Effect of cholesterol diet on reproductive function in male albino rats

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ABSTRACT

Objective: The present study was carried out to investigate the effect of cholesterol diet (400 mg/kg body weight) for 60 days on gonadal function in albino rats.

Methods: The study was conducted in the Animal House Unit at Jordan University of Science and Technology, School of Medicine, Irbid, Jordan between October 2003 and February 2004. Adult male and female albino rats of Sprague Dawley strain were raised under controlled temperature and light. Male rats were divided into: a) control group - rats receiving vehicle (olive oil) for 60 days and treatment group - rats receiving cholesterol diet for a reproductive cycle. Animals were weighed and autopsied 24 hours after the last dose. Biochemical and histological approaches were used to assess fertility in both groups.

Results: The treatment caused significant reduction ($p \leq 0.001$) in sperm motility and density in cauda epididymides and testes. A significant reduction ($p \leq 0.001$) in epithelial cell height of caput, cauda and seminal vesicle was also observed. In the treated group, there was a significant reduction ($p < 0.001$) in seminiferous tubules diameter and Leydig cell nuclear diameter. Spermatocytes (primary and secondary) were significantly decreased ($p \leq 0.01$) and spermatids were significantly reduced ($p \leq 0.001$) in the treatment group. Whereas, the number of degenerating Leydig cells (interstitial cells) increased significantly ($p \leq 0.001$). Serum biochemistry reveals significant increase ($p \leq 0.001$) in cholesterol and triglyceride levels. The intragastric administration of cholesterol diet to male rats for 60 days significantly reduced the number of females impregnated by these males. However, the number of implantations and number of viable fetuses were significantly ($p < 0.01$) decreased in female rats impregnated by males that ingested cholesterol. On the other hand, the number of resorptions was significantly ($p < 0.01$) increased in females impregnated by males that ingested cholesterol. The histometry and histology of reproductive organs confirm these results.

Conclusion: Hyperlipidemia can cause alteration in the biochemistry and histometry of reproductive organs and can cause inhibition of spermatogenesis via the Leydig cell.

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Throughout the world millions of deaths each year are attributed to coronary heart disease.[1-5] Men have higher death rate than women, but male to female ratio declined with increasing age[6] although, mortality rates from coronary heart disease in the elderly have decreased since 1968.[7] Increasing hospitalization rates and utilization of other heart care services emphasize the need for more vigorous efforts on preventing risk factors for development of coronary heart disease.

Number of risk factors have been identified, which can be classified in 2 categories: primary factors, which include those factors that cannot be altered or non-modifiable factors such as age, gender, race and familial causes. Whereas, in the second category includes all those factors that can be controlled or modifiable such as hypertension, obesity, lack of exercise, cigarette smoking, diabetes, renal disease and liver diseases .[8-10]

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A considerable work has been carried out on hyperlipidemia in relation to body metabolism and function status in the animal kingdom,[11] but the relationship between hyperlipidemia and gonadal functions remained unexplored. The present study was undertaken with the object of detailed and intensive investigation into the effect of feeding hyperlipidemia diets (cholesterol mixed with olive oil) on gonadal function in albino rats.

Method. The study was conducted at Jordan University of Science and Technology, School of Medicine, Irbid, Jordan between October 2003 and February 2004. Adult male and female albino rats of Sprague Dawley strain, weighing about 300 gm were raised in the Animal House Unit under controlled temperature of 21 ± 1°C and 12 hours light and 12 hours darkness schedule. Food and water were available ad labitum. Male rats were divided into 2 groups: control group: rats of this group received vehicle (olive oil) for 60 days. The treatment group: rats of this group received cholesterol diet (400 mg/kg body weight) for a reproductive cycle, 60 days.

After 24 hours of the last dose, the animals were weighed and autopsied under light ether anesthesia. Blood was collected through cardiac puncture using a dry and clean syringe for serum studies. Fertility was estimated in adult male rats treated with cholesterol diet and in control males counterparts. Each male was placed in an individual cage with 2 virgin untreated females of the same strain; they were left together for 10 days during which 2 estrous cycles should have elapsed.[12] One week after the removal of the exposed males, females were sacrificed by cervical dislocation under light ether anesthesia and the number of pregnant females, number of implantation sites, number of viable fetuses and number of resorptions were recorded.

To determine the sperm motility and sperm counts, 100 mg of cauda epididymides were minced in 2 ml of physiological saline. One drop of evenly mixed sample was applied to a Neubauer's counting chamber under coverslip. Quantitative motility expressed as percentage was determined by counting both motile and immotile spermatozoa per unit area. Cauda epididymal and testicular sperm counts were made by routine procedure and expressed as million/ml of suspension.[13]

The initial and final body weights of the animals were recorded. The reproductive tract was taken out trimmed free of fat and each organ was weighed separately on electronic balance. The reproductive organs taken into account for study in male included testes, epididymides, ventral prostrate, seminal vesicle, vas deferens. Reproductive organs along with a small piece of liver, heart and kidney were fixed in Bouin's fixative for histological studies. The Bouin's fixed reproductive organs (testes, epididymides, seminal vesicle, ventral prostate, vas deferens) along with liver, kidney and heart muscles were cut into small pieces and processed. The paraffin embedding was followed by section cutting (5 mm) and staining (Harris hematoxylin and eosin).

With the help of Camera Lucida hundred circular appearing seminiferous tubules were traced at x80 and the diameter of each tubule was measured separately. The measurement was expressed in terms of mean of all the traced tubules. Similarly, Leydig cell nuclei were traced at x800. The epithelial cell height of cauda epididymides, caput epididymides and seminal vesicle were also traced at x360. Spermatogenic elements, example, spermatogonia, spermatocytes and spermatids were counted in 5 mm thick cross sections of 10 seminiferous tubules in 10 animals of each group. All raw counts were transformed to true counts by an adaptation of Abercrombie formula from germ cell diameter measurement.[14]

Interstitial cell types such as fibroblast, immature and mature Leydig cells and degenerating cells were estimated, applying a differential count over 200 cells population and statistically verified by the binomial distribution.[19] Total protein, cholesterol, triglycerides, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) using commercial kits from Cis BIO International) Gif sur Yvette, France). Plasma follicle-stimulating hormone (FSH) and testosterone concentrations was measured by radioimmunoassay using 2 commercial kits from Cis BIO International) Gif sur Yvette, France). All the values of body/organ weight biochemical estimation and histometry were expressed in terms of mean value ± standard deviation. The different treatment groups were compared with control group using chi-square test and student's "T" test.[16]

Results. **Table 1** shows that intragastric administration of cholesterol diet can cause increase in body weight, when initial and final body weights were compared in experimental group versus control group. The weight of the testes, epididymides, seminal vesicle, ventral prostate and vas deferens were significantly decreased in treated male rats compared to control group.

The motility of sperm in cauda epididymis was significantly ($p<0.001$) decreased in treated animals that ingested cholesterol in comparison with control. Sperm density in treated animals, the seminiferous tubule diameter and Leydig cell nuclear diameter of treated male was also significantly ($p<0.01$) decreased. Epithelial cell height in epididymides (cauda and caput) and seminal vesicle were significantly ($p<0.01$) decreased (**Table 2**).

Table 1 - Body and organ weights of cholesterol fed male rats.

Treatment	Body weight (gm)		Testes	Epididymides	Seminal vesicle	Ventral prostate	Vas deferens
	Initial	Final	(mg/ 100 gm body weight)				
Control group	304 ± 5.8	319 ± 42.65	895 ± 15.21	389 ± 7.61	376 ± 4.38	226 ± 4.1	68.8 ± 3.36
Treatment group	237 ± 8.55	361 ± 10.6†	789 ± 12.1†	343.1 ± 5.33†	348.1 ± 8.1†	167.8 ± 4.35†	62.2 ± 2.20*

Results are expressed as mean ± SD, 10 rats were included per group.
* $p<0.05$, † - $p<0.01$ - significantly different from control group (Student's t-test).

Table 2 - Histometrical parameters and sperm dynamics of cholesterol fed male rats.

Treatment	Sperm motility	Sperm density million/ml		Seminiferous tubule diameter (μm)	Leydig cell nuclear diameter (μm)	Epithelial height		
	Cauda	Testes	Cauda			Caput (μm)	Caput (μm)	Seminal vesicle (μm)
Control group	74.1 ± 1.94	4.75 ± 0.47	56 ± 1.94	290.6 ± 3.2	6.45 ± 0.96	38.8 ± 0.4	26.08 ± 1.32	17.32 ± 1.17
Treatment group	43.26 ±1.08†	2.88 ± 0.14†	37.185 ± 1.08†	266.27 ± 2.35*	3.79 ± 0.762†	24.68 ± 1.14†	18.4 ± 1.08*	12.45 ± 1.27*

Results are expressed as mean ± SD, 10 rats were included per group.
* - $p< 0.01$,† - $p< 0.001$ significantly different from control group (Student's t- test).

Table 3 - Testicular cell population dynamics of cholesterol fed intact male albino rats.

Treatment	Germinal cell types				Interstitial cell type			
	Spermatogonia	Spermatocyte (primary)	Spermatocyte (secondary)	Spermatids	Fibroblast	Immature Leydig cell	Mature Leydig cell	Degenerating cell
Control group	23.99 ± 2.93	18.85 ± 1.80	64.126 ± 3.51	147.71 ± 4.87	63.83 ± 1.64	65.195 ± 3.47	70.64 ± 1.03	18.34 ± 1.67
Treatment group	17.05* ± 4.44	12.96 ± 2.41†	17.97 ± 3.73‡	9.32 ± 6.82‡	58.66 ± 1.33*	41.66 ± 1.65‡	46.66 ± 1.78†	63.9 ± 1.76‡

Results are expressed as mean ± SD, 10 rats were included per group.
* - $p<0.05$, † - $p<0.01$, ‡ - $p<0.001$ significantly different from control group (Student's t- test).

Table 4 - Serum biochemistry of cholesterol fed intact male albino rats.

Treatment	Glucose	Cholesterol	Triglycerides	Bilirubin	SGOT	SGPT	Testosterone	FSH
	Mmol			μmol	U/L		μmol	IU/L
Control group	7.3 ± 0.212	1.4 ± 0.47	0.7 ± 0.07	3.175 ± 0.142	36.7 ± 1.98	77.7 ± 7.14	14.4 ± 2.53	21.87 ± 2.47
Treatment group	7.86 ± 1.03*	2.3 ± 0.7	1.92 ± 0.05†	3.42 ± 0.22*	83.11 ± 2.22*	92.75 ± 8.87*	7.06 ± 1.88‡	17.83 ± 3.66†

Results are expressed as mean ± SD, 10 rats were included per group.
* - $p<0.05$, † - $p<0.01$, ‡ - $p<0.001$ significantly different from control group (Student's t- test).
SGOT - serum glutamic oxaloacetic transaminase, SGPT - serum glutamic pyruvic transaminase, FSH - follicle stimulating hormone.

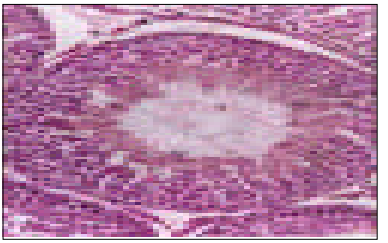

Figure 1 - Microphotograph of testis showing normal spermatogonial elements. Hematoxylin and eosin x200.

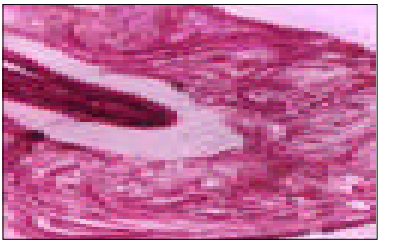

Figure 3 - Microphotograph of cauda epididymis showing normal epithelium. The lumen is full with a large number of mature spermatozoa. Hematoxylin and eosin x200.

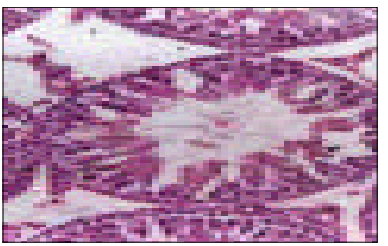

Figure 2 - Microphotograph showing the degenerating spermatogenic cells and reduced seminiferous dimensions. Hematoxylin and eosin x200.

Figure 4 - Microphotograph of cauda epididymis showing reduced in epithelial cell height, few spermatozoa are present in lumen. Hematoxylin and eosin x200.

Administration of cholesterol diet caused a significant decrease in the germinal cell population: spermatocytes: primary (p<0.001) and secondary (p<0.001) and spertmatids were decreased to a significant level (p<0.001). Similarly the immature and mature Leydig cells numbers were also significantly (p<0.001) decreased. However, the degenerating cells number was significantly (p<0.001) increased. Fibroblast and spermatogonia numbers were not significantly altered (**Table 3**).

Results presented in **Table 4** indicate that the glucose and bilirubin levels were within normal range. Total cholesterol and triglycerides levels were significantly (p<0.01) increased. Serum glutamic oxaloacetic transaminase (SGOT) and SGPT were significantly (p<0.01) increased in cholesterol group when compared to control. The levels of plasma FSH and testosterone were significantly (p<0.01) decreased in treated group when compared to control group.

Our results imply that intragastric administration of cholesterol diet at dose (400 mg/kg body weight) for 60 days to male rats had significantly (p<0.01) decreased the number of females (14 out of 20) impregnated by them when compared to those impregnated by controls (18 out of 20). The number of implantations 7.82 ± 3.31 (p<0.01) and number of viable fetuses 6.63 ± 1.54 (p<0.01) were also significantly decreased in females impregnated by males ingested cholesterol (9.62 ± 2.66) in comparison to those impregnated by controls (9.37 ± 1.16). On other hand, the ratio of the number of resorption to the total number of implantation 22/123 (15.4%) was significantly (p<0.05) increased in females impregnated by males ingested cholesterol when compared to those impregnated by controls 4/173 (2.31%).

The testes of control animals (**Figure 1**) present a picture with all successive stages of spermatogenesis, example, spermatogonia, primary and secondary spermatocytes, spermatid and spermatozoa. In the center of the tubule, bunches of spermatozoa can be seen adhered to Sertoli cells. In the intertubular spaces healthy Leydig cells,

connective tissues and blood vessels are present. Control testes showed active spermatogenesis.

Cholesterol diet treated rats **(Figure 2)** show severe degeneration in the germinal epithelium. Few spermatocytes could be seen, and the number was decreased. The seminiferous tubule diameter was reduced to a highly significant level ($p \leq 0.001$). The relative amount of interstitial tissue was decreased. The Leydig cell nuclei were shrunken and the diameter was reduced, and distribution of Leydig cell was abnormal, when compared to controls. Cauda epididymides of control animals (**Figure 3**) show enlarged tubules. The structure is lined with pseudostratified epithelium with low columnar cell. The lumen is filled with a large number of mature spermatozoa.

Cholesterol diet treatment to intact rats caused significant changes in cauda epididymides (**Figure 4**). The epithelial cell height was reduced in comparison to control. Few spermatozoa were present in the lumen.

Discussion. The animal model used in this work has been used previously by several workers to assess the adverse effects on reproductive functions in males.[17,18]

In rats, the whole spermatogenic process requires 53 days out of which spermatozoa spend last for 6 - 7 days in the final transit through epididymides.[19] The cholesterol was administrated for one complete spermatogenic cycle. The present investigation shows that oral administration of cholesterol can caused reduction in fertility in male albino rats. Long-term feeding of cholesterol diet seems to increase the body weight.[20,21] In the present investigation rats showed a slight increase in the body weight in the low dose (400 mg/kg body weight) of cholesterol. It is well established fact that weight, size, histological appearance and secreting functions of the epididymides, seminal vesicle and ventral prostate are closely regulated by androgens. Changes taking place in these organs after castration can be counteracted by administration of testicular hormones, thus, serving as "indicator test" for male hormones.[22-24]

The weights of reproductive organs were markedly decreased. The dose acted on pituitary gland and decreased in main hormones of spermatogenesis FSH and testosterone. The process of spermatogenesis and accessory reproductive organs function are androgen dependent. Decrease androgen production is reflected in decreased number of mature Leydig cells and their functional status. In the present study, the number of degenerating Leydig cells were significantly increased, it reflects the decrease in androgen levels. It is further confirmed by the decrease in the number of spermatocytes (primary and secondary) and spermatids as these stages are completely androgen dependent.[25] The decrease in weight and histometry of reproductive organs further confirm androgen decrease. Significant decrease in sperm motility of cauda epididymis was observed in treated group. This may be due to the effects of cholesterol diet on the activity of the enzymes of the oxidative phosphorylation.[17,26,27]

The results presented in this paper also show that the ingestion of cholesterol diet by adult male rats had no effect on the fertility of females impregnated by treated males, even though, decreased the number of females impregnated by the exposed males. However, the number of implantation sites and the number of viable fetuses were reduced. These losses appear to be due to a decrease in sperm motility and sperm function. Reduction in implantation and fetotoxic effects may be due to genetic or cytotoxic effects, which can result in decreased fertility, failure of preimplantation or postimplantation death.[28] Cytotoxic agents can disrupt pregnancy, possibly by interfering with mitotic division of fetus. The increase in the number of resorption in female rats impregnated by cholesterol-exposed males may be attributed to an increase in the incidence of preimplantation mortality of fertilized ova.

Control of mammalian spermatogenesis by hormones has been studied extensively by various investigators using different parameters and animal species.[29] It has been shown that both gonadotrophins and testosterone are involved in the process of spermatogenesis, although, the extent of involvement of each hormone may vary in different species of animals. It has been demonstrated that the hormone requirement for initiation of spermatogenesis in the immature rat may be different from that required for its maintenance in mature rat.[30,31]

Spermatogonia and primary spermatocytes (meiotic prophase) do not require any hormone inducement and develop independently. The passage of primary spermatocyte (prophase) into metaphase and then into secondary spermatocytes, testosterone is necessary. Moderate amount of gonadotropins is necessary for the formation of spermatids. Immature spermatozoa can be formed if there is abundance of FSH and LH, for the maturation of spermatozoa, testosterone is necessary in adult male rats.[32] Slight different results were obtained by Chowdhary,[33] spermatogonia and primary spermatocytes required partially testosterone and FSH, all other stages of spermatogenesis example meiotic division and spermatogenesis required only testosterone. Further Dym et al[25] reported that adult rats do not need FSH to maintain spermatogenesis. Similar results were also obtained in men by Sherins.[34] Histological quantitative results indicate that cholesterol diet

may affect the intratesticular androgen levels, either by inhibiting Leydig cell function or by inhibiting hypothalamus pituitary axis.

There are 2 possible mechanisms: the treatment may selectively act on the Leydig cells and inhibit the main enzymes of steroidogenesis, example, hydroxysteroid dehydrogenates (HSD's) and thus, depletion in intratesticular androgen levels occurs. Increase in degenerating Leydig cell numbers in all cholesterol diet treatment confirmed the interference of androgen levels.[35] Further, reduction in different germ cell number example spermatogonia, spermatocytes and spermatids in all treatment confirmed the depletion of androgen levels, since these germ cell are partially or completely androgen dependent.[23,32] Second possible mechanism may be that this treatment may block anti-androgenic stimulation of the hypothalamus pituitary axis thus, inhibiting LH synthesis and release. Due to this, inhibition of Leydig cells and significant reduction in different germ cell population in cholesterol diet treatments confirms the above mechanism of action and imbalance of intratesticular androgen. Similar results were also observed in rats by well known anti-androgen, example, cyproterone acetate.[36,37]

It is known fact that small doses of exogenous androgen selectively suppress the LH via hypothalamo pituitary axis, as a result of which, inhibition of gonadal steroid genesis takes place and causes arrest of spermatogenesis.[38-42]

In conclusion, cholesterol diet possesses strong compound or principles that decrease fertility mainly by affecting Leydig cells and impose toxic effects on fertility in male rats.

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