %	52.4	15.3	-0.25
Progressive Sperm Motility %	30.2	10.8	-0.15
Normal Sperm Morphology %	3.3	1.7	-0.31
Vital Sperm (Dye Exclusion) %	72.6	11.8	0.23
Viable Sperm (HOS Test) %	57.8	16.2	-0.02

* Pearson correlation coefficient between the ATP content per 10^6 sperm and all other sperm variables analyzed revealed no significant association ($p < 0.05$).

Table 2: Comparison of ATP content of sperm recovered from the filtrate and retentate following silica wool filtration.

ATP Content per 10^6 Sperm/mL*	**Filtrate**	**Retentate**	**Student's t-test**
Overall Motile Sperm	1214.8 ±741.3	53.4 ±110.5	0.0005
Progressively Motile Sperm	1142.8 ±690.9	36.9 ±81.9	0.0005

*A significant difference in the ATP content of motile sperm recovered from the different filtrates and their respective retentates ($p < 0.05$).

Discussion

There was no significant association between the ATP content and standard sperm parameters or the percent of vital and viable sperm, suggesting that the ATP content is independent of the established traditional semen variables (Table 1). ATP is essential for many of the sperm functions such as sperm motility and cell metabolism including fusion events associated with fertilization and is dependent on the overall sperm morphology [10, 12-15]. The ATP content may therefore represent the effective number of functionally active sperm in an ejaculate.

A poor correlation between ATP content of the sperm and other sperm variables as noted in (Table 1) paradoxically makes it a more desirable assay, because it indicates a different and new parameter is being measured that may reflect the overall quality of sperm in an ejaculate [10]. Many investigators will discount a putative new sperm function assay if a good correlation is not found between the new assay and other established sperm parameters. Such a conclusion is often not valid since if a good correlation is found between the new assay and one or more of the already established assays, then the new assay has no unique diagnostic value other than perhaps being easier or more convenient than other established assays.

An ejaculate, after fractionation via silica wool column filtration, had an initial aliquot sperm population with significantly higher motility, functional membrane integrity, acrosin content, and hamster oocyte penetration potential than subsequent fractions or the original ejaculate itself [13]. Nani and Jeyendran concluded that the sperm with functionally inactive or physically damaged membranes tend to adhere to silica wool thus providing viable sperm of higher quality [16]. To determine whether different populations of sperm may have different ATP content, we analysed the ATP content of sperm obtained in the filtrate and retentate following silica wool column. A highly significant difference in ATP content was observed between the sperm recovered in the filtrate as compared to sperm recovered in the retentate. In other words, even among motile sperm, there is a distinct population of sperm

that have significantly low ATP content which may reflect that the other metabolic functions are compromised.

With a limited number of unselected ejaculates, we have shown that the ATP content of an ejaculate differs from other established semen variables and may identify a population of metabolically active or "effective" sperm in an ejaculate. This rapid, quantitative ATP assay provides new information regarding the overall metabolic function of the ejaculate and is independent of other semen variables. Determination of ATP content of the sperm may represent a new direction for research, which will advance our current knowledge of the role of metabolism and male fertility. Future studies of pregnancy outcome will corroborate whether this assay helps to identify the minimum number of metabolically active sperm needed to classify an ejaculate as fertile, subfertile or infertile.

Funding

None.

Conflicts of Interest

None.

Author Contributions

All authors contributed to the manuscript; research design (RSJ, LF); acquisition, analysis and interpretation of data (RSJ, AL, MI, SL, LF); drafting the paper (RSJ, LF); critical review (RSJ, SL, LF); and approval of submitted and final versions (RSJ, LF).

REFERENCES

1. Khatun A, Rahman MS, Pang MG (2018) Clinical assessment of the male fertility. *Obstet Gynecol Sci* 61: 179-191. [Crossref]
2. Sakkas D, Ramalingam M, Garrido N, Barratt CLR (2015) Sperm selection in natural conception: what can we learn from Mother Nature to improve assisted reproduction outcomes? *Hum Reprod Update* 21: 711-726. [Crossref]
3. Wang C, Swerdloff RS (2014) Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. *Fertil Steril* 102: 1502-1507. [Crossref]
4. Piomboni P, Focarelli R, Stendardi A, Ferramosca A, Zara V (2012) The role of mitochondria in energy production for human sperm motility. *Int J Androl* 35: 109-124. [Crossref]
5. Ferramosca A, Zara V (2014) Bioenergetics of mammalian sperm capacitation. *Biomed Res Int* 2014: 902953. [Crossref]
6. Amaral A, Ramalho-Santos J, St John JC (2007) The expression of polymerase gamma and mitochondrial transcription factor A and the regulation of mitochondrial DNA content in mature human sperm. *Human Reprod* 22: 1585-1596. [Crossref]
7. Stendardi A, Focarelli R, Piomboni P, Palumberi D, Serafini F et al. (2011) Evaluation of mitochondrial respiratory efficiency during in vitro capacitation of human spermatozoa. *Int J Androl* 34: 247-255. [Crossref]
8. World Health Organization (2010) WHO laboratory manual for the examination and processing of human semen, 5th ed. Geneva.
9. Schrader SM, Platek SF, Zaneveld LJ, Perez-Pelaez M, Jeyendran RS (1986) Sperm Viability: a comparison of analytical methods. *Andrologia* 18: 530-538. [Crossref]
10. Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ (1984) Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil* 70: 219-228. [Crossref]
11. Jeyendran RS, Perez-Pelaez M, Crabo BG (1986) Concentration of viable spermatozoa for artificial insemination. *Fertil Steril* 45: 132-134. [Crossref]
12. Gomendio M, Roldan ERS (2008) Implications of diversity in sperm size and function for sperm competition and fertility. *Int J Dev Biol* 52: 439-447. [Crossref]
13. Jeyendran RS, Holmgren WJ, Bielfeld P, Wentz AC (1992) Fertilizing capacity of various populations of spermatozoa within an ejaculate. *J Assist Reprod Genet* 9: 32-35. [Crossref]
14. Jeyendran RS, Zaneveld LJ (1993) Controversies in the development and validation of new sperm assays. *Fertil Steril* 59: 726-728. [Crossref]
15. Lüpold S, Calhim S, Immler S, Birkhead TR (2009) Sperm morphology and sperm velocity in passerine birds. *Proc Biol Sci* 276: 1175-1181. [Crossref]
16. Nani JM, Jeyendran RS (2001) Sperm processing: glass wool column filtration. *Arch Androl* 47: 15-21. [Crossref]