Original Article

# The impact of Ghee on Serum Lipids, Sex Hormones and Spermogram of Wistar rats

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## ABSTRACT

**Background:** The traditional texts designate animal ghee are known as nutritionally important functional food and have health beneficial effects on human health, due to its composition. **Objective:** Our objective was to determine the effects of ghee on the serum lipids, sex hormones and spermogram of Wistar rats. **Methods:** Sixteen adult rats were divided into two groups. The experimental groups for 90 days were as follows; 1) standard diet (Control), and 2) Ghee rats group (treated): orally received 35% of heated ghee (100-120 °C) without restriction. One day after the last treatment, spermatozoa were recovered from epididymis, and blood serum samples were processed for lipid profiles (cholesterol, triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL)) and sex hormone concentrations (Dehydroepiandrosterone (DHEA) and testosterone). **Results:** Results showed that serum levels of cholesterol ($p=0.025$) in traded group was significantly higher than those in the control group, meanwhile DHEA and testosterone concentrations were lower ($p<0.01$). Compared with control group, the administration of ghee reduced ($p>0.05$) LDL and TG, and increased HLD ($p>0.05$). Sperm count was significantly lower ($p<0.001$) in ghee supplemented rats compared to control group, whereas the motility rate was significantly higher ($p<0.01$). In treated animals did not show significant changes in normal/abnormal sperm morphology ($p>0.05$). In summary, animal ghee is clearly detrimental for spermatogenesis and overall sexual hormone functions, whereas lipid profiles can be modified.

**Keywords:** Ghee, Rat, Serum Lipids, Spermatogenesis, Sex hormone.

## Introduction

The health science has faced with major challenges for management of reproductive disorder. Nowadays, in most parts of the world the lifestyle modifications consisted of diets rich in seed plant and low in saturated fat foods, orderly participation in physical activity and avoids the pollutants could affect the reproductive organs function, and common therapeutic methods often results in unsatisfactory results. Also, there is an increasing interest in this topic (animal functional foods), due to the overall sperm damage increase in industrialized countries, but there is still much to know concerning the effect of animal oils consumption on the reproductive system.

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One of the most important factors affecting reproductive health of individuals is diet. A review of literature shows that eating habits, as the main indicator of lifestyle, can significantly affect male fertility [1]. The spread of foods that contain saturated fatty acids (SFAs) and high monounsaturated trans fatty acids (MUFAs) (through decreasing SFAs intake to less than 10% of total energy intake and cholesterol to less than 300 mg/daily [2], and the decrease in consumption of foods that contain antioxidants and essential fatty acids such as fruits, vegetables and sea food can adversely affect male fertility [3].

Animal ghee is an important ingredient of diet in most of countries in south Asia, and include dairy products with high cholesterol and saturated fat content [4]. In Iran, ghee (usually called Kermanshah oil) is produced from milk by traditional techniques, and widely used for medicinal and culinary purposes. In general, it contain about 65% SFAs and 33% MUFAs [5]. Ghee has enhanced the activity of antioxidative enzymes by scavenging the free radicals which ultimately improves the antioxidative enzyme systems [6], thus could improve reproductive health of individuals. However, there is a lack of scientific data regarding

the effect of animal ghee on sperm quality. Meanwhile, given the effects of food on prevention and control of infertility, the present study was conducted for two main objectives: to measure the effect of long-term consumption of animal ghee on A) serum lipids, and B) spermatogenesis in rat.

## Materials and Methods

### Chemicals and reagents

All chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany). The kits (Cosmo Bio Co. Japan) were prepared to evaluate some sex hormones (Dehydroepiandrosterone (DHEA) and testosterone) and lipid profile concentrations (cholesterol, triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL)).

### Animals

Healthy adult male Wistar rats (210±25 g; 8-week-old) were purchased from the Pasteur Research Center (Karaj, Iran). Rats were kept in an air-conditioned animal room under 12 h light:dark cycle under standard environmental conditions (22±1 °C, 52±5% humidity) with free access to tap water and commercial dry pellet diet. Rats were housed in polypropylene cages lined with pine wood husk, changed every day. Experimental protocols were approved by the Ethics Review Committee of Islamic Azad University, Sabzevar (IR. IAU. S. REC. 1399.007).

### Experimental design

Sixteen rats were divided into two groups. The experimental groups for 90 days were as follows; 1) standard diet (control), and 2) Ghee rats group (treated): orally received 35% of heated ghee (100-120 °C) without restriction (according to international food regimens) (Table 1).

**Table 1. Composition and calculated amino acids analysis of animal ghee (%)**

| Raw Protein | Raw Fiber | Ash | PH | NaCl | Moisture | Lysine | Methionine | Methionine+Cysteine | Threonine | Tryptophan | Energy (kcal/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | 3.5-4.5 | Max 10 | 0.65-0.7 | 0.5-0.55 | Max 10 | 1.15 | 0.33 | 0.63 | 0.72 | 0.25 | 16.16-17 |

### Sampling and assay

One day after the last treatment, spermatozoa were recovered from epididymis, and blood serum samples were processed for lipid profiles (cholesterol, TG, LDL and HDL) and sex hormone concentrations (DHEA and testosterone). Blood samples were collected in vacuum tubes early in the morning before treatments. In order to evaluate the serum was separated by centrifugation (×4000g for 10 min) and readily frozen at -20 °C. Samples were assessed in groups by radioimmunoassay. Standard commercial kits were used for analysis and the procedures were adopted as recommended by the manufacturer.

### Epididymal sperm analysis

In order to assess the epididymal sperm count, cauda epididymides were placed in 1 ml of Ham's F10 medium. The epididymis was cut into 2-3 pieces and incubated at 37 °C for few minutes (5% $CO_2$) in order to allow sperm to swim out the epididymal tubules. An aliquot of sperm suspension was diluted 1:20 with Ham's F10 medium and transferred into a Neubauer's hemocytometer. Spermatozoa were counted under a light microscope at ×400 and expressed as million/ml of suspension (World Health Organization, Department of Reproductive Health and Research, 2010).

The amount of 196 µl of the homogenized sperm solution was quickly spread on Neubauer's slide and covered with slide cover. Samples were assessed and the results were recorded separately according to the standard method as A (immotile), B (non-progressive), C (slowly progressive) or D (rapidly progressive) [7, 8]. At least 10 fields were assessed for each sample using a bright-field microscope with a closed diaphragm and the percentage of motile spermatozoa was estimated subjectively.

Sperm morphology was analyzed by eosin-nigrosine staining in 500 spermatozoa. A drop of stained sperm suspension was put on a clean slide and a thin smear was made and allowed for drying. This slide was examined under a light microscope at ×1000 and spermatozoa with white and pink heads were considered as alive or dead, respectively [9].

### Statistical analysis

Our data were analysed by SPSS 17.0 software. In order to compare the treatments, one-way ANOVA, t-test and Duncan test were used for multiple comparisons between groups ($p<0.05$). The results were expressed as the mean ± standard deviation (SD).

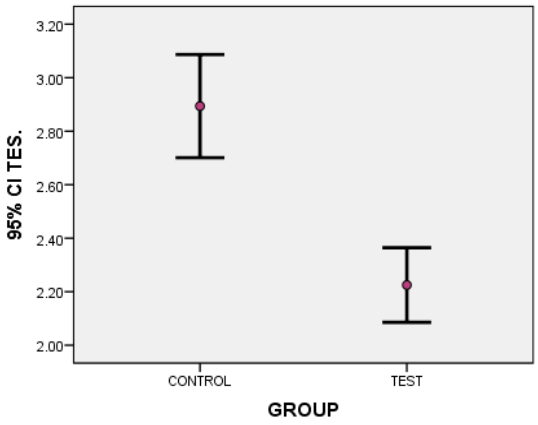
## Results

Based on present results, the difference in lipid profile and sperm quality between control and ghee fed rats is likely attributed to differences in daily diet intake. It was not observed significant difference between control-normal vs animal treated groups for the lipid profile concentrations including TG, HDL and LDL ($p>0.05$; Table 2), but our findings shows that the administration of animal ghee significantly increased serum levels of cholesterol ($p<0.05$). Compared with control group, the administration of animal ghee significantly reduced sex hormone concentrations including DHEA and testosterone ($p<0.05$; Figure 1).

**Table 2. The effect of ghee on lipid profile (mg/dl) in Wistar rats**

| Parameters | Control | Treated | p-value |
|---|---|---|---|
| Cholesterol | 67.2±4.83 | 72.7±3.84 | 0.025 |
| TG | 90.3± 9.54 | 89.1±6.46 | 0.764 |
| LDL | 12.2±1.66 | 10.8±0.83 | 0.056 |
| HDL | 34.3±1.06 | 36.2±3.84 | 0.205 |

TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein. Values are given as means±S.D (n=8)



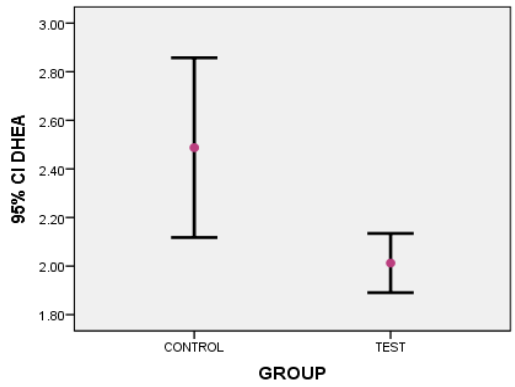




**Figure 1.** The effect of ghee on sex hormones concentrations (nmol/L) in Wistar rats (n=8). (Error bar was used to assess the difference between the two groups using mean and confidence interval of 95%).

Effects of ghee treatments on the spermogram of rats are presented in Figure 2 and Table 3. Given the significant difference between the control and treatment group regarding sperm count ($p<0.001$), the results showed that sperm count was significantly higher in the control group.

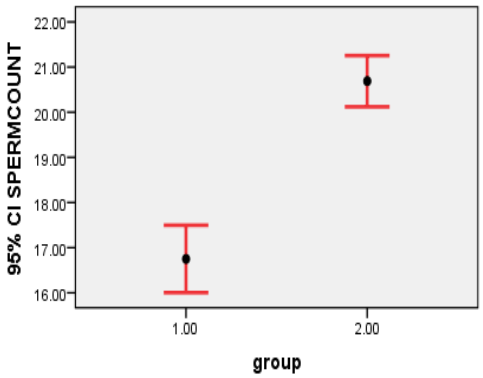



**Figure 2.** The effect of ghee on sperm count ($\times 10^8$) in Wistar rats (n=8)

In treated group did not show significant changes in on sperm morphology and motility, when compared with control group ($p>0.05$; Table 3). The results show that both groups had similar means regarding immotile (A), but the case group showed a higher increase in non-progressive motility (B) and slowly progressive motility (C) categories as compared with the treatment group. This trend was stronger for rapidly progressive (D) category in the treatment group than that in the case group.

**Table 3. The effect of ghee on sperm morphology and motility (%) in Wistar rats**

| Group | Sperm morphology | | | | Motility | | | |
|---|---|---|---|---|---|---|---|---|
| | Normal | Abnormal | Viable | Non-viable | A | B | C | D |
| Control | 94.8±1.2 | 5.12±1.2 | 93.6±1.6 | 5.5±1.3 | 7.5±1.1 | 10.5±2.1 | 10.6±1.9 | 6.25±0.9 |
| Treated | 95±1.5 | 5±1.5 | 92±1.5 | 8±1.5 | 9.5±1.3 | 6.5±0.9 | 6.38±1.1 | 10.75±2.2 |

A (immotile), B (non-progressive), C (slowly progressive) or D (rapidly progressive). Values are given as means±S.D (n=8)

## Discussion

In the recent, years, new technologies and techniques initiate innovation. Meanwhile, further understanding of eating habits and their effects on the reproductive system could help improving infertility treatments in patients.

To the best of our knowledge, this is the first comprehensive experiment exploring the mechanistic basis for the beneficial reproductive health effects of ghee in rat models. However, the effect of ghee on lipid profile, sex hormones and spermogram needs further studies to examine the mechanism of effect and its metabolism in vivo.

Many studies have reported controversial effects of ghee on blood lipid levels as increasing, with no effect or even decreasing [10, 11]. Based on present results, consumption of a diet with 35% animal ghee for 90 day reduced ($p>0.05$) LDL and TG, and increased HLD ($p>0.05$), as compared with the control group, HDL level slightly increased simultaneously with a decrease in LDL. A line with these results, Mohammadifard et al. (2010) examined the effects of ghee on serum lipids profile and reported that ghee oil treatment caused a reduction in TG levels [12]. On the contrary,

Yogita et al (2016) reported that no useful effects in bovine ghee on TG levels [13]. Ghee oil has been reported to be a good source of oleic acid that prevents oxidation of LDL and hence atherosclerosis [14].

Traditionally, animal oils could have commonly been used to treat free radicals, and tissue injured after inflammation. In fact, fats and oils are needed for provision of essential fatty acids and absorption of fat-soluble vitamins. They also have a high calorie, strongly satisfying nature and favorable taste, which is why they are wanted by many people [15]. Meanwhile, ghee oil can have unique physicochemical properties as it has SFAs that increase blood fat, neutral SFAs and MUFA [16].

Our findings clearly showed that long term consumption of ghee could significantly reduce testosterone and DHEA in the treatment group as in the control group in a dose-dependent manner, but this reduction cannot decease fertility in Wistar rat. Together with the results present study in men and women, Mu et al. (2001), and Attaman et al. (2012) showed that oils with SFAs could adversely affect fertility [17, 18].

Reduced testosterone is accompanied with increase cholesterol level, until now its metabolic process is still unknown [19]. No relationship was found between sperm count and total cholesterol or TG in human [20]. However, low cholesterol diet has been shown to cause low motility and low sperm count in mice [21, 22], rats [23], and rabbits [24]. A line with present results, these data shows that cholesterol and lipid hemostasis can be an important factor for infertility in animals.

The increase in blood lipids can disturb production, maturation and function of sperms as it causes damage at the level of testicular cells [25]. Also, increased fat affects spermatogenesis, quality of spermatozoids and sperm motility [26, 27]. In this study, sperm count, motility and morphology were studied. The results showed that the treatment group had a significant decrease in sperm count ($p>0.05$). Motility, viability and sperm count are the most important parameters for assessing fertilizing ability and integrity of the membrane [28]. Rooke et al. (2001) reported that feeding pigs with fish oil significantly increased sperm viability, progressive motility and percentage of sperms with normal acrosome [29]. The results presented in this study demonstrated that supplementation of ghee oil show higher rates of sperms with rapidly progressive motility. Since no study was found on the effects of ghee, the results of the present study can be compared only with the results of studies on other oils.

The toxic products resulting from oxidizing lipoproteins (LDL and HDL) can considerable decrease sperm counts in rat's diets received ghee oil. HDL carries cholesterol from tissues to liver [30, 31]. HDL also contributes to the inhibition of LDL [32]. Feeding of ghee from weaning to sexual maturity of rats lowered lipid peroxidation [6]. Lipid peroxidation is considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death [33]. Hence, it is recommended that future experiment should focus more on exact relations among side effects of toxic products resulting from oxidizing lipid components in the male reproductive system.

Given that ghee is unsaturated, Campos-Silva et al. (2015) studied the effects of pig oil diets (saturated and unsaturated) on metabolism and sexual function of rats and reported that a 16-week diet with 50% pig oil increased the thickness of germinal epithelium in seminiferous tubules of testicles [34]. Meanwhile, rats with a diet of 50% unsaturated fat (canola oil) showed an increase, but the combination of the two (saturated/unsaturated) did not cause this problem. Furthermore, no significant change was observed in the weight of testes, serum level of testosterone, and external diameter of seminiferous tubules in the study groups [34]. Therefore, studies revealed that ghee as a saturated oil can affect sex hormone and sperm count, but cannot significantly change normal and abnormal morphology as compared with the control group.

Examining the sperm morphology showed that normal morphology was observed in 95% of rats fed with ghee as compared with 94% in the control group. Although the difference is not significant ($p>0.005$), there is a slight increase. As for the abnormal morphology, a mean of almost 5.00 in the ghee group was better as compared with a mean of 5.12 in the control group although the difference was not significant (Table 3). However, the results show a slight increase in viable sperms in the control group while the increase in the number of nonviable sperms was significantly different between the two groups ($p>0.005$). Mean non-viability of 8% in the treatment group and 5.5% in the control group shows the increased death rate in the treatment group.

In conclusion, given the higher rate of progressive motility in the treatment group as well as reduced sperm count and increased death in this group, we cannot consider ghee as a positive or negative factor for fertility and spermatogenesis.

## Consent for publication

Author agree to send this manuscript to journal of Advanced Pharmacy Education & Research, are in agreement with its content, and do not have any restriction in order to publish the obtained results.

## Availability of data and materials

This manuscript contains original data and it is not under editorial consideration elsewhere. We have adhered to the ethical guidelines of your journal.

## Competing interests

Authors state no conflict of interest.

## Funding

None declared.

## Authors' contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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## Abbreviations:

SFAs: saturated fatty acids; MUFAs: monounsaturated trans fatty acids; DHEA: Dehydroepiandrosterone; TG: triglyceride; LDL: low density lipoprotein; HDL: high density lipoprotein.

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