



Received on 12 April, 2017; received in revised form, 13 August, 2017; accepted, 17 August, 2017; published 01 December, 2017

DIETARY HYPERCHOLESTEROLEMIA INDUCES OXIDATIVE STRESS CHALLENGING SPERMATOGENESIS IN RAT MODEL: A LINK TO POSSIBLE INFERTILITY

Anupam Biswas^{*1}, Urban J.A. D'souza² and Shankar Bhat³

Department of Physiology¹, Faculty of Medicine, Mahsa University, Kuala Lumpur - 59100, Malaysia.

Department of Physiology², KVG Medical College, Sullia - 574327, Karnataka, India.

Department of Physiology³, Yenepoya Medical College, Yenepoya University, Mangalore - 575022, Karnataka, India.

Keywords:

Hypercholesterolemia, Epidemic, Sperm morphology, Semen quality, Sperm count, infertility

Correspondence to Author:

Anupam Biswas

Senior Lecturer,
Faculty of Medicine
Department of Physiology,
MAHSA University, Jalan Elmu,
Off Jalan University, Kuala Lumpur-
59100, Malaysia.


E-mail: anupambiswas111@yahoo.co.in

ABSTRACT: Background: Hypercholesterolemia is rapidly becoming a worldwide epidemic that affects children and adults. Some attempt had made to generalize a relationship between hypercholesterolemia and infertility, but until now it remains scanty. Thus, the aim of the present study was initiated. **Methods:** Male Wistar rats were divided into four groups of six each (n=6). The first group was control and second, third and fourth groups were fed with pure cholesterol and cholic acid orally once a day for 30 days. Body weights were noted weekly. At the end of treatment duration all animals were sacrificed by cervical dislocation. Epididymis and testis were carefully removed and the organ weights were evaluated. Left epididymis was used for sperm count and sperm morphology. Blood sample were separated and used for determination of MDA level. Serum was used to determine the Total cholesterol (TC), Triglyceride (TG), HDL levels. LDL, VLDL levels were calculated. **Results:** Cholesterol-fed animals showed significant increases in obesity index and blood TABARS levels. TC and TG and LDL levels were also increased significantly. HDL level were decreased. Reproductive organ weights and sperm counts in the epididymis were significantly decreased. Furthermore, sperm abnormality was increased in high cholesterol fed animals as evidenced by a trend toward reduction in fertility potential. **Conclusion:** In conclusion, our data showed that diet-induced hypercholesterolemia adversely affects semen quality by reducing sperm count and a rise in altered sperm morphology. The low sperm quality may cause a reduction in fertility potential and lead to impairment in male fertility.

INTRODUCTION: Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. Cholesterol is a steroid lipid found in the cell membranes and transported in the blood plasma of all animals.

Cholesterol is an essential component of mammalian plasma membranes where it is required to establish proper membrane permeability and fluidity.¹ Within the plasma membrane, cholesterol has also been implicated in cell signaling processes.² Changes in the organization of membrane lipids can have profound consequences on cellular functions such as signal transduction and membrane trafficking.^{3,4}

However excessive levels of dietary cholesterol in blood circulation are strongly associated with progression of atherosclerosis which leads to cardiovascular diseases.⁵⁻⁸

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.8(12).5065-71</p>
<p>Article can be accessed online on: www.ijpsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8(12).5065-71</p>	

Hypercholesterolemia is a risk factor for non-insulin-dependent diabetes, osteoarthritis, some types of cancer, and certain reproductive and metabolic disorders.⁹ It is also associated with disturbance in the hormonal milieu that can affect the reproductive system, which is clear in women who present reproductive disorders when obese.¹⁰¹¹ However, in men this relationship is poorly characterized, due to the lower number of studies in the literature.^{12, 13}

In recent years, some studies have associated the body mass index (BMI) with reproductive parameters in men, showing that increased BMI is related to poor semen quality¹⁴, decreased sperm concentration,¹⁵ decreased normal-motile sperm cells and increased DNA fragmentation index.¹⁶ Experimental animals fed with saturated fat-enriched diets raise their plasmatic cholesterol levels and this would have impact on the cell-specific lipid equilibrium between cholesterol and phospholipids that organize the plasma membrane. The later modification could affect cellular functions as signal transduction pathways coupled to membrane cholesterol. Sperm membrane lipids are highly responsive to dietary manipulation.¹⁷ Lipid peroxidation is an important factor that may induce morphological changes in the spermatozoa.¹⁸

Cholesterol-rich diets have been shown to produce a decrease in sperm kinetics,¹⁹ and detrimental effects on leydig and sertoli cell secretory capacity in rabbits.²⁰ Moreover, previous works showed that human male infertility might be associated with altered lipid metabolism in seminal plasma.²¹ The present study aimed at investigating the effects of diet induced hypercholesterolemia on rat's sperm morphology.

MATERIALS AND METHODS:

Ethics statement: The animal studies described here were reviewed and approved by the Institutional animal ethical committee of KVG Medical College, Sullia. As per the guidelines of Committee for the control and supervision of experiments on animals (CPCSEA) and Animal Welfare Division.

Chemicals: Pure cholesterol purchased from Merck Specialities Private Ltd., Mumbai, cholic

acid, trichloro acetic acid and thiobarbituric acid purchased from Loba Chemie Private Ltd., Mumbai. All other reagents and chemicals used in the present study were of analytical grade (AR). Edible quality coconut oil was purchased from local market. Only double distilled water was employed for preparing the reagents.

Animals: Wistar strain male albino mature (12 - 15 weeks old) rats of approximately same age group, having body weight 170 - 220 grams were used from animal house of K.V.G. Medical College, Sullia, D.K. All animals were housed in polypropylene cages in groups of 6 to provide sufficient space and allowed to acclimatize for 2 weeks before study at laboratory conditions. Rats were maintained as per the standard condition with pellet diet (Gold Mohur-Lipton India Ltd., Mumbai) and water *ad libitum*.

Induction of Experimental Hypercholesterolemia: In order to induce hypercholesterolemia the method reported by Bopanna *et al.*, was followed.²² Hyperlipidemia has been induced by feeding pure cholesterol and cholic acid orally mixing with coconut oil with three different doses (*i.e.* 0.25%, 0.5% and 1%) pure cholesterol and cholic acid, the ratio between cholesterol and cholic acid was 2:1 for 30 days.

Experimental Groups and Study Design: After the acclimatization period 24 Male Wistar rats(170-220gm) were randomly divided into two groups. Experimental (18 rats) and control (6 rats) groups. The experimental groups animals were given pure cholesterol and cholic acid with 2:1 ratio daily once, orally with the help of oral gavage for 30 days. First treatment day with cholesterol was considered treatment day 1. Body weight of each rat was noted down end of every week. Control group of animals received normal diet and water only. After 24 hrs of last treatment, on 31st day the animals were sacrificed by cervical dislocation. Thorax was opened and heart exposed and blood sample was collected by cardiac puncture. Portions of blood sample were centrifuged and plasma were separated and used for determination of TBARS level. Estimation of Lipid peroxidation marker-Thiobarbituric acid reactive substances (TBARS) levels in the plasma were determined by the method described by Okhawa *et al.*²³

The remaining blood was allowed to clot in a test tube and serums were collected. The serum was used to determine the total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) levels enzymatically, using commercially available kits E-Coline (Merck). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels were estimated indirectly by using the formula: $VLDL = TG/5$, $LDL = TC - HDL - VLDL$. Simultaneously, the epididymis and testis were carefully removed and weights were noted. One epididymis from each rat was used for sperm count and sperm morphological examination.

Sperm Morphology Assay: The assay were conducted by the methods reported by D'Souza et al.²⁴ Left epididymis was cut into small pieces with 1ml PBS (Phosphate Buffer Saline, pH 7.2) and suspension were prepared after filtering the buffer solution with 80 micron nylon mesh, this suspension were stained with (10:1) eosin Y and smears were made on the cleaned glass slides, these slides were used to evaluate the sperm morphology. From each slide two hundred sperms were examined with 200/400X magnifications.

Epididymal Sperm Count: The epididymal sperm count were conducted by the procedure described by D'Souza et al.²⁵ The same suspension was used for the sperm count analysis. It was calculated with the help of haemocytometer. The suspension was pipette out up to 0.5 marks and diluted with the buffer solution up to 11 marks. The dilution factor was 1:20. After charging, all 8 square (1mm^2) were counted. Total sperm count/ epididymis were determined by multiplying with 5×10^6 .

Statistical Analysis: All the data was expressed as Mean \pm SEM. Analyzed by using one - way ANOVA, followed by Bonferoni post - hoc test. The statistical test was obtained from statistical package of social sciences (SPSS) version 19, 2007. Values of $P < 0.05$ were considered as statistically significant.

RESULTS:

Changes of Body Weights: As shown in the Table 1, feeding normal rats on basal diet supplemented with different doses cholesterol and cholic acid for four 30 days, highly significantly ($p < 0.001$) increased body weight as compared to the control

rats in the 0.5% and 1% cholesterol fed groups. In the 0.25% cholesterol fed group, body weight was increased significantly ($p < 0.05$) as compared to the control group animals.

TABLE 1: BODY WEIGHT GAINS DURING THE HYPERCHOLESTEROLEMIA INDUCTION

Group	Cholesterol fed for 30 Days	
	Initial(g)	Final(g)
Control	191.33 \pm 2.29	215 \pm 3.22
Hyperchol with 0.25%	200.66 \pm 3.48	286 \pm 1.93*
Hyperchol with 0.5%	199.5 \pm 2.68	298.66 \pm 3.48**
Hyperchol with 1%	191 \pm 1.77	300 \pm 1.15**

The values are expressed in Mean \pm SE

*The mean difference is significant at the ($p < 0.05$) level, ** The mean difference is highly significant at the ($p < 0.001$) level.

Changes of Reproductive organ weights: Table 2 showed that the testis weight and epididymal weight were decreased in all the cholesterol fed groups animals, but no significant changes were noticed as compared to control group rats.

TABLE 2: EFFECT OF REPRODUCTIVE ORGAN WEIGHT IN CONTROL AND EXPERIMENTAL GROUPS ANIMALS

Group	Testis weight (gm)	Epididymis weight (gm)
Control	1.32 \pm 0.0640	0.282 \pm 0.01
0.25% cholesterol fed	1.27 \pm 0.016	0.262 \pm 0.001
0.5% cholesterol fed	1.26 \pm 0.011	0.259 \pm 0.002
1% cholesterol fed	1.2 \pm 0.02	0.247 \pm 0.002

The values are expressed in Mean \pm SE

Effects on Lipid profile: Hypercholesterolemia (1%) induced animals showed highly significant ($p < 0.001$) levels of elevated total serum cholesterol with respect to control animals. Whereas 0.25% and 0.5% cholesterol fed groups animals showed significant ($p < 0.05$) rise in total cholesterol levels. Hypercholesterolemia induced animals showed an increased level of triglyceride as compare to normal control animals but it was statistically insignificant.

In all the hypercholesterolemic groups HDL level descended. VLDL levels were slightly increased in all the cholesterol fed groups. There was highly significant ($p < 0.001$) increased serum LDL level in all hypercholesterolemic rats. Lipid peroxidation marker level was highly significantly ($p < 0.001$) increased in all the hypercholesterolemic groups as compared to the control group animals (Table 3).

TABLE 3: EFFECT OF LIPID PROFILE IN CONTROL AND EXPERIMENTAL GROUPS

Lipid profile	Control	0.25% Chol	0.5% Chol	1% Chol
TC	91.79±4.75	181.23±3.46*	184.06±2.32*	199.92±4.51**
Trig	225.27±31.11	239±13.39	240.01±8.92	235.13±11.91
HDL	22.6±1.55	22.13±0.9	21.48±0.99	20.89±0.97
VLDL	45.1±6.23	47.79±2.68	47.99±1.78	47.02±2.38
LDL	25.78±5.54	111.3±4.97**	114.58±2.16**	132±2.29**
MDA	350.63±17.9	720.81±31.2**	770.06±46.18**	860.02±41.72**

The values are expressed in Mean ± SE

* The mean difference is significant at the (p<0.05) level, ** The mean difference is highly significant at the (p< 0.001) level.

Effects of hypercholesterolemia on sperm count and sperm morphology: Experimentally produced hypercholesterolemic animals showed insignificant reduced sperm count in all cholesterol fed groups as compared to the control rats. The rats in the

control group had more number of normal sperms. Hypercholesterolemic groups showed less normal sperms and more abnormal sperms dose dependently (**Table 4**).

TABLE 4: EFFECT OF HYPERCHOLESTEROLEMIA ON SPERM COUNT AND SPERM MORPHOLOGY

Group	Sperm Count x10 ⁶	Sperm morphology (%)	
		Normal sperm (%)	Abnormal sperm (%)
Control	49.6±2.56	80.5±0.56	19.5±0.56
0.25% cholesterol fed	41.67±2.04	73.33±1.42	26.66±1.42
0.5% cholesterol fed	39.74±1.95	62.5±2.52	37.5±2.52
1% cholesterol fed	38.98±1.33	53.33±1.42	46.66±1.42

The values are expressed in Mean ± SE

Normal sperm morphology (**Fig. 1**). Hypercholesterolemia induces different types of head abnormality on rat sperms. Head deformities were double head; head less, hookless, amorphous, microcephaly and macrocephaly sperm (**Fig. 2**). Hypercholesterolemia also caused tail deformity in

the sperm morphology. Tail abnormality was double tail, coiled, bent, looped, folded tail, broken and tail less sperm. (**Fig. 3 and 4**). **Fig. 5** showed hypercholesterolemia leads both head and tail abnormality to the sperm morphology.

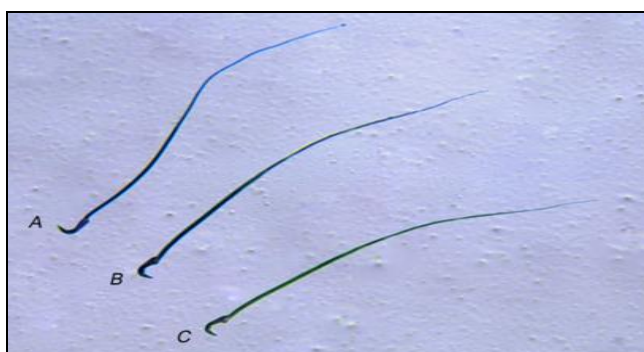


FIG. 1: PHOTOMICROGRAPH OF NORMAL RAT SPERM. (ORIGINAL MAGNIFICATIONS 400X)

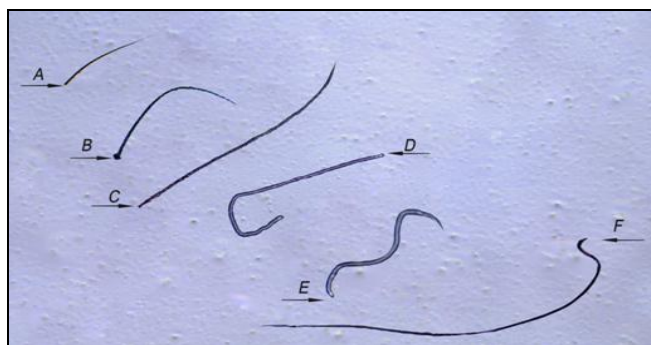


FIG. 2: PHOTOMICROGRAPH OF ABNORMAL SPERMS WITH HEAD ABNORMALITY. HEADLESS (A, C, D, E), AMORPHOUS (B), BANANA SHAPED (F), (ORIGINAL MAGNIFICATIONS 400X)

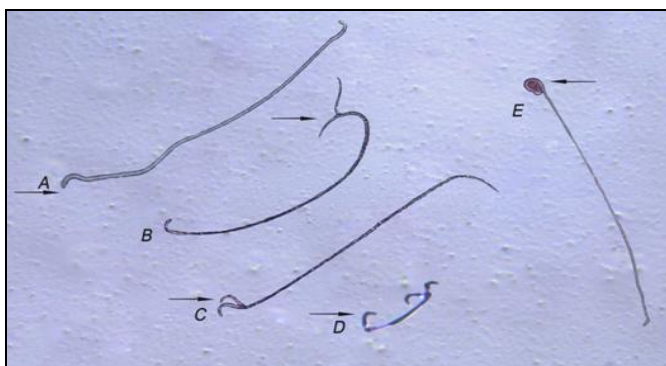


FIG. 3: PHOTOMICROGRAPH OF DOUBLE HEAD (A, C, D) ABNORMAL SPERM, B WITH DOUBLE TAIL, E WITH AMORPOUS HEAD. (ORIGINAL MAGNIFICATIONS 400X)

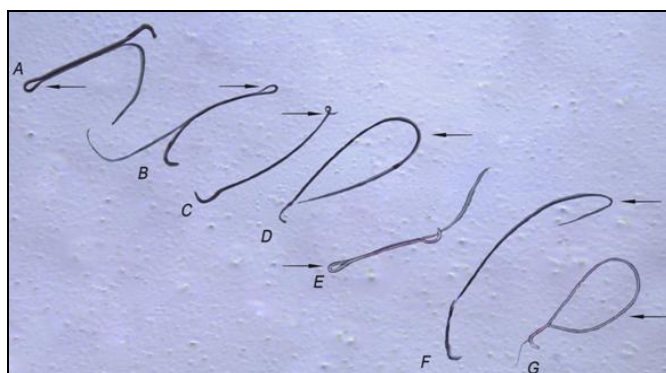


FIG. 4: PHOTOMICROGRAPH OF SPERMS WITH COILED TAIL ABNORMALITY A, B, C, E, G. D AND F WITH BEND TAIL. (ORIGINAL MAGNIFICATION 400X)

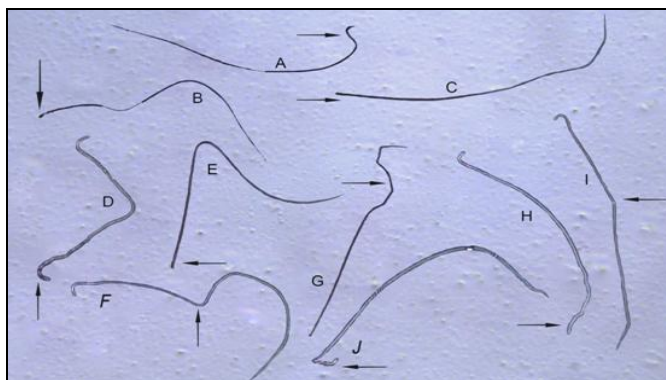


FIG. 5: PHOTOMICROGRAPH OF DIFFERENT SPERMS WITH HEAD AND TAIL ABNORMALITIES. (ORIGINAL MAGNIFICATION 400X)

DISCUSSION AND CONCLUSION: Cholesterol the precursor of all sex steroid hormones, has a pivotal function in all areas of male and female reproductive physiology, from sex differentiation to gamete production.²⁶ It is believed that with the increasing prevalence of sedentary lifestyles and dietary changes, hypercholesterolemia is emerging, in turn, as an important cause of adverse health outcomes, including male infertility.²⁷

Data from different population studies show an inverse relationship between BMI (body mass index) and fertility.²⁸ Although the mechanism by

which fertility is affected is still unclear.²⁹ The high-fat diet used in the present study was effective in promoting hypercholesterolemia, as demonstrated by an increased lipid levels in association with a higher body weight. This phenomenon favors the deposition of lipids such as triacylglycerol in adipose tissue, leading to an increase in body weight.^{30, 31} An important sperm parameter for evaluating male fertility is sperm morphology because it may indicate cytotoxic events.³² The morphological alterations in hypercholesterolemic animals indicate a high probability of adversely affect spermatogenesis.

It has been reported that phospholipids and fatty acid composition of spermatozoa are altered in men with infertility.³³ It has been very difficult to pinpoint a strong correlation between the serum lipids concentrations and variations in seminal parameters. The lipid composition and testicular function in mammals showed high lipid levels exerted direct adverse effects at the testicular levels.³⁴

Another study showed that high lipid levels were common in patients with azoospermia. The incidence of hypercholesterolemia and hypertriglyceridemia among infertile men is 65%.³⁵ There was a correlation between abnormal semen quality with high levels of cholesterol and triglyceride. Our results showed that high level of triglyceride and cholesterol were related with abnormal sperm morphology. Triglycerides, which are metabolic energy sources in spermatozoa, have substrates to produce glycerol in mature mammalian sperm.

However, elevation in triglycerides concentration may have deleterious effects on spermatogenesis. Hypercholesterolemia and hypertriglyceridemia was related with decreased capacity of sperm acrosome reaction in rabbits.³⁶ Hypercholesterolemia also may have a detrimental effect on sperm capacity to penetrate and fertilize oocytes in rabbit.³⁷ It has been shown that epididymal dysfunction in hypercholesterolemic animals may have detrimental effects on the cytostructural modifications and biochemical changes that occur during sperm epididymal maturation and may result in increase sperm morphometric abnormality.³⁸

In conclusion, the results showed that the serum lipids are adversely related with sperm physiology in hypercholesterolemic rats. The molecular pathways involved thus need further investigation and could yield new insights into the concept of lipid-related sperm maturation.

Further studies with unexplained etiology will help to elucidate the underlying mechanism and probably lead to therapeutic options. This subject needs further investigations, in complement of the research made on the testis, and this field of research will without any doubt bring new insights in the understanding of the complex outcome of

male infertility, a point emphasized by the growing proportion of males in their reproductive age, with dyslipidemia, having difficulties to conceive. In conclusion, diet induced hypercholesterolemia results in elevated oxidative stress and affects the sperm parameters in a negative way possibly affecting the fertility of male population. Clinical studies on obese males may support these findings.

ACKNOWLEDGEMENT: We thank our colleagues from KVG Medical College who provided valuable insight and expertise that greatly assisted this research work. We thank to KVG Ayurveda Medical College for supporting us during the hibiscus flower extraction procedure.

CONFLICT OF INTEREST: The authors are declared that there is no conflict of interest exists.

REFERENCES:

1. Yeagle PL: Cholesterol and the cell membrane. *Biochim. Biophys Acta* 1995; 9: 267–87.
2. Simons K, Eehalt R: Cholesterol, lipid rafts and disease. *J Clin Invest* 2002; 110: 597–603.
3. Visconti PE, Ning X, Fornes MW, Alvarez JG, Stein P: Cholesterol efflux-mediated signal transduction in mammalian sperm: cholesterol release signals an increase in protein tyrosine phosphorylation during mouse sperm capacitation. *Dev Biol* 1999;2: 429–43.
4. Kannel WB, Castelli WP, Gordon T: Cholesterol in the prediction of atherosclerotic disease: new perspectives based on the Framingham Study. *Ann Intern Med* 1979; 90: 85–91.
5. Eyster KM: The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist. *Adv Physiol Educ* 2007; 31: 5–16.
6. Asashina, M Sato and Imaizumi K: Genetic analysis of diet induced hypercholesterolemia in exogenously hypercholesterolemic (ExHC) rats. *J. Lipid. Res* 2005; 46: 2289 - 2294.
7. Rerkasen, K Gallagher PJ, Grimble RF, Calder PC and Shearman CP: Managing hypercholesterolemia and its correlation with carotid plaque morphology in patients undergoing carotid endarterectomy. *Vascular Health Risk Manage* 2008; 6:1259-1264.
8. Ramachandran HD, Narasimhamurthy K and Raina PL: Modulation of cholesterol induced hypercholesterolemia through dietary factors in Indian desert gerbils (*Meriones hurricinae*). *Nutr. Res* 2003; 23: 245-256.
9. Mayes JS, Watson GH: Direct effects of sex steroid hormones on adipose tissues and obesity. *Obes Rev* 2004; 5:197-216.
10. Pasquali R, Pelusi C, Genghini S, Cacciari M, Gambineri A: Obesity and reproductive disorders in women. *Hum Reprod Update* 2003; 9:359-372.
11. Robker RL: Evidence that obesity alters the quality of oocytes and embryos. *Pathophysiology* 2008; 15:115-121.
12. Hammoud AO, Wilde N, Gibson M, Parks A, Carrell DT, Meikle W: Male obesity and alteration in sperm parameters. *Fertil Steril* 2008; 90:2222-2225.

13. Chavarro JE, Toth TL, Wright DL, Meeker JD, Hauser R: Body mass index in relation to semen quality, sperm DNA integrity and serum reproductive hormone levels among men attending an infertility clinic. *Fertil Steril* 2010; 93: 2222-2231.
14. Magnúsdóttir EV, Thorsteinsson T, Thorsteinsdóttir S, María Heimisdóttir M, Ólafsdóttir K: Persistent organochlorines, sedentary occupation, obesity and human male subfertility. *Hum Reprod* 2005; 20: 208-215.
15. Jensen TK, Andersson AM, Jørgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE: Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril* 2004; 82:863-870.
16. Kort HI, Massey JB, Elsner CW, Mitchell-Leaf D, Shapiro DB, Witt MA, Roudebush WE: Impact of Body Mass Index Values on Sperm Quantity and Quality. *J Androl* 2006; 27:450-452.
17. Diaz Fondetvilla M, Bustos-Obregon E: Cholesterol and polyunsaturated acid enriched diet: Effects on Kinetics of the acrosome reaction in rabbit spermatozoa. *Molecular Reproduction and Development* 1993; 35: 176-186.
18. Sanchez ET, ML Marquette, DB Brown and NH Ansari: The effect of oxidative stress on human sperm morphology. *Fertil. Steril* 2006; 861.
19. Diaz Fondetvilla M, Bustos-Obregon E, Fornes MW: Distribution of filipin-sterol complexes in sperm membranes from hypercholesterolemic rabbits. *Andrologia* 1992; 24: 279-283.
20. Yamamoto Y, Shimamoto K, Sofikitis N, Miyagawa I: Effects of hypercholesterolaemia on Leydig and Sertoli cell secretory function and the overall sperm fertilizing capacity in the rabbit. *Human Reproduction* 1999; 6: 1516-21.
21. Sebastian SM, Selvaraj S, Aruldas M, Govindarajulu P: Pattern of neutral phospholipids in the semen of normospermic, oligospermic and azospermic men. *Reprod. Fertil* 1987; 79: 373-378.
22. Bopanna KN, Bhagyalakshmi N, Rathod SP, Balaraman R, Kannan J. Cell culture derived Hemidesmus indicus in the prevention of hypercholesterolemia in normal and hyperlipidemic rats. *Ind J Pharmacol* 1997; 19:105-6.
23. Okhawa H, Qohishi N, Yagi K: Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979; 95:351-358.
24. D'souza UJ: 5-Fluorouracil-induced sperm shape abnormalities in rats. *Asian Journal of Andrology* 2003; 5: 353-356.
25. D'Souza UJ: Effect of tamoxifen on spermatogenesis and tubular morphology in rats. *Asian Journal of Andrology* 2004; 6: 223-226.
26. Fabrice Saez, Aurelia Ouvrier and Joel R Drevet. Epididymis cholesterol homeostasis and sperm fertilizing ability. *Asian Journal of Andrology* 2011; 13:11-17.
27. Hammoud AO, Gibson M, Peterson CM, Hamilton BD, Carrell DT: Obesity and male reproductive potential. *J Androl* 2006; 27:619-626.
28. Magnúsdóttir EV, Thorsteinsson T, Thorsteinsdóttir S, María Heimisdóttir M, Ólafsdóttir K: Persistent organochlorines, sedentary occupation, obesity and human male subfertility. *Hum Reprod* 2005; 20:208-215.
29. Viguera-Villasenor RM, Rojas-Castaneda JC, Chavez-Saldana M, Gutierrez- Perez O, Garcia-Cruz ME, Cuevas-Alpuche O, Reyes-Romero MM, Zambrano E: Alterations in the spermatid function generated by obesity in rats. *Acta Histochem* 2011; 113:214-20.
30. Schrauwen P, Westerterp KR: The role of high-fat diets and physical activity in the regulation of body weight. *Br J Nutr* 2000; 84:417-427.
31. Tentolouris N, Pavlatos S, Kokkinos A, Perrea D, Pagoni S, Katsilambros N: Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat. *Metabolism* 2008; 57:313-320.
32. Plassmann S, Urwyler H: Improved risk assessment by screening sperm parameters. *Toxicol Lett* 2001; 119:157-171.
33. Gulaya NM, Margitch VM, Calcada L: Phospholipid composition of human sperm and seminal plasma in relation to sperm fertility. *Arch Androl* 2001; 46: 169-175.
34. Padron RS, Mas I, Boston P: Lipid and testicular function. *Int Urol Nephrol* 1989; 21: 515-519.
35. Ramirez-Torres MA, Carrera A, Zambrana M: High incidence of hyperestrogenemia and dyslipidemia in a group of infertile men. *Ginecol Obstet Mex* 2000; 68: 224-229.
36. Diaz-Fontdevilla M, Bustos-Obregon E: Cholesterol and polyunsaturated acid enriched diet: Effects on kinetics of the acrosome reaction in rabbit spermatozoa. *Mol Reprod* 1993; 35: 176-180.
37. Yamamoto Y, Shimamoto K, Sofikitis N, Miyagawa I: Effects of hypercholesterolaemia on leydig and sertoli cell secretory function and the overall sperm fertilizing capacity in the rabbit. *Hum Reprod* 1999; 14: 1516-1521.
38. Glander HJ, Schiller J, Süß R, Paasch U, Grunewald S, Arnhold J: Detioration of spermatozoal plasma membrane is associated with an increase of sperm lysophosphatidylcholines. *Andrologia* 2002; 34: 360 - 366.

How to cite this article:

Biswas A, D'souza UJA and Bhat S: Dietary hypercholesterolemia induces oxidative stress challenging spermatogenesis in rat model: a link to possible infertility. *Int J Pharm Sci Res* 2017; 8(12): 5065-71. doi: 10.13040/IJPSR.0975-8232.8(12).5065-71.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)