**Diet with peanuts and wild Jujubier honey can affect male hamster spermatogenesis?**

Ibtissam CHAKIR¹, ³*, Mohssine FATIHI², Omar EL BLIDI², Lahcen TAMEGART³, Omar CHOKAIRI², Malika BARKIYOU²

¹ Laboratory of Genie of Biology, Faculty of Sciences & Technics. Department of Biology Faculty polydisciplinary. University of Sultane Moulay Slimane, Beni Mellal, Morocco
² Histology-Embryology & Cytogenetics Laboratory, Mohammed V University, Faculty of Medicine and Pharmacy, Rabat, Morocco.
³ Laboratory of Biology and health, faculty of Science. Department of Biology. University of Abdelmalek Essadi, Morocco.

**ARTICLE INFO**

Received March 1st, 2023  
Received in revised form May 2nd, 2023  
Accepted May 2nd, 2023

**ABSTRACT**

Infertility is a multifaceted issue, involving male and female factors, along with modifiable and non-modifiable elements. Our investigation delved into the effects of daily peanut and Jujubier wild honey consumption on reproductive function and heightened seminal secretions in 21-day-old male hamsters (n=12), divided into three groups. By scrutinizing gonad, seminal vesicle size, and body weight, we observed positive influences of diets containing peanut and/or honey, notably on body weight and testis size in comparison to control groups. This preliminary study underscores the potential impact of diet on sperm production in prepubescent male hamsters, offering early insights into dietary strategies for potentially addressing infertility. As a topical concern, infertility’s intricate dynamics warrant further exploration, and our study contributes to the growing understanding of the interplay between diet and reproductive health.

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**Keywords:** Nutrition, Jujubier honey, Fertility, Spermatogenesis.

1. Introduction:
In today's context, fertility has emerged as a significant medical and scientific concern, particularly in Morocco. A staggering one in eight Moroccan couples experiences fertility challenges, with infertility affecting a substantial 15% to 17% of couples in their reproductive years. This equates to a significant number, totaling over 825,000 individuals, as reported by the Moroccan Reproductive Medicine Institute [1-2]. Infertility is defined as the absence of pregnancy after a year of regular and unprotected sexual intercourse. The ability of both partners to conceive is crucial for successful reproduction (SMMR, 2015). Yet, infertility is not solely a medical matter; it carries profound social, economic, and psychological repercussions that can be just as impactful as physical health concerns [3-4]. Fertility is a complex issue influenced by various factors, and nutrition holds a prominent place among the socio-economic elements that can impact human health, either as a source of prevention or treatment [5]. Diet plays a dual role; it can not only influence fertility but also serve as a defense against the adverse effects of the environment. The influence of diet on fertility extends beyond the present generation, potentially impacting the health of future offspring [6]. Importantly, the risks of infertility can pertain to either the woman, the man, or both partners in a couple. Despite common perceptions, male factors contribute significantly to infertility. A recent study conducted at the University Hospital of Rabat unveiled a surprising finding: 40% of infertility cases in Morocco are of male origin. The study delved into abnormalities in sperm parameters, revealing teratospermia, oligospermia, asthenospermia, and necrozoospermia as frequent issues among infertile men [7].

(*) Corresponding author:  
Tel.: + 212 6 61 82 33 73  
E-mail address: ibtissam.chakir@usms.ac.ma
The nexus between diet and fertility is now firmly established. Researchers are increasingly exploring natural therapeutic agents, considering their potential to yield positive outcomes without the side effects associated with synthetic drugs. Notable studies have highlighted specific micronutrients that enhance sperm quality. For instance, the regular consumption of nuts has been shown to boost sperm vitality, motility, and morphology. Our investigation sought to examine the potential effects of peanuts and wild honey on reproductive function. Peanuts contain essential elements such as arginine, zinc, selenium, and vitamins C and E, all of which are known to influence fertility. Similarly, honey, recognized for its overall health benefits, is rich in nutritional factors that impact fertility, including arginine, selenium, zinc, and vitamins [8-9].

In sum, the issue of fertility is of paramount importance in contemporary society, with Morocco facing a significant prevalence of infertility cases. Recognizing the multifaceted nature of fertility and its links to nutrition, our study endeavors to shed light on the potential benefits of natural dietary components like peanuts and honey. By understanding the role of diet in reproductive health, we hope to contribute to improved strategies for addressing fertility challenges and promoting overall well-being.

2. Materials and method:
Animals were housed under a natural light-dark cycle within controlled temperature conditions (22±2°C). Both food and water were provided without restriction. We utilized 12 hamsters at 21 days of age. For the diet, we used wild Jujubier honey sourced from the Nador region in Morocco, along with a commercial peanut source. The honey was administered in a total volume of 250ml, with 14g of honey, and the seed mix contained 60g of peanut seeds within the 140g mixture. Animals had ad-libitum access to food. They were categorized into three groups: GT - the control group, G1 - the group with additional peanuts in its diet, and G2 - the group with both peanuts and honey added to its diet. Parameters such as weight, body size, rib cage size, and testis size were measured. At the next step of the experimental procedures, animals were euthanized under terminal anesthesia. Each animal underwent transcardial perfusion. Hamsters were anesthetized using sodium pentobarbital (6%, 1ml/kg, Sigma-Aldrich, St Louis, MO, USA), followed by aortic infusion with 80ml of heparinized NaCl 0.9%. This was immediately followed by infusion of 150ml of cooled 4% paraformaldehyde (PAF) in 0.1M saline phosphate buffer (PBS) with a pH of 7.4. Testes were then extracted, post-fixed overnight at 4°C in PAF, dehydrated in gradually increasing alcohol concentrations (two baths of 2 hours at 70%, two baths of 2 hours at 95%, and one bath of 1 hour at 100%), and finally in butanol for 30 minutes. Subsequently, the tissues were coated in polyethylene glycol [10]. The testicles were sectioned into 30μm thick slices using a vibratome (Leica VT 1000E), and these sections were placed on gelatin-coated slides in 0.1M PBS. The experimental design aimed to minimize animal usage and reduce their distress, adhering to institutional animal ethics protocol approval. Figure 1 depicts a representative sample illustrating the alterations in physiological parameters observed throughout the course of this study.
3. Results:
Figure 2 illustrates the histogram depicting the progression of animals’ body weight in grams (g) across different time intervals within each group. The measurement of hamster body mass showcased variations in weight gain based on the assigned groups. Notably, a notable rise in weight was observed across all three groups on day 4. This increase in weight was particularly pronounced in the group consuming a diet comprising peanuts and honey, and it subsequently reached a stable level. Overall, our observations indicated that weight gain is more substantial when the diet includes peanuts and/or honey, in contrast to the control group whose diet consists of a seed mixture.

Figures 3 and 4 display the measurements of body size, revealing distinct variations in animal size across different groups and diets. Specifically, animals within the treated group exhibited larger sizes compared to those in the control group. Notably, the G2 group, which consumed a diet abundant in peanuts and honey, displayed the most considerable increase in size. This consistent pattern is also observed when analyzing rib cage size.

Figure 5 presents the findings regarding gonad size averages influenced by the designated groups and diets. The assessment of gonad diameter, encompassing both length and width measurements, revealed substantial distinctions among the groups under study. Notably, the group consuming a diet abundant in peanuts and honey exhibited significantly larger gonad diameters compared to the group consuming a diet rich in peanuts alone. In comparison, the control group displayed the smallest gonad diameter in contrast to the other two groups.

Figure 2. Histogram shows the development of the body weight of animals in gram (g) in each group according to time.

Figure 3. Histogram shows the body size in mm by group.

Figure 4. Histogram shows the height of rib cage in mm by group.
4. Discussion:
The mass and body size of organisms, along with the diameter and weight of their gonads, serve as essential indicators of physiological changes that can occur in response to variations in feed intake. The findings from our study underscore the significance of such indicators in understanding the impact of dietary choices on animal physiology. Specifically, we sought to investigate the effects of incorporating peanuts and wild honey into the diet of hamsters, and our results offer valuable insights into the physiological responses to these dietary changes. Our observations reveal a dynamic relationship between feed intake and physiological parameters. Following an altered feed regimen during the initial week, a noticeable increase in body mass becomes evident. This initial surge is succeeded by a gradual yet consistent weight gain in the subsequent weeks, which continues until the conclusion of the experiment. Of note, the control group, which adhered to a standard seed mixture diet, exhibited the least substantial weight gain. This outcome is in line with established literature that highlights the impact of dietary composition on body mass regulation. Interestingly, our findings echo the results reported by Foucaut et al. (2019) [11], who demonstrated that various doses of honey can lead to significant increases in body weight. These parallel underscores the relevance of our study and its consistency with existing scientific knowledge [12-13].

The distinct weight gain patterns observed among the different groups can be attributed to the specific dietary compositions, specifically the inclusion of peanuts, honey, or a combination of both. Intriguingly, the most pronounced weight gain was observed in the group consuming a diet rich in both peanuts and honey. This highlights the synergistic effect of these dietary components on physiological responses. Moreover, the consistency of our results with previous literature supports the notion that dietary elements can play a pivotal role in modulating physiological changes [14-17]. Further analyses of body size, including measurements of body dimensions and rib cage size, further elucidated the impact of dietary variations. These measurements exhibited patterns that mirrored the trends in weight gain, reinforcing the influence of feed intake on overall body development. The observed differences in body size among the groups underscore the significance of dietary factors in shaping not only weight but also physical dimensions.

A particularly compelling finding emerged from our examination of gonad size. Notably, there were distinct differences in gonad diameter among the groups, suggesting a direct influence of dietary components on reproductive function. Specifically, the group consuming a diet rich in peanuts and honey exhibited significantly larger gonad diameters compared to the group consuming only peanuts. These results resonate with prior research demonstrating the influence of diet on reproductive physiology. Literature supports the idea that dietary choices can impact reproductive function, further underscoring the intricate interplay between nutrition and physiological outcomes [18]. In essence, our preliminary study underscores the far-reaching impact of dietary choices on various physiological parameters. The short-term alteration of feed intake, particularly the regular consumption of elements rich in microelements and nutrients, was found to exert substantial effects on body weight, body size, and gonad diameter in male hamsters. Notably, the combination of honey and peanuts yielded particularly pronounced results in terms of reproductive function. This observation aligns with the well-established role of zinc, a nutrient abundant in both honey and peanuts, in testosterone production and its implications for reproductive health. Moreover, our findings draw attention to the potential benefits of a Mediterranean-style diet, rich in whole grains, fruits, vegetables, legumes, and nuts, in addressing aspects of sexual health and function [19-20].

The implications of our study extend beyond the realm of animal physiology, pointing to a broader connection between dietary choices and physical well-being. Our findings resonate with the growing field of micronutrition, which highlights the impact of diet on a range of health issues, including chronic fatigue, mood disorders, digestive problems, joint pain, headaches, obesity, and even fertility problems [21-22]. This study serves as a stepping stone, emphasizing the need for further research to unravel the intricate mechanisms underlying these observed physiological changes. As we delve deeper into the realm of nutrition and its impact on health, we inch closer to a more comprehensive understanding of the intricate relationship between diet and physiological well-being.
5. Conclusion:
Our initial inquiry emphasizes the influence of diet on reproductive function in prepubescent male hamsters. Despite the histological analysis of the testes yielding minimal disparities, apart from the seminiferous tubule lumen diameter, our study underscores the potential of diet to counteract environmental stressors. This points to diet's potential to not just impact general well-being but also to significantly shape fertility outcomes.

Acknowledgment:
The Faculty of medicine and pharmacy supported this research. Authors thank Mohammed FELAHI for his help and the assistance at Histology – Embryology & Cytogenetics Laboratory (Mohammed V University, Faculty of medicine and Pharmacy, Rabat, Morocco).

Funding: This research received no external funding.
Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.
Data Availability Statement: Data is contained within the article.
Conflicts of Interest: The authors declare no conflict of interest.

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