CURRENT BIOCHEMISTRY
ISSN: 2355-7877
e-ISSN: 2355-7931
Journal homepage: http://journal.ipb.ac.id/index.php/cbj
Journal E-mail: current.biochemistry@apps.ipb.ac.id

Sprague Dawley Rat Spermatozoa Quality Due to Bee Pollen Consumption

(Kualitas Spermatozoa Pada Tikus Akibat Pemberian Bee-Pollen)

Akhmad Endang Zainal Hasan[1*], Moerfiah[2], Septa Fitri Handayani[2]

[1]*Department of Biochemistry, IPB University, Bogor, 16680, Indonesia*
[2] *Pharmaceutical Study Program, Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor-16143, Indonesia .*

Received: *26 October 2022 ;* Accepted: *27 December 2022*

Corresponding author : Akhmad Endang Zainal Hasan, Departemen Biokimia FMIPA IPB;
e-mail: pakzainalhasan@gmail.com

ABSTRACT

Bee Pollen is a food that has a complete nutritional content including as a source of steroid hormones, helping to improve concentration and brain performance. This study aims to determine the effect of bee pollen on the sperm quality of male rats. Administering 1 ml of water as control (-), 1 mg/kg of Eurycoma longifolia capsule as control (+), 1 mg/kg of nano bee pollen, and bee pollen with concentrations of 0.33 mg/kg, 0.66 mg/kg, 1 mg/kg, and 1.33 mg/kg 34 times in Sprague Dawley male white rats. The mice were then operated on and examined for testicular weight, epididymal weight, sperm pH, sperm motility, sperm concentration, and sperm abnormalities. The results showed that bee pollen and nano bee pollen did not significantly affect the quality of rat spermatozoa. In examining the testicular weight of rats, 0.33 mg/kg of bee pollen had an average testicular weight greater than the control group. For sperm weight and pH of the rat epididymis, the administration of bee pollen showed the average weight and pH were lower than the control. In the examination of sperm motility, 0.33 mg of bee pollen had a higher percentage than the control, while 1 ml of nano bee pollen had the lowest percentage of motility. The results of the concentration and abnormalities of rat spermatozoa from a dose of 1 mg/kg of bee pollen had an average sperm count higher than the control which was 2,129.7 million/ml and the lowest percentage of sperm abnormalities was 8.73%.

Keywords*: Bee pollen, quality, rat, spermatozoa*

1. INTRODUCTION

Traditional medicine in Indonesia is known as native Indonesian medicine or jamu. One of the herbs that are widely circulated and consumed by the public, especially men, is an herbal medicine that can increase vitality and fertility *(*Kigen *et al*. 2017). Since the first, many studies have been carried out linking male fertility with sperm quality such as the number, motility, and morphology of spermatozoa (Kumar & Singh 2022).

Bee Pollen is a food that has complete nutrition; because it contains almost all the nutrients needed by our bodies *(Khalifa et al.* 2021). About 50% of the protein content is in the form of amino acids that can be used directly by the body (Church *et al*. 2020).

Bee Pollen is known as a source of steroid hormones that can increase sperm count in men, as an antioxidant, can increase energy and stamina, increase endurance and performance potential, accelerate healing and help improve concentration and brain performance (Khalifa *et al.* 2021). Based on previous research, pollen can improve growth and health, as well as increase the fertility and egg production of birds and hens (Saleh *et al.* 2021). Bee pollen contains compounds that can improve the quality of spermatozoa such as protein, vitamins, minerals and flavonoids (Hashem *et al*. 2021).

Based on the information, this study aimed to assessed the effect of bee pollen on the sperm quality of white male rats of the *Sprague-Dawley* strain in vivo with different concentrations of bee pollen.

2. METHODOLOGY

The materials used were Bee Pollen powder, *Eurycoma longifolia* capsules, Nano Pollen capsules, *Sprague-Dawley* male White Rats weighing between 160-250 g, and Rat Feed. The material consists of acetonitrile, methanol, phenol, borate buffer, and 15 amino acid mixed standard solutions (10, 25, 100, 250 and 1000 nmol cm^{-3}), HCl, sodium phosphate monobasic, and nitrogen gas.

Amino acid analysis was carried out by the HPLC method (Babu *et al.* 2002). A total of 21 rats were adapted in cages for 2 weeks, after which they were randomly divided into 4 groups. Group 1 was given drinking water, group 2 was given pollen with each dose consisting of 0.33 mg/kg, 0.66 mg/kg, 1 mg/kg, and 1.3 mg/kg; group 3 was given nanopollen 1 mg/kg; group 4 was given the *Eurycoma*. The treatment was carried out orally for 2 months every 3 days (Cochereau *et al*. 2007). During the study, rats were given food and drink *et-libitum*. After 2 months of treatment, the rats were euthanized. Then surgery was performed and the testis and epididymis were collected for weighing.

Macroscopic observations were made on sperm color, sperm consistency, and sperm pH (Ismawatie *et al.* 2021). The quality of spermatozoa was observed using a microscope by counting the number of motile spermatozoa in several areas of the field of view using a light microscope at 40x magnification, by estimating the progressively moving spermatozoa from the entire field of view and the estimated area and expressed as a percentage of motile (%).

Calculations on the concentration of spermatozoa were carried out using a Counting Chamber with a dilution of 1: 500 to formol saline (Ismawatie *et al.* 2021). The morphology of spermatozoa was observed by staining with Eosin Nigrosin viewed under a light microscope at 40x magnification. Observations were made on the overall shape of the head to tail of the spermatozoa and calculated the percentage of abnormal sperm.

3. RESULT

Table 1 shows the results of the amino acid analysis of bee pollen. The most common type of amino acid is glutamic acid and the smallest is methionine. Table 2 shows the results of testicular and epididymis weights. Table 2 shows the results of testicular weights where the highest weight was obtained from treatment with bee pollen at a dose of 0.33 mg/kg. However, statistically, it was not significant. The weight of the epididymis of rats given water as a control (-) was higher than that of rats treated with Eurycoma as a control (+), bee pollen, and nano bee pollen. However, the average weight of the epididymis in all treatments was relatively not much different.

Figure 1 Bee pollen powder

Table 1. Amino Acid Content in Bee Pollen

No.	Types of Amino Acids	Level (%)
1	Aspartate	1,37
2	Glutamic Acid	1,87
3	Serine	0,75
4	Histidine	0,39
5	Glycine	0,64
6	Threonine	0,67
7	Arginine	0,80
8	Alanine	0,91
9	Tyrosine	0,43
10	Methionine	0,33
11	Valin	0,78
12	Phenylalanine	0,78
13	Isoleucine	0,69
14	Leucine	0,99
15	Lysine	0,97

From these results, it can be seen that the average testicular weight for Eurycoma as a control (+) has an average of 2.99 g, while in water as a control (-) has an average of 2.7 g. In the administration of nano bee pollen, the average weight was smaller than the control (+), which was 2.78 g. In the administration of 0.33 ml of bee pollen, the average testicular weight was greater than the other treatment groups, which was 3.15 g. For the administration of 0.66 ml bee pollen, the average weight was 2.82 g and it was smaller than the control (+) and 0.33 ml of bee pollen but larger than the control (-), nano bee pollen and bee pollen at concentrations of 1 ml and 1.33 ml. The administration of 1 ml of bee pollen has an average weight of 2.62 g, in this case, the average value is greater than the administration of 1.33 ml of bee pollen and smaller than the control (+), control (-), nano pollen and bee pollen 0.33 ml and bee pollen 0.66 ml. In the administration of 1.33 ml of bee pollen, the average weight was smaller than the other treatment groups, which was 2.56 g.

The results of the effect of bee pollen on spermatozoa pH, motility, concentration, and spermatozoa abnormalities are in Table 3. The results of the sperm motility examination obtained showed almost the same value so the data did not spread. However, as if seen in Table 3, the administration of 0.33 ml of bee pollen has the highest average percentage of motility, which is 80%. In the administration of Eurycoma as control (+), 0.66 ml, 1 ml, and 1.33 ml of bee pollen had the same average spermatozoa motility of 76.67%. Water has average sperm motility of 77.5% and nano pollen has average sperm motility of 73.33%. The results of statistical tests for sperm motility showed that there was no

Table 2. Testis and Epididimis weight

Group of Treatment	Sample	Testis weight (g)	Epididymis weight (g)
Eurycoma sp.	3	2,99±0,19[a]	1,18±0,13 [a]
Water	2	2,7±0,68 [a]	1,24± 0,45 [a]
Nano Pollen	3	2,78±0,23 [a]	1,19±0,19 [a]
Bee Pollen 0,33 ml	3	3,15±0,25 [a]	1,22±0,45 [a]
Bee Pollen 0,66 ml	3	2,82±0,04 [a]	1,11±1,45 [a]
Bee Pollen 1 ml	3	2,62±0,34 [a]	1,14±0,23 [a]
Bee Pollen 1,33 ml	3	2,56±0,54 [a]	1,03±0,14 [a]

Description: a = not significant

Table 3. Effect of Bee Pollen treatment on Spermatozoa Quality

Group of Treatment	Number of samples	Spermatozoa pH	Spermatozoa Motility (%)	Spermatozoa Concentration (million/ml)	Spermatozoa Abnormality (%)
Eurycoma	3	6,7±0 [a]	76,67±2,89 [a]	1795,83±508,42 [a]	11,04±3,44 [a]
Water	2	7,0±0 [a]	77,5±3,54 [a]	1743,75±486,14 [a]	9,08±2,6 [a]
Nano Pollen	3	7,0±0 [a]	73,33±2,89 [a]	1700±195,26 [a]	9,68±0,63 [a]
Bee Pollen 0,33 ml	3	6,9±0,17 [a]	80±0 [a]	1679,17±555,69 [a]	10,88±0,74 [a]
Bee Pollen 0,66 ml	3	6,9±0,17 [a]	76,67±2,89 [a]	2104,17±239,25 [a]	10,56±3,03 [a]
Bee Pollen 1 ml	3	6,87±0,29 [a]	76,67±2,89 [a]	2129,17±52,04 [a]	8,73±0,98 [a]
Bee Pollen 1,33 ml	3	6,7±0 [a]	76,67±2,89 [a]	1958,33±31,46 [a]	10,11±0,76 [a]

Description : a = not significant

significant difference in the effect of each treatment.

From the results of the examination of the number of spermatozoa in the cauda epididymis treated with water as control (-), Eurycoma capsule as control (+), nano bee pollen 1 ml, and bee pollen with concentrations of 0.33 ml, 0.66 ml, 1 ml, and 1.33 ml for 34 treatments, the average value for control (-) was 1743.75 million/ml, control (+) were 1795. 83 million/ml, Nano Pollen 1 ml at 1700 million/ml, and bee pollen with a concentration of 0.33ml at 1679.17 million/ml, 0.66 ml bee pollen at 2104.17 million/ml, bee pollen 1 ml at 2129.17 million/ml, and 1.33 ml of bee pollen of 1958.33 million/ml.

From Table 3, it can be seen that the highest average pH of spermatozoa was in the nano bee pollen group of 1 ml and in the water group, which was 7. In the Eurycoma and bee pollen groups of 1.33 ml also had the same average of 6, 7, the 0.33 ml bee pollen group and 0.66 ml bee pollen also had the same average spermatozoa pH of 6.9, while the 1 ml bee pollen group had an average of 6.87. These results also showed that there was no significant difference between treatments.

Table 3 shows changes in the mean value of abnormal spermatozoa morphology from all treatment groups. Based on these data, it can be seen that if the mean abnormal morphology in the Eurycoma or control group (+) is compared to other treatment groups, it can be seen

that there is a decrease in the mean in the water group as the control (-), bee pollen and nano bee pollen. The mean abnormal morphology of the 0.33 ml bee pollen group compared to the 0.66 ml bee pollen group increased, but the average decreased again in the 1 ml bee pollen group and again increased in the 1.33 ml bee pollen and nano bee pollen groups. The lowest mean abnormal morphology value was in the 1 ml bee pollen group with a value of 8.73% and the highest abnormal morphology average in the Eurycoma group as a control (+) with a value of 11.04%.

4. DISCUSSION

Bee pollen results from drying and grinding shows a uniform shape. In measuring the concentration of amino acids, it turns out that all amino acids are found in bee pollen. The essential amino acids contained in bee pollen consist of Isoleucine, Lysine, Leucine, Valine, Threonine, Histidine, Methionine, Phenylalanine, and Tryptophan. With the presence of these amino acids, bee pollen is a bee product that contains complete essential amino acids. Thus, if bee pollen is consumed, the body's energy regulation, immune system, and hemoglobin production will be available properly (Komosinska-Vassev *et al.* 2015). The body's hormones and enzymes in the body will work properly and blood sugar will be regular (Khalifa *et al.* 2021). It can maintain a healthy heart, and liver, and improve the function of the immune system and the central nervous system (Mărgăoan *et al.* 2019). Several amino acids play an important role in absorbing the minerals zinc and selenium from food and play a role in the formation of other amino acids that are also needed by the body and help the formation of tyrosine and dopamine which are important for brain function and help the synthesis of serotonin in the brain (Khadka 2021). The presence of histidine makes bee pollen very important for the fulfillment of essential amino acids for children (Taha *et al.* 2019).

The testis is a place to produce spermatozoa cells continuously in large numbers. Androgen hormones (testosterone produced by Leydig cells) influence the process of formation of spermatozoa (Suede *et al.* 2022). The rate at which testosterone is produced by Leydig cells is determined by the level of LH in the blood. In contrast, secretion by the pituitary is controlled by the effect of testosterone levels on the pituitary and hypothalamus as well as the influence of the biphasic nature of testosterone (Oduwole *et al.* 2018).

When puberty arrives, the seminiferous tubules will work optimally to produce sperm and reproductive hormones such as testosterone and androgens. At that time, indirectly required a large capacity of the seminiferous tubules that will increase the weight and volume of the testis to support the process. The development and increase in sperm production is something that goes hand in hand with the development of testicular weight (Gunes *et al.* 2016). Smith & Walker (2014) added that the arrangement of the testis, which consists of 90 % of the seminiferous tubules, will affect the weight of the testis of adult animals.

From these results, there is no significant effect between treatments. At the time of testicular retrieval, the age of the mice at that time was about 16 weeks. This allows the development of the testicles to have decreased so that the weight of the testicles obtained is not at its highest point. The decrease in testicular volume occurs due to the loss of epithelial germ cells that

cannot regenerate due to age (Matzkin *et al.* 2021).

The epididymis is a long tube that attaches to the testicle from top to bottom which is at the back of the testicle. The epididymis will channel sperm (produced by the testis) to the outside. Sperm must travel quite a long journey and the epididymis is the site of sperm maturation after being produced in the testis (Sullivan & Mieusset 2016).

The size of the epididymis weight of rats given water as a control (-) was on average greater than that of rats treated with Eurycoma as a control (+), bee pollen, and nano bee pollen. However, the average weight of the epididymis in all treatments was relatively not much different. The size of the epididymis weight of rats from the study is presented in Table 3. The administration of bee pollen and nano bee pollen can reduce the weight of the epididymis. However, the administration of bee pollen and nano bee pollen did not interfere with the development of the epididymis. This can be seen from the measurement results which show that there is relatively no difference in the size of the epididymis weight between control rats and mice that received bee pollen and nano bee pollen. The development of the epididymis goes hand in hand with the development of reproduction itself. From these results, there is no significant effect between treatment groups on the weight of the epididymis of rats.

In this study, the results obtained showed that the pH of the sperm decreased with the increase in the concentration of bee pollen given. For the pH of the spermatozoa to be acidic and there was a possibility that it was an X-chromosome-carrying sperm so that if the sperm fertilized the ovum, it was likely to produce a female child. In each spermatogonium, one of the 23 pairs of chromosomes contains the genetic information that determines the sex of each child. This pair consists of one X chromosome, called the female chromosome, and one Y chromosome; called the male chromosome. During meiotic division, the male Y chromosome goes to a spermatid which then becomes a sperm carrying the Y chromosome; and the female X chromosome to another spermatid which will become the sperm carrying the X chromosome. The sex of a child will be determined by the two types of sperm that fertilize the ovum (Hall & Hall 2020).

From the results of this study, it was shown that the administration of bee pollen was able to increase the motility of spermatozoa but decreased with increasing bee pollen concentration. When compared with rats that were only given water, the provision of bee pollen and nano bee pollen did not have a significant effect on the motility of spermatozoa, because it was seen from the percentage of sperm motility which was relatively the same.

Based on conducted by Taha *et al.* (2019) state that bee pollen contains various minerals such as Zn, Cu, and Fe. Endogenous factors that affect spermatozoa motility include energy supply (ATP), spermatozoa maturation, and cell membrane integrity, while exogenous factors from nutrients that affect spermatozoa motility are the availability of inorganic ions (Tirpák *et al.* 2021). Therefore, Zn is one of the important minerals needed for spermatozoa motility. Zn is reported to be involved in lipid catabolism which is the main source of energy required for the movement of spermatozoa (Fallah *et al.* 2018).

The spermatozoa maturation process requires the main ingredients consisting of ions (Ca, Na, K, Cl), substrates (protein, sialic acid, glycogen, lactic acid, glycerol

phosphorylcholine), and enzymes, all of which are produced by the lumen of the epididymis (James *et al.* 2020). If these three elements are available in sufficient quantities, the process of spermatozoa maturation is safe and the quality of spermatozoa will increase (Skoracka *et al.* 2020).

In this study, there was no significant effect between treatments, possibly due to decreased testicular development due to age and the mineral and protein content in bee pollen was not sufficient to improve the quality of spermatozoa. The average number of abnormal spermatozoa did not show a significant difference in each treatment. The insignificant results may be due to the observation time being too long so that the spermatozoa may have been damaged and the age factor of the rats also affects testosterone levels in the epididymis and affects the process of spermatozoa maturation. Based on statistical tests, it is known that there is a difference between the control group (+) and the experimental group. In this case, the control (+) had the greatest spermatozoa abnormality. This indicates an increase in the number of abnormal spermatozoa.

In the process of spermatozoa maturation in the epididymis, motility development occurs, as changes in tail structure, changes in acrosome morphology, loss of cytoplasmic droplets, and changes in the plasma membrane. The abnormal spermatozoa found in this study were probably due to the presence of cytoplasmic droplets due to the imperfect maturation process in the epididymis. Maturation in the epididymis requires an environment that is highly dependent on testosterone levels. In this case, the possibility of testosterone levels in plasma is below the threshold so that the epididymis is unable to stimulate the process of spermatozoa maturation and consequently the percentage of abnormal spermatozoa numbers will increase (Alahmar 2019).

Examination of the morphology of spermatozoa is necessary in assessing its quality. Each ejaculate contains several abnormal spermatozoa. Semen with a high proportion of abnormalities gave low fertility results. In this study, the range of abnormalities due to treatment was between 8.73%-11.04%. According to Skoracka *et al.* (2020), spermatozoa with morphological abnormalities (abnormalities) of less than 20% are still considered normal. Referring to these criteria, the seven groups of treated rats had spermatozoa in the normal category.

Chemical compounds that are thought to increase the number of spermatozoa are sterols and phenolic compounds. The phenolic compounds found in bee pollen are flavonoids that have estrogenic activity. Flavonoids can increase testosterone levels in the blood (Martin & Touaibia 2020). Flavonoids inhibit enzymes that catalyze the conversion of androgens to estrogens which will increase the hormone testosterone. According to Skoracka *et al.* (2020), testosterone is a primary reproductive hormone that has a role in the process of spermatogenesis (spermatozoa formation).

The administration of bee pollen and nano pollen did not have a significantly different effect on testicular weight, rat epididymis weight, and spermatozoa motility. The effect on the pH of spermatozoa by giving bee pollen is more acidic while the administration of nano pollen with sperm pH is normal. The greatest concentration of spermatozoa was found in the administration of 1 ml of bee pollen, which was 2,129.17 million/ml.

ACKNOWLEDGMENT

To the Chancellor of IPB University and the Ministry of Education and Culture, Technology Research and Higher Education of the Republic of Indonesia for the research funding support the authors would like to thank.

REFERENCES

Alahmar, A. T. (2019). Role of Oxidative Stress in Male Infertility: An Updated Review. *Journal of Human Reproductive Sciences*, *12*(1), 4–18. https://doi.org/10.4103/jhrs.JHRS_150_18

Babu, S. V. S., Shareef, M. M., Shetty, A. P. K., & Shetty, K. T. (2002). HPLC method for amino acids profile in biological fluids and inborn metabolic disorders of aminoacidopathies. *Indian Journal of Clinical Biochemistry : IJCB*, *17*(2), 7–26. https://doi.org/10.1007/BF02867967

Church, D. D., Hirsch, K. R., Park, S., Kim, I. Y., Gwin, J. A., Pasiakos, S. M., Wolfe, R. R., & Ferrando, A. A. (2020). Essential amino acids and protein synthesis: Insights into maximizing the muscle and whole-body response to feeding. *Nutrients*, *12*(12), 1–14. https://doi.org/10.3390/nu12123717

Cochereau, I., Goldschmidt, P., Goepogui, A., Afghani, T., Delval, L., Pouliquen, P., Bourcier, T., & Robert, P.-Y. (2007). Efficacy and safety of short duration azithromycin eye drops versus azithromycin single oral dose for the treatment of trachoma in children: a randomised, controlled, double-masked clinical trial. *The British Journal of Ophthalmology*, *91*(5), 667–672. https://doi.org/10.1136/bjo.2006.099275

Fallah, A., Mohammad-Hasani, A., & Colagar, A. H. (2018). Zinc is an Essential Element for Male Fertility: A Review of Zn Roles in Men's Health, Germination, Sperm Quality, and Fertilization. *Journal of Reproduction & Infertility*, *19*(2), 69–81.

Gunes, S., Hekim, G. N. T., Arslan, M. A., & Asci, R. (2016). Effects of aging on the male reproductive system. *Journal of Assisted Reproduction and Genetics*, *33*(4), 441–454. https://doi.org/10.1007/s10815-016-0663-y

Hall, J. E., & Hall, M. E. (2020). *Guyton and Hall Textbook of Medical Physiology* (14th Edition (ed.)). Elsevier Ltd. https://www.elsevier.com/books/guyton-and-hall-textbook-of-medical-physiology/hall/978-0-323-59712-8

Hashem, N. M., Hassanein, E. M., & Simal-Gandara, J. (2021). Improving reproductive performance and health of mammals using honeybee products. *Antioxidants*, *10*(3), 1–24. https://doi.org/10.3390/antiox10030336

Ismawatie, E., Adhipireno, P., Suyono, S. S., Purwanto, E., Santoso, B., & Limijadi, E. K. S. (2021). Differences of Spermatozoa Concentration Analysis Between Manual and Automatic Methods. *Indonesian Journal of Medical Laboratory Science and Technology*, *3*(2), 122–134. https://doi.org/10.33086/ijmlst.v3i2.1961

James, E. R., Carrell, D. T., Aston, K. I., Jenkins, T. G., Yeste, M., & Salas-Huetos, A. (2020). The role of the epididymis and the contribution of epididymosomes to mammalian reproduction. *International Journal of Molecular Sciences*, *21*(15), 1–17. https://doi.org/10.3390/ijms21155535

77

Khadka, Y. R. (2021). Amino Acid-Essentiality to Human Body. *Patan Pragya*, *8*(01), 196–206. https://doi.org/10.3126/pragya.v8i01.42437

Khalifa, S. A. M., Elashal, M. H., Yosri, N., Du, M., Musharraf, S. G., Nahar, L., Sarker, S. D., Guo, Z., Cao, W., Zou, X., Abd El-Wahed, A. A., Xiao, J., Omar, H. A., Hegazy, M. E. F., & El-Seedi, H. R. (2021). Bee pollen: Current status and therapeutic potential. *Nutrients*, *13*(6), 1–15. https://doi.org/10.3390/nu13061876

Kigen, G., Kipkore, W., Wanjohi, B., Haruki, B., & Kemboi, J. (2017). Medicinal Plants Used by Traditional Healers in Sangurur, Elgeyo Marakwet County, Kenya. *Pharmacognosy Research*, *9*(4), 333–347. https://doi.org/10.4103/pr.pr_42_17

Komosinska-Vassev, K., Olczyk, P., Kaźmierczak, J., Mencner, L., & Olczyk, K. (2015). Bee pollen: Chemical composition and therapeutic application. *Evidence-Based Complementary and Alternative Medicine*, *2015*. https://doi.org/10.1155/2015/297425

Kumar, N., & Singh, A. K. (2022). Impact of environmental factors on human semen quality and male fertility: a narrative review. *Environmental Sciences Europe*, *34*(1). https://doi.org/10.1186/s12302-021-00585-w

Mărgăoan, R., Stranț, M., Varadi, A., Topal, E., Yücel, B., Cornea-Cipcigan, M., Campos, M. G., & Vodnar, D. C. (2019). Bee collected pollen and bee bread: Bioactive constituents and health benefits. *Antioxidants*, *8*(12), 1–33. https://doi.org/10.3390/antiox8120568

Martin, L. J., & Touaibia, M. (2020). Improvement of Testicular Steroidogenesis Using Flavonoids and Isoflavonoids for Prevention of Late-Onset Male Hypogonadism. *Antioxidants (Basel, Switzerland)*, *9*(3). https://doi.org/10.3390/antiox9030237

Matzkin, M. E., Calandra, R. S., Rossi, S. P., Bartke, A., & Frungieri, M. B. (2021). Hallmarks of testicular aging: The challenge of anti-inflammatory and antioxidant therapies using natural and/or pharmacological compounds to improve the physiopathological status of the aged male gonad. *Cells*, *10*(11). https://doi.org/10.3390/cells10113114

Oduwole, O. O., Peltoketo, H., & Huhtaniemi, I. T. (2018). Role of follicle-stimulating hormone in spermatogenesis. *Frontiers in Endocrinology*, *9*(December), 1–11. https://doi.org/10.3389/fendo.2018.00763

Saleh, M., Kokoszyński, D., Abd-Allah Mousa, M., & Abdel-Kareem Abuoghaba, A. (2021). Effect of date palm pollen supplementation on the egg production, ovarian follicles development, hematological variables and hormonal profile of laying hens. *Animals*, *11*(1), 1–13. https://doi.org/10.3390/ani11010069

Skoracka, K., Eder, P., Łykowska-Szuber, L., Dobrowolska, A., & Krela-Kaźmierczak, I. (2020). Diet and Nutritional Factors in Male (In)fertility-Underestimated Factors. *Journal of Clinical Medicine*, *9*(5). https://doi.org/10.3390/jcm9051400

Smith, L. B., & Walker, W. H. (2014). The regulation of spermatogenesis by

androgens. *Seminars in Cell & Developmental Biology*, *30*, 2–13. https://doi.org/10.1016/j.semcdb.2014.02.012

Suede, S., Malik, A., & Sapra, A. (2022). Histology, Spermatogenesis. *In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*, 553142.

Sullivan, R., & Mieusset, R. (2016). The human epididymis: its function in sperm maturation. *Human Reproduction Update*, *22*(5), 574–587. https://doi.org/10.1093/humupd/dmw015

Taha, E. K. A., Al-Kahtani, S., & Taha, R. (2019). Protein content and amino acids composition of bee-pollens from major floral sources in Al-Ahsa, eastern Saudi Arabia. *Saudi Journal of Biological Sciences*, *26*(2), 232–237. https://doi.org/10.1016/j.sjbs.2017.06.003

Tirpák, F., Greifová, H., Lukáč, N., Stawarz, R., & Massányi, P. (2021). Exogenous Factors Affecting the Functional Integrity of Male Reproduction. *Life (Basel, Switzerland)*, *11*(3). https://doi.org/10.3390/life11030213