Original Article

KETOGENIC DIET PHYSIOLOGICAL EFFECT ON SPERM PROFILE IN INDUCED STARVATION MALE WISTAR RATS

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Abstract

Background: Stress refers to a condition of mental and physiological strain that occurs when the body is exposed to demanding circumstances. A ketogenic diet, which is high in fat and low in carbohydrates, forces the body to utilise fat rather than carbohydrates for energy, leading to the production of ketone bodies by the liver as an alternative energy source.

Objective: This study investigated the physiological effects of a ketogenic diet on the sperm profile of starvation-induced male Wistar rats.

Methods: Sixty male rats were divided into six groups of ten animals each and observed for 42 days, excluding a two-week acclimatisation period. Semen samples were collected and analysed using the haemocytometer method.

Results: Rats subjected to 9- and 18-hour starvation periods and fed ketogenic diets had slightly higher sperm counts (503.0 and 503.5 mm^3 respectively) compared with the control group (481.3 mm^3). Progressive sperm motility showed a non-significant decrease in starvation-induced groups relative to the control, whereas non-progressive motility increased significantly. A significant reduction in immotile sperm count was observed in the 9-hour starvation group (20.50 mm^3) compared with the 18-hour group (25.25 mm^3) and the control group (25.00 mm^3). Conversely, rats subjected to starvation but fed a standard diet showed a significant reduction in total sperm count compared with the control group, although progressive motility increased.

Conclusion: Prolonged fasting may influence sperm parameters either positively or negatively depending on its duration and dietary context. Extended fasting combined with a standard diet may significantly reduce total sperm count, suggesting that men intending to conceive should exercise caution when engaging in prolonged fasting practices.

Keywords: Diets, Sperm count, Starvation, Rats

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INTRODUCTION

Stress is a state of mental and emotional strain resulting from demanding circumstances. Physiologically, a ketogenic diet, which is rich in fat and low in carbohydrates, compels the body to utilise fat rather than carbohydrates as its primary source of energy. This metabolic shift promotes the production of ketone bodies by the liver, which serves as an alternative energy source for the body. Increased fat intake and reduced carbohydrate consumption alter metabolism, leading to the production of ketones that influence ion channels

while replacing carbohydrates as the main source of energy supply [1].

Stress involves complex immunological and biochemical mechanisms, and the human body responds through the fight-or-flight reaction. Prolonged and deliberate starvation, often associated with fasting, may affect gonadotropic hormones and testosterone levels in fertile males. These effects may occur through alterations in the hypothalamo–pituitary–testicular axis or through direct effects on the testes and spermatogenesis. The quality of spermatozoa is a critical determinant of male fertility [2, 3, 4].

The ketogenic diet has gained popularity as a therapeutic approach for weight reduction and the management of epilepsy. In studies involving fasting in conjunction with ketogenic diets, approximately 80% of patients experienced a marked reduction in seizure activity, characterised by decreased abrupt electrical excitations in clinical trials [5].

Sperm quality is commonly assessed through parameters such as sperm count, motility, and morphology, typically measured using counting chambers such as the haemocytometer and Makler chamber. Although automated optical systems are now available for assessing sperm quality, manual methods remain widely used [6, 7].

Previous studies have reported that ketogenic diets may significantly reduce sperm count, morphology, and motility [8]. In addition, factors such as environmental conditions, stress, and socioeconomic status can influence sperm parameters. Maintaining a balanced diet with appropriate nutritional proportions may help improve sperm quality.

Infertility concerns often arise when a couple engages in unprotected sexual intercourse for over one year without achieving pregnancy. Several factors may contribute to male infertility, including exposure to toxins, cryptorchidism, smoking, drug use, and other lifestyle factors [9, 10].

Interestingly, the ketogenic diet has also been shown to mimic the physiological state of fasting, potentially reducing inflammation and improving metabolic health while serving as an alternative energy source for the nervous system [1].

Materials and Methods

Experimental Animals

Sixty male Wistar rats weighing between 140 g and 210 g were obtained from the Animal House of the Department of Physiology, University of Port Harcourt, Nigeria. The animals were housed in six cages (ten rats per cage) under standard laboratory conditions with adequate ventilation and free access to water ad libitum. The rats were allowed to acclimatise for two weeks prior to the commencement of the experiment.

The experiment lasted for 42 days, after which the animals were sacrificed. The study was conducted using a randomised controlled experimental design.

Diet Preparation

The experimental diets were obtained from Mile 3 Market, Port Harcourt, and prepared according to the method described by [11]. The ketogenic diet was formulated by thoroughly mixing 1 kg of margarine with 1 kg of standard rat feed to obtain a high-fat diet.

Experimental Design

The sixty rats were randomly divided into six groups of ten animals each, organised into two experimental phases.

Phase I: Standard Diet

- Group 1 (Control A): Rats received standard diet and water for 24 hours daily with no starvation.
- Group 2: Rats received standard diet and water for 15 hours daily, followed by 9 hours of starvation.
- Group 3: Rats received standard diet and water for 6 hours daily, followed by 18 hours of starvation.

Phase II: Ketogenic Diet

- Group 4 (Control B): Rats received ketogenic diet and water for 24 hours daily with no starvation.
- Group 5: Rats received ketogenic diet and water for 15 hours daily, followed by 9 hours of starvation.
- Group 6: Rats received ketogenic diet and water for 6 hours daily, followed by 18 hours of starvation.

Sample Collection

At the end of the 42-day experimental period, the rats were anaesthetised and sacrificed humanely. The abdominal cavity was carefully opened through a midline incision to expose the reproductive organs. The testes and epididymis were carefully excised and placed in a clean Petri dish containing normal saline to maintain tissue viability.

The cauda epididymis was isolated and gently lacerated using a sterile blade to allow the semen to be expressed into the saline solution. The semen was then collected and immediately used for sperm analysis, including the assessment of sperm count, motility, and viability, using the haemocytometer method.

All samples were processed promptly to prevent deterioration and to ensure accurate evaluation of sperm parameters.

Semen Analysis

The semen samples were analysed using the haemocytometer method. The epididymis was carefully lacerated to allow the semen to be expressed. The semen was then emulsified with 0.5% eosin stain and examined under a light microscope using ×10 and × 40 objective lenses.

Approximately 10 – 12 microscopic fields were examined to identify viable sperm cells, which were determined as the percentage of unstained cells relative to stained cells. The same procedure was used to assess actively motile sperm cells, non-progressive motile cells, and dead sperm cells.

Sperm count was determined using a haemocytometer counting chamber with a dilution ratio of 1:20, using normal saline as the diluent. The counting chamber was properly assembled and filled with the diluted semen sample. Four sets of sixteen squares (4 × 16) were counted under the microscope, and the final sperm count was obtained by multiplying the number of cells counted by 100,000.

Thus, the sperm concentration was calculated using the formula:

Sperm count = Number of cells counted × 100,000.

Statistical Analysis

All data obtained from the study were analysed using the Statistical Package for the Social Sciences version 24.0 (IBM Corporation, Chicago, IL, USA)**.** The results were presented as mean ± standard error of the mean (SEM)**.** Differences among the experimental groups were evaluated using one-way analysis of variance**.** Where significant differences were observed, appropriate post hoc multiple comparison tests were applied to determine the specific groups responsible for the observed differences. Statistical significance was considered at a p-value less than 0.05 ($p < 0.05$)**.**

RESULTS

The results obtained from the analysis of semen parameters in starvation-induced male Wistar rats fed with ketogenic and standard diets are presented in this section. The study evaluated sperm count, progressive motility, non-progressive motility, and immotile sperm across the different experimental groups. Table 1 show the sperm profile obtained from the experimental animals used for this study.

Table 1: Mean values of sperm analysis in Wistar rats fed with ketogenic diet.

Parameters	Appearance	Control	9 Hours Starvation	18 Hours Starvation	P – values
Sperm count (cells/mm^3)	Milky	481.3 ± 22.21	503.0 ± 6.988	503.5 ± 9.734	0.4954
Progressive motility	Milky	60.50 ± 0.645	58.00 ± 0.913	59.00 ± 1.826	0.3935
Non-Progressive motility	Milky	15.50 ± 1.323	21.50 ± 1.756	15.75 ± 1.548	0.0385

$P < 0.05$ indicates significant different.

Ketogenic diet has almost the same effect on both 9- and 18-hours period fasting compared with control group. Immotile sperm among group exposed to 18 hours starvation increases significantly above other groups. The above table shows a decrease in immotile sperm compared with the control. (Table 2)

Table 2: Effect of sperm profile regarding control and experimental test groups treated with ketogenic diet.

Parameters	Treatments	Control	9 Hours Starvation	18 Hours Starvation
Total sperm count (cells/mm^3)	Standard Diet	555.2 ± 18.36	519.9 ± 3.274	551.7 ± 14.13
	Ketogenic Diet	481.3 ± 22.21	503.0 ± 6.988	503.5 ± 9.734
	P – values	0.0438	0.0519	0.0278
Progressive motility	Standard Diet	61.87 ± 0.92	65.52 ± 2.243	64.490 ± 0.763
	Ketogenic Diet	60.50 ± 0.645	58.00 ± 0.913	59.00 ± 1.826
	P – values	0.0803	0.0019	0.0153
Non-Progressive motility	Standard Diet	11.02 ± 1.100	10.60 ± 0.877	12.30 ± 1.02
	Ketogenic Diet	15.50 ± 1.323	21.50 ± 1.756 *	15.75 ± 1.548
	P – values	0.0863	0.0022	0.1088
Immotile sperm	Standard Diet	25.10 ± 0.866	21.50 ± 0.957	23.30 ± 1.320
	Ketogenic Diet	25.00 ± 1.080	20.50 ± 2.02	25.25 ± 1.652
	P – values	0.6535	0.5796	0.1040

**P < 0.05 indicates significant different*

Effect of hours of starvation on *total sperm count* of Wistar rats on ketogenic diets

There were no significant changes among those starved for 18 hours and 9 hours compared with control respectively (Figure 1).

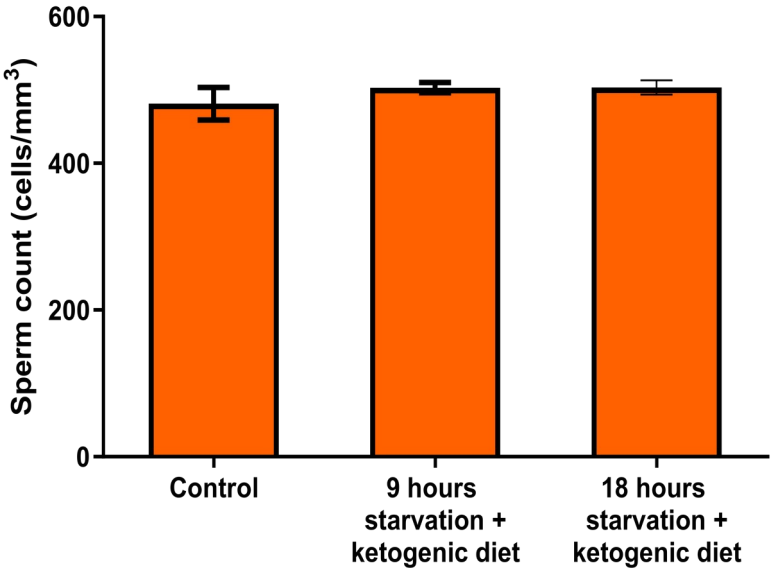

Figure 1: Effect of hours of starvation on *total sperm count* of Wistar rats on ketogenic diets

Effect of hours of starvation on *sperm cells progressive motility* of Wistar rats on ketogenic diets

There were no significant changes in those starved for 18hours and 9hours compared with control respectively (Figure 2).

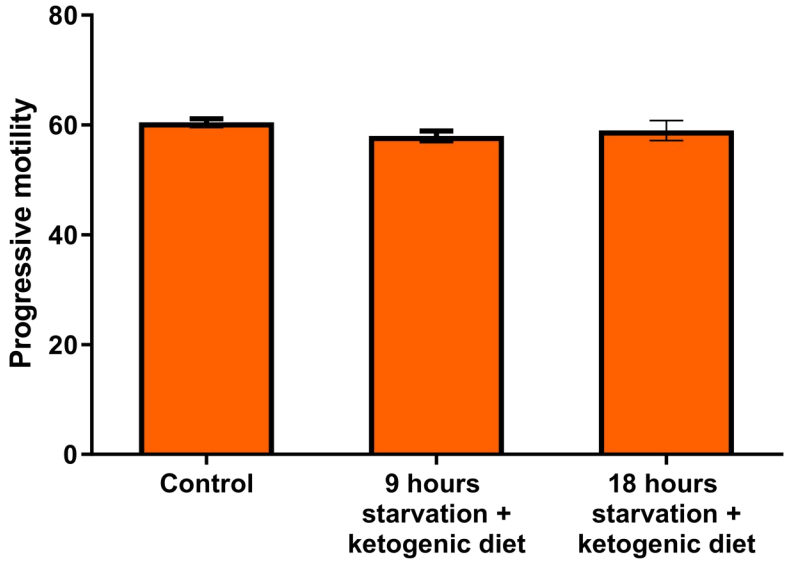

Figure 2: Effect of hours of starvation on *sperm cells progressive motility* of Wistar rats on ketogenic diets

Effect of hours of starvation on *sperm cells non-progressive motility* of Wistar rats on ketogenic diets

There was a significant increase in those starved for 9 hours compared with control and 18 hours starvation respectively; however, there was no significant change in 18 hours starvation compared with control (Figure 3).

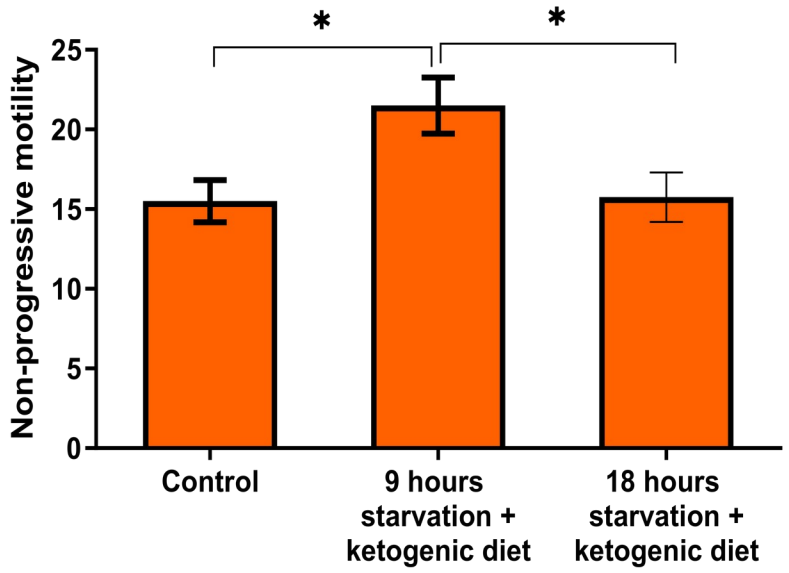

Figure 3: Effect of hours of starvation on *sperm cells non-progressive motility* of Wistar rats on ketogenic diets

Effect of hours of starvation on *immotile sperm* of Wistar rats on ketogenic diets.

There was a significant decrease in 9 hours' starvation compared with control and 18 hours starvation group, but there was no significant difference in 18 hours starvation compared with control (Figure 4).

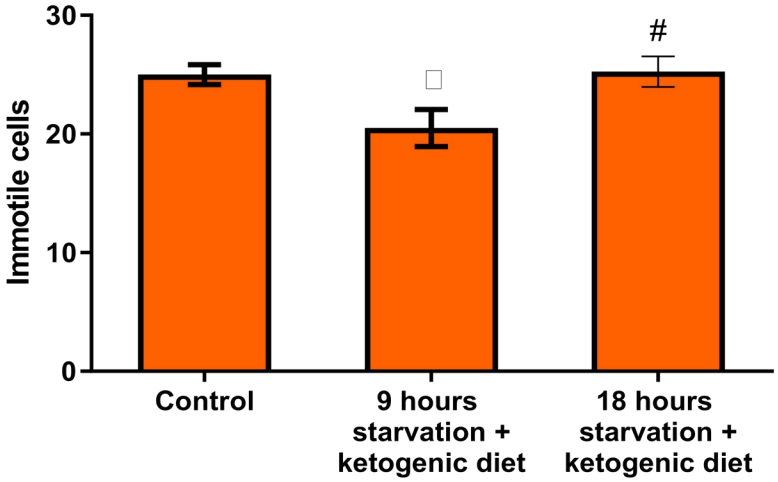

Figure 4: Effect of hours of starvation on *immotile sperm* of Wistar rats on ketogenic diets.

Effect of comparing hours of starvation of Wistar rats fed with ketogenic diets to those fed with standard diets on total sperm count.

There were significant decreases in ketogenic diet group compared with standard diet group for control and 18 hours' starvation, though, there was no significant change in group of ketogenic diet for 9 hours' starvation group compared with standard diet.* indicate significant (Figure 5).

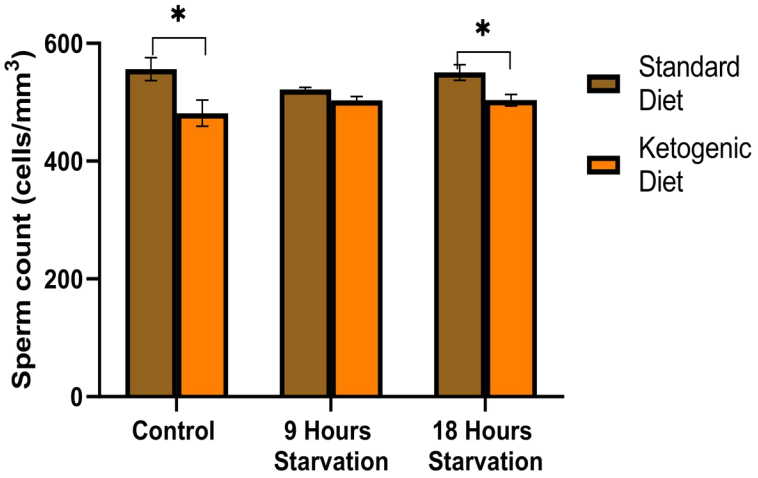

Figure 5: Effect of comparing hours of starvation of Wistar rats fed with ketogenic diets to those fed with standard diets on total sperm count.

Sperm morphology of some groups

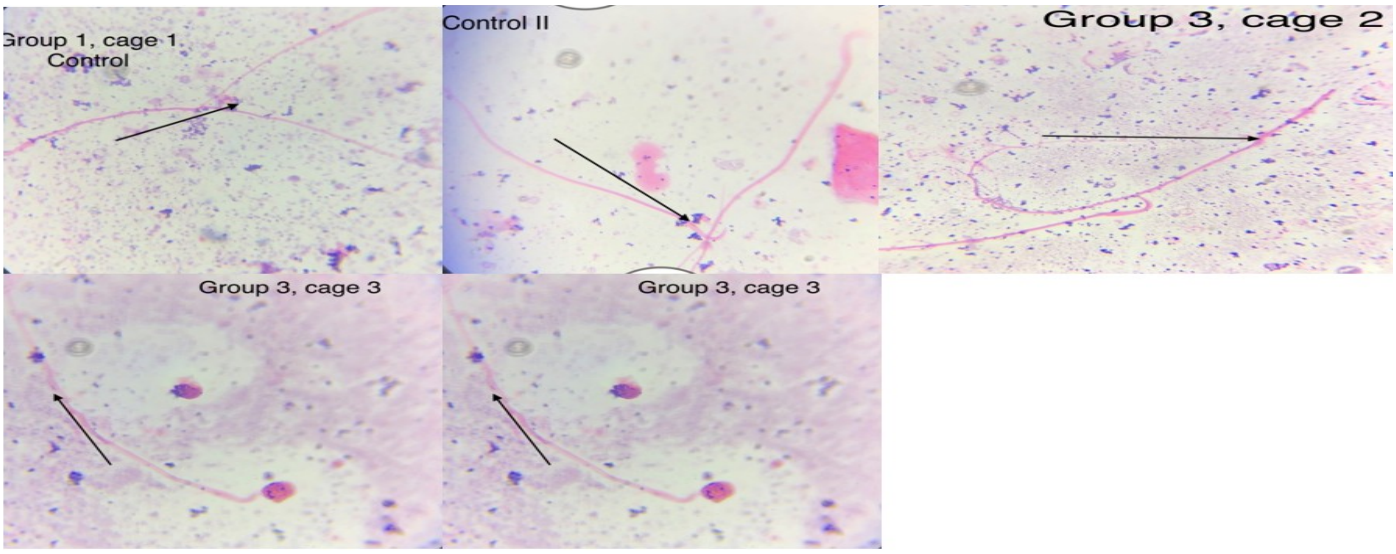

Plate 1: showing sperm morphology with normal head and tails across the cages, Plate 3, 4 and 5: The sperm morphology in cage 1, cage 2 of group3 shows sperm morphology with headless sperm cells

Figure 6: Sperm morphology of some groups.

DISCUSSION

There was an absence of significant changes in both 9- and 18-hour starvation groups with respect to sperm count and progressive motility when the rats were fed a ketogenic diet. This implies that both moderate and prolonged starvation had no significant effect on sperm count and progressive motility under ketogenic dietary conditions. However, a significant increase in non-progressive motility was observed in the 9-hour starvation group compared with the control group, indicating that moderate starvation combined with a high-fat diet may increase the proportion of non-progressive sperm cells.

The 18-hour starvation group showed no significant difference when compared with the control group, but demonstrated a significant decrease when compared with the 9-hour starvation group, suggesting that prolonged starvation combined with a high-fat diet may promote progressive sperm motility [5,12]. A significant decrease in immotile sperm cells was observed in the 9-hour starvation group, indicating that moderate starvation with a high-fat diet reduces the number of immotile sperm cells and may therefore enhance sperm motility compared with the control group. However, the absence of significant change in the 18-hour starvation group suggests that prolonged starvation does not significantly influence the reduction of immotile sperm cells.

Comparison of the mean values of sperm parameters between the normal diet and ketogenic diet groups revealed a significant decrease in sperm count in the control and 18-hour starvation groups. The results presented in Table 1 are consistent with the findings of Hilal et al. [13], who reported that ketogenic diets improved spermatogenesis and may influence male fertility. This effect may be related to changes in testosterone production and mobilisation during testicular spermatogenesis in the ketogenic diet groups compared with the control groups [14]. However, no significant effect was observed in the 9-hour starvation group.

Testosterone has long been recognised as a critical hormone involved in spermatogenesis and Leydig cell function [15]. Furthermore, decreased sperm count may be associated with reduced glycogen content in the testes, which can limit the energy supply required for spermatogenic activity. The significant decrease in progressive sperm motility observed in both 9- and 18-

hour starvation groups when ketogenic diets were compared with the normal diet may be attributed to abnormalities in sperm production and maturation. The epididymis plays a crucial role in sperm maturation, leading to the development of progressive motility and fertilising ability of spermatozoa. In addition, epididymal function is androgen-dependent [16].

CONCLUSION

Significant increase and decrease were also found in the non-progressive motility and immotile sperm cells respectively for prolong starvation with ketogenic diets. However, the findings from this study show an increase in total sperm count among groups treated with ketogenic diet.

Conflict of Interest: None declared.

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