
Original article

Relationship Between Sperm Quality and Male Reproductive Hormones Among Male Partners with Fertility Complications: Attending CHUB

Herbert Mapira Tendayi^{1*}, Jerome Ndayisenga¹, Jean Claude Nshutiyimana¹, Solange Nyiramahirwe¹, Jacqueline Mukanshuti¹, Valens Karenzi¹, Robert Rutayisire¹

¹*Biomedical Laboratory Sciences Department, School of Health Sciences, College of Medicine and Health Sciences, University of Rwanda, Kigali, Rwanda*

***Corresponding author:** Herbert Mapira Tendayi, Biomedical Laboratory Sciences Department, School of Health Sciences, College of Medicine and Health Sciences, University of Rwanda, Kigali, Rwanda. Email address: mapiraherbert@gmail.com

Abstract

Background

Infertility remains a highly prevalent global condition in the second decade of the new millennium. Reproductive hormones determine sperm quality as they initiate and maintain spermatogenesis. Hormonal imbalance can cause abnormal sperm quality that can be treated by hormonal replacement therapy.

Objective

To assess the relationship between sperm quality and male reproductive hormones among male partners with fertility complications attending CHUB.

Methods

The study was a descriptive cross-sectional, and a convenient sampling strategy was used to recruit subjects at CHUB. Sixty-two male subjects with fertility complications provided both semen and blood sample to analyze sperm quality and reproductive hormones. Descriptive statistics were used to analyze data.

Results

Both FSH and LH showed a strong negative correlation with sperm count which is more profound with FSH ($r = -0.722$) than LH ($r = -0.545$). Testosterone showed a strong positive correlation with sperm count ($r = 0.712$). FSH and LH showed a negative correlation with sperm motility which is more profound in FSH ($r = -0.312$) than LH ($r = -0.302$). Testosterone also showed a positive correlation with sperm motility ($r = 0.360$).

Conclusion

Our study found a correlation between sperm quality and male reproductive hormones. We further suggest other studies to investigate predictive power of male reproductive hormones.

Rwanda J Med Health Sci 2020;3(3):315-328

Keywords: sperm quality, reproductive hormones, male fertility complications

Background

Infertility remains a highly prevalent global condition in the second decade of the new millennium, affecting about 186 million worldwide.[1] For the several last decades, there is an increase of infertility and clinical presentation suggests the causes to be hormonal in nature.[2] WHO defines the term infertility as a failure to conceive a child after frequent and unprotected sexual intercourse for 12 months.[3,4] Many scientists prefer the term sub fertility because half of couples conceive without intervention in the following 12-24 months.[5] Reproductive hormones are important determinant of sperm quality as they initiate and maintain spermatogenesis.[6] Testosterone participate in spermatogenesis by acting on sertoli cells in seminiferous tubules and surrounding peritubular cells which increase sperm motility, and cause negative feedback to the LH and FSH.[7] Contrary, the levels of FSH and LH are inversely proportional to the sperm morphology, motility and concentration.[6]

Globally, infertility is estimated to be 10 %- 12% in couples. Female factors related to 88.9% of the cases while male factors related to 66% of the cases.[8] The highest prevalence found in Africa and Central/East of Europe.[9] The study done by Dhont et al (2011) about infertility in Rwanda showed that male factors contribute to 16%, female factors contribute 31% and combination of both male and female factors contribute to 50% while 3% was unexplained.[10] Multiple factors like

genetics, environment, dietary factors and infections might cause the infertility.[4] A study done in Nigeria found the prevalence of endocrine abnormalities of 7.3% in men investigated for infertility, hormonal abnormalities found in 22% of oligospermic males while 43% of hormonal abnormalities found in azoospermic males.[11]

The sperm cells are formed by process of spermatogenesis. It starts with mitotic division of stem cells (spermatogonial stem cells) located near to the tubules' basement membrane that produces two types of cells: type A cells that replenish the stem cells, and type B cells that differentiate into spermatocytes. The primary spermatocyte divides by mitosis to produce two secondary spermatocytes; and then secondary spermatocyte divides by meiosis II into two equally haploid spermatids that transform into spermatozoa by spermiogenesis. Semen are formed by mixing of whitish fluid called seminal fluid produced by seminal vesicles and prostate gland with sperm during ejaculation.[12,13]

Endocrine factors from brain highly regulate testicular functions, and hypothalamus- pituitary- testis circuit is core unit for maintaining endocrine balance and fertility. Hypothalamus secretes Gonadotropin Releasing Hormone that stimulates pituitary gland to produce gonadotropic hormones: LH and FSH. Within testis, FSH and LH have synergic effect to develop and maintain testicular function; LH regulates the synthesis of testosterone in Leydig cells while

FSH control spermatogenesis and spermyogenesis.[14] FSH acts on seminiferous tubules directly and stimulate production of leydig cells in immature testis. FSH in combination with LH and HCG potentiate leydig cell's testosterone production in immature testis, but mediating factors are yet known.[15-17]

Different techniques are used to diagnose both male and female factors of infertility. Female factors should be diagnosed by hysteroscopy, pelvic ultrasound, antral follicle count and hormonal measurement. Tests of male factors include antisperm antibodies screening, genetic tests, testicular biopsy, hormonal measurement and semen analysis. Semen analysis is the major test ordered firstly by clinician after consultation of male partner, and reveal sperm count, motility, morphology, volume, viscosity and presence of leucocytes. The normal results according to WHO reference ranges predict that the male may not be responsible for infertility, and abnormal results for semen analysis suspect male partner for being infantile.[18,19]

WHO proposes the standardization of semen analysis and its reference values based on population-based data. Clinical laboratory should check the quality of semen analysis test, follow standard procedure and reference value for proper diagnosis of infertility. Some infertility factors are not tested by semen analysis. For instance, the process of sperm capacitation that occur during sperm passage through female reproductive tract involves mechanisms that

would lead to infertility if they are impaired, they are not assessed by semen analysis. Other clinical information of sperms that are not tested by semen analysis include the presence of sperm surface proteins responsible for binding to the zona pellucida, penetration and egg fertilization ability.[20]

The infertility of couple is associated with negative social implication in developing countries. Most parts of Sub-Saharan Africa still have strong desire for children, and infertility can cause mental suffering.[1] In Rwanda, men suffer as much as women at community level and there is positive correlation between those suffering and low socio-economic status. Men and women with high-income status were reported to have less suffering than low-income couples.[10] WHO consider infertility as major global health issue, and has issued international standards for infertility laboratory testing and diagnosis.[1,21]

Male reproductive hormones determine sperm quality through initiating and maintaining spermatogenesis.[6] Hormonal imbalance can impair spermatogenesis and result in abnormal sperm qualities including sperm concentration, motility and morphology. Sperm abnormalities cause male infertility which is a frustrating and emotionally devastating health condition.[22] Infertility caused by hormonal abnormalities can be treated by manipulation of hormonal axis.[23] However, no research done to reveal the relationship between sperm

quality and male reproductive hormones in Rwanda. There is more data on infertility in high-income countries, but in developing countries, more research on infertility are highly needed to generate information important for planning fertility interventions.(4) This research aims to assess the relationship between sperm quality and male reproductive hormones for evidenced- based management of male infertility.

Methods

Study design

This study was cross sectional study on the relationship between sperm quality and male reproductive hormones among male partners with fertility complications attended CHUB, in Huye district, Southern province, Rwanda from 18th June to 06th September 2019.

Participants recruitment

A study recruited 62 adult male patients attended Butare University Teaching Hospital presenting with fertility complications. Convenient sampling strategy was used to recruit participants.

Data collection

The participants requested not to have sexual intercourse or masturbate for 2-7 days prior to the sample collection. The blood sample was collected in red top tube with no additive, and the participants provided semen sample. The semen produced by having sexual intercourse with partner or

masturbation in private room. The whole semen collected into plastic container and brought to the lab within 1 hour.

Laboratory testing

Semen were analyzed for count, motility and morphology according to WHO laboratory manual for the examination and processing of human semen, fifth edition. Semen incubated for 30 minutes at 37°C. The sperms were counted using Neubauer counting chamber. The dilution of 1:20 was used and prepared by adding 50 µl of semen to the 950 µl diluent. The diluent was prepared adding 50 grams of sodium carbonate and 10 ml of 35% formalin to the distilled water to make 1000 ml. Zero sperm count were considered as azoospermia, less than 20×10^6 were considered as oligozoospermia, and at least 20×10^6 were considered as normal sperm count.

To examine the sperm morphology, semen specimens were stained with MGG and spermatozoa stained into dark blue purple hue. Normal sperm morphology for man was deliberated at normal morphology greater than or equal to 4% of total sperms morphology.

To examine motility, the semen was examined microscopically to estimate motility of sperm cells. 10 µl of semen was put on glass slide and covered with 22x22 mm coverslip. The slide was then examined microscopically using 40x objective lens. Motility was reported and graded as rapid progressive motility, slow progressive motility, non-

progressive motility or immotility. Normal motility for man was deliberated at total motile greater than or equal to 40%, and progressive motility greater than or equal to 32% of total motility.[24]

Testosterone, LH and FSH were tested using solid phase enzyme-linked immunosorbent assay (ELISA) for quantitative determination of hormones in serum by following manufacturer's protocol. LH and FSH were reported in mIU/ml while testosterone was reported in ng/ml.

Data analysis

Collected data were analyzed using SPSS 25.0 to generate descriptive and inferential statistics. Data was firstly entered in Microsoft excel 2016 and imported in SPSS 25.0. Pearson's r was used to determine the correlation between semen quality and male reproductive hormones. Statistical significance was deliberated at $P < 0.05$. The graphs were plotted using Microsoft excel 2016.

Ethical consideration

Data was collected after obtaining ethical clearance from University of Rwanda, College of Medicine and Health Sciences and data collection approval from the ethical committee of Butare University Teaching Hospital. Only patients who signed informed consent form were included in the study. Confidentiality maintained by using code numbers and data only accessed by authorized personnel.

Results

Sperms were counted using Neubauer counting chamber, and normal sperm count deliberated at sperm count greater or equal to 20×10^6 . Out of 62 tested subjects, 19 (31%) were azoospermic (with no sperm). 16 (26%) were oligozoospermic (with sperm count less than 20×10^6). 28 (43%) had normal sperm count (with sperm count greater or equal to 20×10^6).

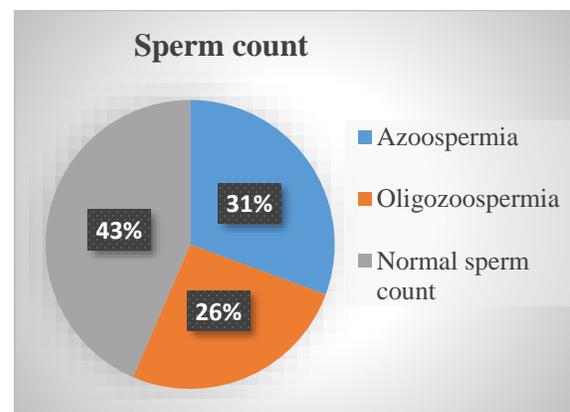


Figure 1. Sperm quality categories according to sperm count

Sperm motility was tested, and among tested subjects, 18 (29%) were asthenozoospermic. 44 subjects (71%) had normal sperm motility.

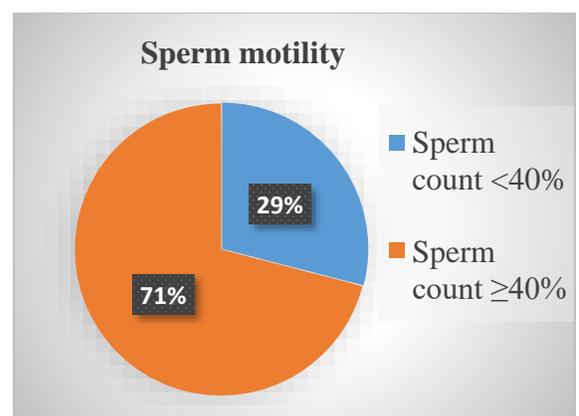


Figure 2. Sperm motility

Sperm morphology were analyzed microscopically after staining with May Grunwald Giemsa. Abnormal sperm morphology in subjects were deliberated at normal sperm morphology less than 4% of total sperm seen. All subjects had no normal sperms greater than 4%; however, 19 (31%) were azoospermic with no any sperm in semen. The rest of subjects, 43 (69%) had normal sperm morphology.

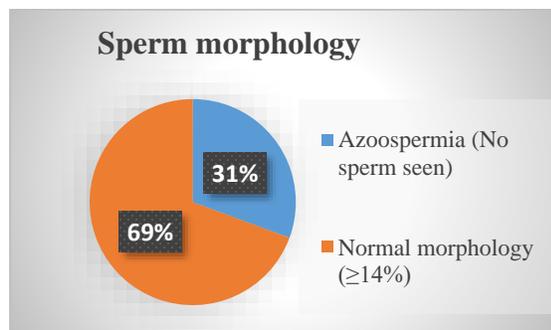


Figure 3. Sperm morphology

The semen and male reproductive hormones were tested in 62 male subjects. Among them, 18 (31%) were azoospermia; 15 (24%) were oligozoospermia; 17 (27%) were asthenozoospermia, 10 (16%) were normozoospermia and 1 (2%) was oligo- asthenozoospermia. Referring to the morphology, all tested subjects had normal sperm morphology according to the WHO semen analysis manual handbook fifth edition 2010 (Greater than 4% of normal sperm count).

One subjects (estimated to be 2%) had both oligozoospermia and asthenozoospermia (few sperm count with less motility). We categorized sperm quality of all tested subjects

according to the sperm count and sperm motility into 5 categories: azoospermia, 19 (31%); oligozoospermia, 15 (24%); asthenozoospermia, 17 (27%); normozoospermia, 10 (16%); and oligo-asthenozoospermia, 1 (2%).

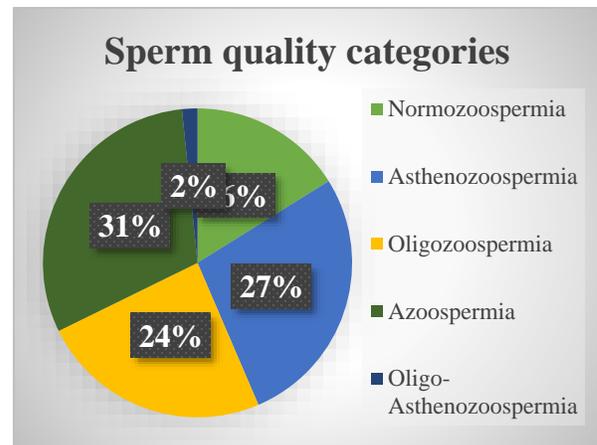


Figure 4. Sperm quality categories

FSH and LH showed strong negative correlation with sperm count which is more profound with FSH ($r = -0.722$, and $p < 0.05$) than LH ($r = -0.545$, and $P < 0.05$). Testosterone showed strong positive correlation with sperm count ($r = 0.712$, and $p < 0.05$).

FSH and LH showed a negative correlation with sperm motility which is more profound in FSH ($r = -0.312$, and $p < 0.05$) than LH ($r = -0.302$, and $p < 0.05$). Testosterone showed a positive correlation ($r = 0.360$, and $p < 0.05$).

FSH have strong positive correlation with LH ($r = 0.843$, and $p < 0.05$); while both FSH and LH showed a negative correlation with testosterone which is more profound in FSH ($r = -0.648$,

and $p < 0.05$) than LH ($r = -0.512$, and $p < 0.05$). Moreover, Sperm count was

positively correlated with sperm motility ($r = 0.540$ and $p < 0.05$).

Table 1. Correlations between sperm count, motility, FSH, LH and testosterone

VARIABLE		COUNT	TESTO	LH	FSH	MOTILITY
COUNT	Pearson Correlation	1	.712**	-.545**	-.722**	.540**
	Sig.(2-tailed)		.000	.000	.000	.000
	N	62	62	62	62	62
TESTOSTERONE	Pearson Correlation	.712**	1	-.512**	-.648**	.360**
	Sig.(2-tailed)	.000		.000	.000	.004
	N	62	62	62	62	62
LH	Pearson Correlation	-.545**	-.512**	1	.843**	-.302*
	Sig. (2-tailed)	.000	.000		.000	.017
	N	62	62	62	62	62
FSH	Pearson Correlation	-.722**	-.648**	.843**	1	-.312*
	Sig. (2-tailed)	.000	.000	.000		.013
	N	62	62	62	62	62
MOTILITY	Pearson Correlation	.540**	.360**	-.302*	-.312*	1
	Sig.(2-tailed)	.000	.004	.017	.013	
	N	62	62	62	62	62

****.** Correlation is significant at the 0.01 level (2-tailed).

*****. Correlation is significant at the 0.05 level (2-tailed).

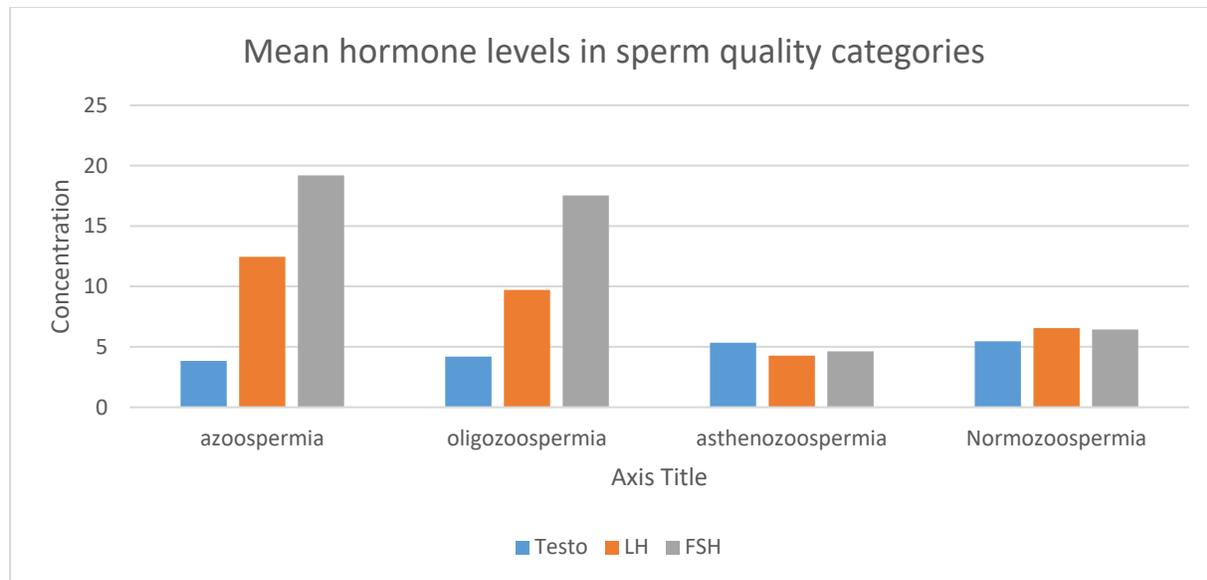


Figure 5. The mean levels of testosterone, LH and FSH in sperm quality categories

In azoospermic subjects, the mean level of gonadotropins were 19.20mIU/ml and 12.45mIU/ml for FSH and LH respectively. The mean level of testosterone was 3.83 ng/ml. In oligozoospermic subjects, the levels of FSH and LH were 17.53 mIU/ml and 9.71mIU/ml respectively, and testosterone was 4.19 ng/ml. In asthenozoospermic subjects, the levels of FSH and LH were 4.62mIU/ml and 4.27 respectively while the level of testosterone was 5.34 ng/ml. In normozoospermic subjects, the levels of FSH and LH were 6.43 mIU/ml and 6.55 mIU/ml while the level of testosterone was 5.46ng/ml.

Discussion

Male reproductive hormones initiate and maintain reproductive function, and they are associated with sperm quality.[6] In this study, found strong

negative correlation between sperm count and gonadotropins; FSH ($r = -0.712$, and $p < 0.05$), and LH ($r = -0.512$, and $p < 0.05$). Our study agreed with a study done by Ismael et al. (2017) that found significant negative correlation between serum FSH and sperm count, and elevated serum LH in azoospermia and oligozoospermia compared to the normozoospermia.[25] However, Dale et al. (2005) found no detectable endocrinopathy, and reported normal serum level of FSH, LH and Testosterone in most infertile men, but the study was done only in male with abnormalities in seminiferous tubule.[26]

FSH and LH are synergic to develop and maintain testicular function; LH regulates the synthesis of testosterone in Leydig cells while FSH control spermiogenesis and spermatogenesis.[14] Samal et al.

(2012) found that high concentration of FSH and LH in azoospermia and oligozoospermia are to stimulate leydig and sertoli cells for proportionate synthesis and secretion of testosterone to enhance spermatogenesis.[27] Abdalla et al. (2009) proved the evidence of increasing of FSH in azoospermia due to the impairment of sertoli cells functions, and result in decrease of testicular activity. Decrease in testicular activity cause alteration of the normal feedback mechanism regulating hormones secretion between testes and hypothalamic-pituitary axis.[28]

The study found a strong positive association between sperm count and serum testosterone ($r= 0.712$, and $p<0.05$). Our findings are supported by a study done by Muhammad et al. (2008) found a significantly decreased levels of testosterone in azoospermia and oligozoospermia compared to fertile group.[5] The results are opposed to the findings of Smith et al. (1985) who found normal testosterone levels in infertile men with Sertoli cell syndrome,(29) and Ismael et al. (2017) who found permissible levels of testosterone in azoospermia and oligozoospermia with the control group of normozoospermia.[25] A high level of testosterone is required for sperm production, improved motility and epididymis function. A failure of pituitary gland to secrete and excrete FSH and LH disrupt testicular function leading to infertility.[16,25]

The study found negative correlation between sperm motility and

gonadotropins, FSH ($r= -0.312$ and $P<0.05$), and LH ($r=-0.302$ and $p<0.05$). The results are supported by a study done Panali (2017) found a negative correlation between gonadotropins and sperm motility.[30] A study found positive correlation between testosterone and sperm motility ($r= 0.36$ and $p<0.05$). The results are supported by a study done by Meeker et al. (2013) who suggested a positive association between sperm motility and testosterone.[6]

Regarding hormones, a study found strong positive correlation between FSH and LH ($r= 0.84$ and $p<0.05$). This result is supported by a study done by Turek et al. (1995) who found increased serum levels of FSH and LH (gonadotropins) that might have disrupted spermatogenesis leading to infertility caused by decreased sperm count.[31] The results are also supported by a study done by Panali (2017) that found a correlation between FSH and LH.[30] A study found positive correlation between sperm count and sperm motility ($r= 0.54$ and $p<0.05$). This result is supported by a study done by Parinaud et al (2009) who found progressive linear correlation between sperm count and sperm motility.[32]

Jungwirth et al. (2015) suggested that screening of FSH, LH and testosterone in infertile male should be limited to abnormal semen parameters;[33] However, our study found significant correlation between FSH, LH and testosterone with both sperm count and sperm motility. Meeker et al. (2013) suggested use of

male reproductive hormones to predict semen quality in epidemiological studies due to the very low participation rates of semen quality studies.[6] Treatment with hormone replacement therapy may be effective for infertile men since it can stimulate tubular function, and hormonal measurement should predict response to a treatment.[14] Other studies should be done to investigate effectiveness of hormonal replacement therapy in Rwanda.

Limitations

A study was done in one referral hospital in Rwanda located in Southern province (Butare University Teaching Hospital), and should not be generalized to whole country.

Conclusion

Our study found correlation between sperm quality and male reproductive hormones. Gonadotropins negatively correlated with sperm count and sperm motility while testosterone showed positive correlation. Gonadotropins positively correlated with testosterone, and sperm count positively correlated with sperm motility. The previous studies had suggested use of reproductive hormones to predict semen quality in epidemiological studies, and in investigation of neuro-endocrine cause of infertility. However, an increase of endocrinopathy in infertile male rise the use of hormone replacement therapy. We recommend other study to investigate predictive power of male reproductive hormones measurement to predict

response to hormonal replacement therapy.

Conflict of interest

All authors declare there aren't the conflict of interest.

Authors' contribution

HMT, JN, JC N, S N, J M, V K and RR contributed with conception, design of the manuscript, interpretation, data analysis, and writing of the manuscript.

Acknowledgments

The authors are thankful to Mr. Ngirinshuti Vincent for his specific advices to the project.

This article is published open access under the Creative Commons Attribution-NonCommercial NoDerivatives (CC BYNC-ND4.0). People can copy and redistribute the article only for noncommercial purposes and as long as they give appropriate credit to the authors. They cannot distribute any modified material obtained by remixing, transforming or building upon this article. See <https://creativecommons.org/licenses/by-nc-nd/4.0/>

References

1. Inhorn MC, Patrizio P. Infertility around the globe: New thinking on gender, reproductive technologies and global movements in the 21st century. *Hum Reprod Update*. 2014;21(4):411–26.
2. Cook J. Reproductive Endocrinology in Infertility. *Lab Med*. 2004;35(3):558–69.
3. Sharma A. Male Infertility; Evidences, Risk Factors, Causes, Diagnosis and Management in Human. *Ann*

- Clin Lab Res. 2017;05(03):1–10.
4. Elhussein OG, Ahmed MA, Suliman SO, Yahya leena I, Adam I. Epidemiology of infertility and characteristics of infertile couples requesting assisted reproduction in a low-resource setting in Africa, Sudan. *Fertil Res Pract.* 2019;5(1):7–11.
5. Khan MS, Ali I, Tahir F, Khan GM. Simultaneous analysis of the three hormones involved in spermatogenesis and their interrelation ratios. *Pak J Pharm Sci.* 2008;21(4):344–9.
6. Meeker JD, Godfrey-Bailey L, Hauser R. Relationships between serum hormone levels and semen quality among men from an infertility clinic. *J Androl.* 2007;28(3):397–406.
7. Jordan K, Fromberger P, Stolpmann G, Muller JL. The role of testosterone in sexuality. *J Sex Med.* 2011;8(11).
8. Masoumi S. An Epdemologic Survey On The Causes Of Infertility In Patien Referred To Infertility Center In Fatimieh Hospital In Hamadan. *Iranian Journal Reproductive Medicine.* 2015;13(8):13 (8), 513–6. Available from: [https://www.ncbi.nlm.nih.gov/Pmc/Articles/PMC4637117/](https://www.ncbi.nlm.nih.gov/Pmc/Articles/PMC4637117/Pdf/Ijrm%0A-%0A13%0A-%0A513.Pdf%0A)
9. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol* [Internet]. 2015;13(1):1–9.
10. Dhont N. Clinical, epidemiological and socio-cultural aspects of -infertility in resource-poor settings. Evidence from Rwanda. *Facts, views Vis ObGyn.* 2011;3(2):77–88.
11. Abarikwu SO. Causes and Risk Factors for Male-Factor Infertility in Nigeria : A Review. 2013;17(December):150–66.
12. Griswold MD. Spermatogenesis: The commitment to Meiosis. *Physiol Rev.* 2016;96(1):1–17.
13. D. Chu DS. Spermatogenesis. Springer link. 2012;1(1):15–26.
14. Foresta C, Selice R, Ferlin A, Arslan P, Garolla A. Hormonal treatment of male infertility: FSH. *Reprod Biomed Online* [Internet]. 2007;15(6):666–72. Available from: [http://dx.doi.org/10.1016/S1472-6483\(10\)60533-0](http://dx.doi.org/10.1016/S1472-6483(10)60533-0)

15. Smith LB, Walker WH. The regulation of spermatogenesis by androgens. *Semin Cell Dev Biol.* 2014;30:2–13.
16. Appasamy M, Muttukrishna S, Pizzey AR, Ozturk O, Groome NP, Serhal P, et al. Relationship between male reproductive hormones, sperm DNA damage and markers of oxidative stress in infertility. *Reprod Biomed Online* [Internet]. 2007;14(2):159–65. Available from: [http://dx.doi.org/10.1016/S1472-6483\(10\)60783-3](http://dx.doi.org/10.1016/S1472-6483(10)60783-3)
17. Ahn HS, Park CM, Lee SW. The clinical relevance of sex hormone levels and sexual activity in the ageing male. *BJU Int.* 2002;89(6):526–30.
18. Hamidpour R, Rahan L. The Novel Remedy Useful for Maintaining Male Fertility. *Transl Biomed.* 2017;08(03):1–2.
19. Aksoy E, Aktan TM, Duman S, Cuce G. Assessment of Spermatozoa Morphology under Light Microscopy with Different Histologic Stains and Comparison of Morphometric Measurements. *Int J Morphol.* 2012;30(4):1544–50.
20. Wang C, Swerdloff RS. Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. *Fertil Steril* [Internet]. 2014;102(6):1502–7. Available from: <http://dx.doi.org/10.1016/j.fertnstert.2014.10.021>
21. Olooto W. Infertility in Male; Risk Factors, Causes and Management- A Review. *J Microbiol Biotechnol Res.* 2012;2(4):641–5.
22. Hamidpour R. *iMedPub Journals The Novel Remedy Useful for Maintaining Male Fertility.* 2017;1–2.
23. Khourdaji I, Lee H, Smith RP. Frontiers in hormone therapy for male infertility. *Transl Androl Urol.* 2018;7(I):S353–66.
24. WHO. WHO laboratory manual for the Examination and processing of human semen. Fifth edti. Vol. 238. Switzerland: WHO Library; 2010.
25. Ismael ZK, Al-Anbari LA, Mossa HAL. Relationship of FSH, LH, DHEA and testosterone levels in serum with sperm function parameters in infertile men. *J Pharm Sci Res* [Internet]. 2017;9(11):2056–61.
26. M. Dale MF. Endocrinology of male fertility and infertility. *Curr Opin Obstet Gynecol.* 1990;2(3):412–7.
27. Samal S, Dhadwe K. Indian Medical Gazette Original Study

- Epidemiological Study of Male Infertility. *Ind Med Gaz.* 2012;
28. Abdalla MI, Ibrahim II, Rizk AM, El Agouz WT, Girgis SM, Etriby AA, et al. Endocrine studies of azospermia. I. Serum steroid levels in sertoli cell only syndrome. *Syst Biol Reprod Med.* 1979;2(3):253–6.
29. Smith R, Thompson SG, Haines AP, Jeffcoate SL, Hendry WF. Plasma concentrations of pituitary and testicular hormones of fertile and infertile men. *Clin Reprod Fertil.* 1985;3(1):37–48.
30. Palani A. Impact of Male Reproductive Hormones on Sperm Activity via Regulation of Semen Antioxidant Levels. *J Garmian Univ.* 2017;4(ICBS Conference):497–504.
31. Turek PJ, Kim M, Gilbaugh JH, Lipshultz LI. The clinical characteristics of 82 patients with Sertoli cell-only testis histology. *Fertil Steril* [Internet]. 1995;64(6):1197–200. Available from: [http://dx.doi.org/10.1016/S0015-0282\(16\)57984-9](http://dx.doi.org/10.1016/S0015-0282(16)57984-9)
32. Parinaud J, Vieitez G, Moutaffian H, Milhet P. *Human Reproduction* vol . 11 no . 6 pp . 1240-1243 , 1996 Relationships between motility parameters , morphology and acrosomal status of human spermatozoa. 2019;2019.
33. Jungwirth A, T. Diemer, G.R. Dohle et al. Male Infertility EAU Guidelines on [Internet]. EAU Guidelines. European Association of Urology; 2016. 1–46 p. Available from: <https://uroweb.org/wp-content/uploads/EAU-Guidelines-Male-Infertility-2016-2.pdf>