Songklanakarin J. Sci. Technol.
46 (1), 31–37, Jan. – Feb. 2024

http://www.sjst.psu.ac.th

Original Article

Effect of royal jelly as supplementation in feed on sperm progressive motility and histology of testis for rabbits

Fathin Athirah Mohd Sabri[1], Mohd Nor Imran Mohd Azman[2], Mohammad Mijanur Rahman[1], and Raja Ili Airina Raja Khalif[1, 3*]

[1] *Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, Jeli, Kelantan, 17600 Malaysia*

[2] *Faculty of Bioresource and Food Security, Universiti Sultan Zainal Abidin, Besut Campus, Besut, Terengganu, 22200 Malaysia*

[3] *Department of Internal Medicine, Reproduction and Population, Faculty of Veterinary Medicine, Ghent University, Salisburylaan, Merelbeke, Belgium*

Received: 1 August 2023; Revised: 1 October 2023; Accepted: 29 January 2024

Abstract

Royal jelly (RJ) was applied as a supplement in feed to examine its impact on rabbit buck fertility. The aims were to evaluate the effect of RJ on sperm progressive motility and on histology of testis in rabbits. Twenty male rabbits were divided into four groups, each consisting of five bucks: Control (0mg), T1 {100mg RJ/kg body weight (BW)}, T2 (200mg RJ/kg BW), and T3 (300mg RJ/kg BW), with dietary treatment administered for 8 weeks. Sperm progressive motility (%), testis weight (g) and diameter of seminiferous tubule (µm) were measured. T1 group showed significantly higher ($P<0.05$) sperm progressive motility and seminiferous tubule diameter compared to the other groups, while an excessive concentration of RJ had a negative impact on the sperm quality, potentially because its components may result in an imbalance in redox reactions due to antioxidative activity. It is concluded that an appropriate dose of RJ improved the reproductive characteristics, but the results do depend on the dose administered to the rabbit.

Keywords: royal jelly, rabbit, progressive motility, testis

1. Introduction

In early 2020- 2021, farmers faced difficulties in recovering their losses from the two-year lockdowns, which required prompt actions to guarantee that food supply and demand were balanced (Hashim, Mohd Aminuddin, Idris, & Prihandono, 2023). Malaysia has to discover alternative food sources due to the current issues with a shortage of white meat, particularly from the chicken sector (Saidon *et al.*, 2023). In order to meet the growing demand for high-protein white meat, the cuniculture or rabbit farming industry had to grow its stock (Hashim *et al.*, 2023).

*Corresponding author
Email address: airina@umk.edu.my

Assisted reproduction technology, such as artificial insemination (AI) and *in vitro* fertilization, improve the reproduction performance of rabbits. It can help the farmer choose the best genetics for rabbits to produce high-quality meat. Farmers can increase the production of rabbit without reliance on natural mating. This can eliminate the costs of buying a male rabbit and its maintenance. AI is by definition the method or process of collecting sperm cells from male animals and manually depositing them into the reproductive tract of female animals. Royal jelly was chosen as a supplement for the rabbits to improve the sperm progressive motility and histology of testis.

The biological field of histology studies the structure and organization of plant and animal tissues in relation to their unique activities. The mediastinum testis, a

conical mass of connective tissue, projects into the testis from the tunica albuginea, a thick capsule that surrounds the testis (Lendvai, 2019). Androgens and spermatozoa are both made by a pair of testes. The fluid components of semen are generated by several accessory glands. The sperm is kept in long tubes until delivery to the penis. Spermatogenic cells and Sertoli cells are present in the seminiferous tubules' germinal (seminiferous epithelium) (Lendvai, 2019). Spermatogenesis takes place in the testis, which contains a group of u-shaped seminiferous tubules. Sertoli cells that help germ cells in various phases of development line the seminiferous tubules (Lendvai, 2019).

The RJ is composed of 67% water, 12.5% crude protein, 11% simple sugars (monosaccharides), and a comparatively high quantity of fatty acids (5%) (Ahmadnia, Sharifi, Alizadeh, Kamalati, & Marjan, 2015). It was assumed that the polyunsaturated fatty acids and phospholipids in RJ serve as a potential physiological mechanism for its effects when acting to protect the sperm cell membrane from oxidative damage, interact with testosterone receptors, promote cell growth, and elevate testosterone levels (Tasdogan *et al.*, 2020). Al-sanafi and Abdulla (2007) reported that RJ can significantly improve semen motility, and testosterone level which is important for motility of the sperm. RJ consumption can increase the seminal parameters, especially the motility and sperm count (Ahmadnia *et al.*, 2015).

The addition of RJ to semen extenders has a variety of effects on the quality of the sperm, as well as the ability to maintain sperm viability, motility, and fertility. It also interacts with the activity of hormones, such as luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosteron (Abdelnour *et al.*, 2020). Some studies have concluded that RJ can probably serve as a treatment to improve fertility (Ghanbari *et al.*, 2018). El-Sherbiny (2014) conducted research on the impact of RJ and bee honey added to semen extender on rabbit semen fertility capacity at room temperature. The addition of RJ and bee honey can preserve the fertility capability and semen quality, including progressive motility, viability, and abnormal spermatozoa, for at least two days, when the semen is stored at room temperature (El-Sherbiny, 2014). The objective of this study was to evaluate the effects of royal jelly on sperm progressive motility and histology of testis in rabbits.

2. Materials and Methods

2.1 Experimental animals

In total twenty male rabbits were used in the study. The study was conducted at Rabbit House, Jeli Campus, Universiti Malaysia Kelantan (UMK), Malaysia. Each treatment group consisted of five bucks. There were four groups in the study: control group was given no royal jelly (C), treatment 1 had 100 mg of RJ + 0.5 mL/kg of body weight (T1), treatment 2 had 200 mg of RJ + 0.5mL/kg of body weight (T2), and treatment 3 had 300 mg of RJ + 0.5 mL/kg of body weight (T3). Oral administration was given once a day, three times a week for 8 weeks, with 2 weeks adaptation period. Semen collection was performed every 2 weeks and the samples were pooled by groups. All the experimental procedures involving animals were conducted in accordance with Institutional Animal Care guidelines of UMK.

2.2 Semen collection

The semen collection was performed using an artificial vagina (AV) that was designed especially for rabbits (Bredderman *et al.*, 1964). The temperature of the AV was around 45–49 °C. The semen collection was performed during the day between 7:00 and 10:00 a.m. The collection was carried out every two weeks over the period of eight weeks.

2.3 Preparation of extender

The control extender was used with the formula tris-citric acid-fructose-egg yolk (TCFY). The TCFY consisted of 3.028 g of tris, 1.675 g of citric acid, 1.250 g of fructose, 20ml of egg yolk, and 0.1g of penicillin (Ng, Mohd Sabri, Mohd Azman, Rahman & Raja Khalif, 2022). The semen extender was prepared for the semen dilution.

2.4 Sperm progressive motility

3 µL of diluted semen was put on the warm glass slide. A coverslip was put on the warm slide. The glass slide was observed under a compound microscope with a 40X objective. Three measurements were made, each with a count of 100–200 sperm (Ng *et al.*, 2022). The sperm was graded based on its movement. There were four grades of sperm motility:

- Grade A: Rapid progressive motility, in which sperm can swim fast in a straight line.
- Grade B: Slow or Sluggish or Non-linear progressive motility, in which the sperm move forward but in a curved or crooked motion.
- Grade C: Non-progressive motility or vibrate, in which sperm move their tail but do not move forward.
- Grade D: Immotile, which fail to move at all.

2.5 Histopathology of testis

For sampling, two rabbits were slaughtered from each group and the testis was taken to a preservation process. The testicles of the slaughtered bucks were preserved using 10% buffered formalin. The samples were brought to the histopathology lab on the Pengkalan Chepa Campus, UMK, for histopathology with standard operating procedures, after being preserved. The specimen for histopathology was cut into small pieces. The tissue must be sliced to a maximum thickness of 3 mm. The specimen was then put on a cassette with a label. The tissue processing and embedding process was then carried out overnight on the tissue cassette using an automated tissue processor.

Following tissue preparation and embedding, tissue sectioning was carried out. To ensure that the wax was cool enough for sectioning to generate a thin, smooth, and consistent thickness of tissue section ribbon, FFPE tissue blocks were put on a – 5°C cold plate for 15 minutes. Then, the blade and blade holder's angle were fixed to 5°. To prevent significant tissue loss, the tissue block was positioned in the object clamp in the same orientation as the last tissue

block. The tissue block was thinned out to a thickness of 10 to 30 µm so that sectioning could provide a representative section. The tissue block's surface was polished with a few light strokes to remove the rough trimming. The slides for staining were obtained using a subsequent segment with a thickness of 3–4 µm. Following that, the cohesive ribbon will float on the spotless 45°C tissue flotation. Until they were sufficiently flattened, sections were left floating on the water. A slide was slowly dipped into the water bath to allow attachment of the tissue section. The slide was set up on a wooden support.

Cryostat sample handling was the next stage. For urgent biopsies, continuing surgery, neurohistology samples, demonstrating fibrofatty tissue, and donor transplant samples, frozen sections were used in cryostat sample handling. The specimen was kept in the cryochamber overnight with 30% sucrose. The auto-stainer equipment was then used to execute the Harris haematoxylin and eosin (H&E) staining procedure. Finally, mounting, decalcification, and Masson trichrome were carried out.

2.6 Experimental design

There were 20 bucks selected for the research. They were divided into 4 treatment groups, receiving by oral administration 0, 100, 200, or 300 mg/kg of RJ. Each group consisted of 5 bucks. The bucks were given a 2-week adaptation period before the oral administration. Semen collection was performed for each buck every 2 weeks over the treatment period of 8 weeks. In every collection the samples were pooled and mixed with control extenders for the sperm evaluation. Sperm quality, such as sperm viability and sperm motility (4^{Th} Asia Pacific Regional Conference on Food Security, n.d.), and sperm progressive motility, were observed in each extender. The evaluation was made after 2 hours in chilled temperature. In week 8, two bucks from each treatment group were selected to be slaughtered. The testicles were taken and preserved for histopathology. Testicle characteristics such as testicle weight and seminiferous tubule diameter were recorded.

2.7 Statistical analysis

Percentage data of sperm progressive motility and histology of testis such as testis weight (g) and diameter of seminiferous tubule (µm) were analyzed with one-way ANOVA using SPSS software. On comparing treatments, a P-value of $P<0.05$ was considered significant. The data are expressed as mean ± standard error of mean (SEM).

3. Results and Discussion

3.1 Sperm progressive motility

Table 1 shows the effects of RJ on sperm progressive motility during 8 weeks of treatment. Based on the results, T1 showed a significant difference ($P<0.05$) from the others receiving RJ, while there was no significant difference ($P>0.05$) between T1 and control group. T1 showed the highest sperm progressive motility (11.33), while T3 showed the lowest sperm progressive motility below that of control group (1.47 vs. 6.87). Table 2 shows the effects of royal jelly on sperm motility during the 8 weeks (4^{Th} Asia Pacific Regional Conference on Food Security, n.d).

A similar pattern of results was obtained in Al-sanafi and Abdulla (2007) and in Khadr *et al.* (2015). Al-sanafi & Abdulla (2007) showed that patients receiving RJ for infertile males at doses of 25, 50, and 100 mg/day experienced a substantial ($P<0.01$) increase in active sperm motility. According to other study findings, the ejaculate volume, sperm concentration, sperm progressive motility percentage, and seminal fructose content were all significantly ($P<0.05$) increased by RJ or honey treatments (Khadr *et al.*, 2015).

This may be attributed to the impact of RJ, which is known to enhance sperm motility by reducing phosphodiesterase activity and contains motility stimulants including adenosine and adenosine monophosphate ((AMP) N (1)-oxide). This enhances the phosphorylation of both cAMP/calcium-responsive element-binding protein and mitogen-activated protein kinase, and elevates cAMP at the level of the sperm tail (Nawar, Al-dujaily, Alwachi & Hatem, 2015). Due to the high concentration of calcium ions in RJ, it may entirely yield sperm cells to increase motility (Nawar *et al.*, 2015). Consequently, a rise in cAMP causes a steady increase in sperm motility. Through its impact on glycolysis, cAMP contributes significantly to the sperm's glycolytic pathway. It may affect the energy needed to generate motility in the sperm (Nawar *et al.*, 2015).

The ability of antioxidant protein components and the presence of micronutrients with beneficial impacts on the health and metabolism of bucks may be significant factors in the enhancement of sperm motility in bucks consuming RJ (Abdelnour *et al.*, 2020; Asadi, Kheradmand, Gholami, M., Saidi, & Mirhadi, 2019; Park *et al.*, 2019). The antioxidative activity of RJ has been proven by Nagai & Inoue (2004), who studied the antioxidant properties of RJ in enzymatic hydrolysates, water, and alkaline extracts. Kanbur *et al.* (2009) and Silici, Ekmekcioglu, Eraslan and Demirtas (2009) have also proven that antioxidative activity of RJ can protect against oxidative stress in animals. Pizzino *et al.* (2017) stated that a biological system's ability to detoxify these reactive products and the production and accumulation of reactive oxygen species (ROS) in cells and tissues create an imbalance that leads to the phenomenon known as oxidative stress.

The T3 group showed the lowest sperm progressive motility (1.47) throughout the treatment. In this case, there seems to be excessive antioxidant activity due to the high concentration of RJ. A redox imbalance develops when oxidants and antioxidants are no longer in balance (Liu *et al.*, 2017). ROS, such as hydroxyl radicals, directly damage cells, tissues, and blood vessels. The tissue response to somatotopic injury appears to be characterized by ROS activation and its subsequent capacity to trigger several transcription factors. Numerous genes, including those responsible for the production of pro-inflammatory cytokines, are up-regulated as a result of transcription factors such as nuclear factor-B (Watanabe *et al.*, 2013). Pro-inflammatory cytokines spread inflammation by concentrating on cells. The research makes it abundantly evident that the somatic cells of the male reproductive tract were responsible for the initial release of the pro-inflammatory cytokine. Inflammation-related changes in immunological homeostasis can result in male infertility (Sadia *et al.*, 2023). Other than that, our study also investigated sperm quality such as sperm viability and sperm

Table 1. Effects of royal jelly on sperm progressive motility during 8 weeks of treatment (Mean ± SEM)

Sperm quality	Week	Experimental group			
		C	T1	T2	T3
Progressive sperm (%)	0	2.67 ± 0.33	3.67 ± 0.33	2 ± 0.58	3 ± 0.58
	2	7.67 ± 2.91	4.17 ± 2.09	4.33 ± 2.85	3 ± 1.15
	4	4.33 ± 1.45	11.67 ± 4.80	3.33 ± 1.76	0.33 ± 0.33
	6	5 ± 2.52	28.33 ± 5.61	2 ± 0	1 ± 0.58
	8	14.67 ± 2.91	15.67 ± 3.93	0.33 ± 0.33	0 ± 0
Overall mean ± SEM		6.87 ± 1.41^{ab}	11.33 ± 2.45^{b}	2.40 ± 0.68^{a}	1.47 ± 0.42^{a}

Note: SEM, Standard error of Mean. ^{ab} Means with different superscripts in a same row differ significantly ($p<0.05$).
C = Control Group, T1 = 100mg/kg of Royal Jelly, T2= 200mg/kg of Royal Jelly, T3 = 300mg/kg of Royal Jelly

Table 2. Effects of royal jelly on sperm motility during 8 weeks of treatment

Sperm quality	Week	Experimental group				Overall mean ± SEM
		C	T1	T2	T3	
Sperm motility (%)	0	71	64	66	66	66.75±0.91^{a}
	2	61	62	31	44	51.20±3.38^{a}
	4	77	85	64	15	63.07±6.65^{a}
	6	67	86	71	23	62.00±7.17^{a}
	8	81	82	22	22	51.00±9.28^{a}
Overall mean ± SEM		71.5±1.99^{c}	73.1±2.43^{c}	50.20±5.82^{b}	34.07±5.02^{a}	

Note: SEM, Standard error of Mean. ^{ab} Means with different superscripts in a same column or row differ significantly ($p<0.05$).
C = Control Group, T1 = 100mg/kg of Royal Jelly, T2= 200mg/kg of Royal Jelly, T3 = 300mg/kg of Royal Jelly.
Table in 4Th Asia Pacific Regional Conference on Food Security, n.d.

Table 3. Effects of royal jelly on the testis of rabbit after 8 weeks of treatment (Mean ± SEM)

Experimental group	Parameters	
	Testis weight (g)	Seminiferous tubule diameter (µm)
Control	2.6±0.1^{a}	194.72±1.66^{a}
T1	4.4±0.8^{a}	246.03±1.92^{c}
T2	3.3±0.6^{a}	212.33±1.96^{b}
T3	3.8±0.1^{a}	196.42±1.45^{a}

Note: SEM, Standard error of Mean. ^{ab} Means with different superscripts in a same column differ significantly ($p<0.05$).
C = Control Group, T1 = 100mg/kg of Royal Jelly,
T2 = 200mg/kg of Royal Jelly, T3 = 300mg/kg of Royal Jelly

motility (unpublished on 4Th Asia Pacific Regional Conference on Food Security, 2023).

3.2 Histology of testis

Based on the Table 3, there were non-significant differences ($P>0.05$) in testis weight between groups. However, T1 showed a higher testis weight than the other groups (4.4±0.8). Nonetheless, the average seminiferous tubule diameter in T1 was significantly higher ($P<0.05$) than in the other groups, while T2 showed a significant difference from control and T3 ($P<0.05$). Table 3 and Figure 2 show that T1 had the highest seminiferous tubule diameter (246.03±1.92) and T3 showed the lowest seminiferous tubule diameter (196.42±1.45). Figure 1 shows photomicrographs of transverse section of rabbit's testis, while Figure 2 shows differences in seminiferous tubules of rabbit's testis. A indicates control group, B indicates T1, C indicates T2, and D indicates T3. Figures 1 and 2 show that B has the biggest seminiferous tubules among the treatment groups.

These basic findings are consistent with research showing that there is no significant difference ($P>0.05$) in testis weights among the four groups. However, oral administration of RJ does seem to improve the testis weight of the rabbit. The present results agree with Kohguchi *et al.* (2004) and Waykar and Alqadhi (2020). Kohguchi *et al.* (2004) showed that histological observations between three treatment groups (0, 50 and 500 µg of RJ/g diet) showed no changes in the testicular weight of the hamster. According to Waykar and Alqadhi (2020), when honey and royal jelly were given orally to rats (honey 500 mg/kg/day + Royal jelly 100 mg/kg/day), it was found that these rats' sperm function parameters-such as sperm count, sperm viability, sperm motility, and weight of the epididymis and testis-showed a non-significant rise in comparison to the control group.

Even though there was no significant difference ($P>0.05$) in the testis weight of the rabbits, the addition of RJ increased the testis weight. This is in line with Ghanbari *et al.* (2015), who found that male rats treated with RJ (100mg RJ/kg orally) had significantly increased testicular weight. Other studies have also found that, regarding the means for body weight and testicular weights, there was a significant difference between the diabetic group receiving RJ (400 mg/kg/day BW) treatment and the untreated diabetic group ($P<0.05$) (Karaca *et al.*, 2015). Shi *et al.* (2019) also found

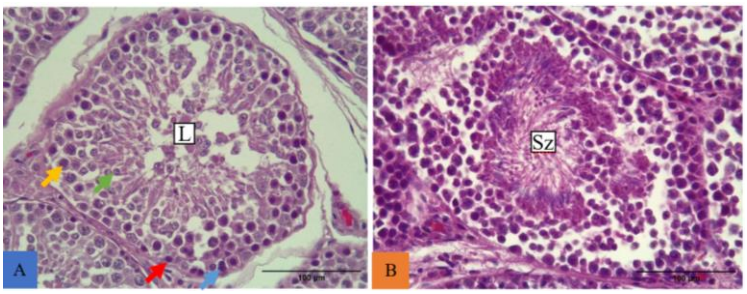

Figure 1. Photomicrographs of transverse section of rabbit's testis. A normal seminiferous tubule has numerous Sertoli cells (blue arrow), spermatogonium (red arrow), spermatocytes (yellow arrow), spermatids (green arrow), and spermatozoa (Sz) in the lumen of tubule (L). Seminiferous tubules of (A) control group, and (B) T1 group

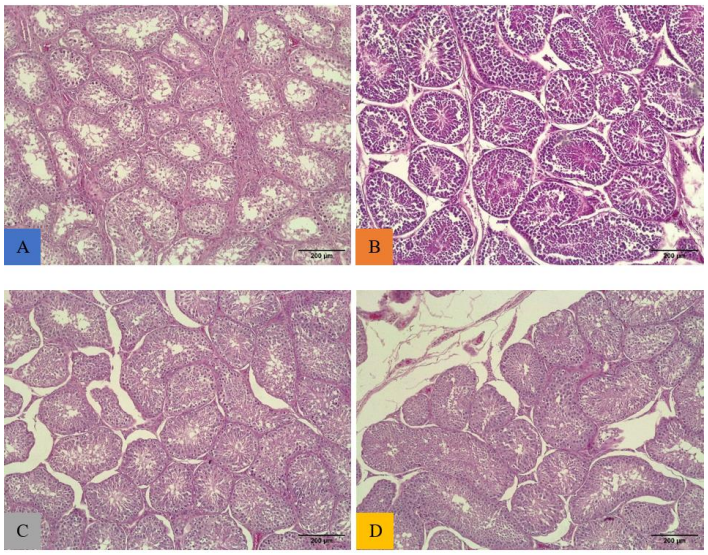

Figure 2. Seminiferous tubule of rabbit's testis. Spermatozoa in the lumen of tubule (L). (A) control group showed less spermatozoa in the lumen. (B) T1 group and (C) T2 group showed a normal seminiferous tubule. (C) T3 group showed less spermatozoa in seminiferous tubule

that at postnatal day 14, oral administration of a moderate dose of RJ (250 mg/kg/day) significantly ($P<0.05$) enhanced the testis weight, the diameter of the seminiferous tubules, and the height of the seminiferous epithelium in the offspring mice. However, seminiferous tubule width was lowered by high dose of RJ (500 mg/kg/day). High doses of RJ decreased testicular weight and size (seminiferous tubule diameter and seminiferous epithelium height). This was included to verify that a higher dosage of RJ can have a negative effect on the male reproductive organs.

However, a similar conclusion was reached by Karaca, Demirtaş, Karaboğa and Ayvaz. (2015) and Raafat and Hamam (2012) for seminiferous tubule diameter. Raafat and Hamam (2012) showed that the mean diameter of the seminiferous tubules increased significantly ($P<0.05$) in the RJ-treated group when compared to the cisplatin group, but not significantly ($P>0.05$) when compared to the control group. According to earlier findings, the diabetic group receiving RJ treatment had larger seminiferous tubules and a higher Johnsen score than the diabetic group not receiving treatment. When compared to the control group, these values were significantly lower in the untreated diabetes group (Karaca *et al.*, 2015).

El-Hanoun, Elkomy, Fares and Shahien (2014) explained that RJ had positive impacts on male reproductive capabilities because it boosted semen production from seminiferous tubules, which in turn produced complete, highly mobile sperm. Furthermore, FSH is necessary for the growth of the seminiferous tubule epithelium, maintenance of the mitotic stages of spermatogenesis, and increases production of androgen binding protein, which promotes proliferation in the spermatogenesis process (Inoue *et al.*, 2003), although RJ contains L-arginine and carnitine amino acids, which are essential for spermatogenesis (Nagai & Inoue, 2004), the blood testis barrier (BTB) will evolve as a result if specific protein expression and/or assembly are changed, because this allows toxins to more easily penetrate the seminiferous epithelium (Abdel-Kawi, 2021).

4. Conclusions

In conclusion, 100 mg of RJ has been proven to increase the seminiferous tubule diameter, but the treatment effects vary depending on the dose given to the rabbit. Therefore, oral administration of 100 mg of RJ was successful in enhancing the quality of rabbit testis compared to control,

and this dose slightly increased the sperm progressive motility. RJ has been demonstrated to be helpful when used as supplement in rabbit feed.

Acknowledgements

The authors would like to thank the Ts. Tengku Halimatun Sa'adiah Binti T. Abu Bakar, owner of rabbit house for allowing use of the place. We also like to thank Encik Ahmad Fadli Bin Ahmad Sanusi and Puan Amirah Baharin for help in histopathology test. The study was funded by Animal Reproduction Biotechnology Lab and Services (ARBiLs), R/INV/A0700/01355A/003/2022/01047.

References

Abdel-Kawi, S. H. (2021). The prophylactic effect of oyal jelly in amelioration of 6-mercaptopurine - induced testicular damage in albino rats (Histological and Immunohistochemical study). *Journal of Medical Histology*, *5*(1), 48–59. Retrieved from https://doi.org/10.21608/jmh.2022.125328.1096

Abdelnour, S. A., El-Hack, M. E. A., Alagawany, M., Taha, A. E., Elnesr, S. S., Elmonem, O. M. A., & Swelum, A. A. (2020). Useful impacts of royal jelly on reproductive sides, fertility rate and sperm traits of animals. *Journal of Animal Physiology and Animal Nutrition*, 1–11. Retrieved from https://doi.org/10.1111/JPN.13303

Ahmadnia, H., Sharifi, N., Alizadeh, S., Kamalati, A., & Marjan, S. (2015). Wonderful effects of royal jelly on treatment of male- factor related infertility. *Austin Journal of Reproductive Medicine and Infertility*, *2*(6), 1–3.

Al-Sanafi, A. E., Mohssin, S. A., & Abdulla, S. M. (2007). Effect of royal jelly on regeneration. *Thi-Qar Medical Journal (TQMJ)*, *1*(1), 1–12.

Asadi, N., Kheradmand, A., Gholami, M., Saidi, S. H., & Mirhadi, S. A. (2019). Effect of royal jelly on testicular antioxidant enzymes activity, MDA level and spermatogenesis in rat experimental Varicocele model. *Tissue and Cell*, *57*, 70–77. Retrieved from https://doi.org/10.1016/j.tice.2019.02.005

Bredderman, P. J., Foote, R. H., & Yassen, A. M. (1964). An improved artificial vagina for collecting rabbit semen. *Journal of Reproduction and Fertility*, *7*, 401–403. Retrieved from https://doi.org/10.1530/jrf.0.0070401

El-Hanoun, A. M., Elkomy, A. E., Fares, W. A., & Shahien, E. H. (2014). Impact of royal jelly to improve reproductive performance of male rabits under hot summer conditions. *World Rabbit Science*, *22*(3), 241–248. Retrieved from https://doi.org/10.4995/wrs.2014.1677

El-Sherbiny, A. M. (2014). Effect of bee honey and royal jelly addition to extender on rabbit semen fertilizing capacity at room temperature. *Egyptian Journal of Rabbit Science*, *24*(1), 119–134. Retrieved from https://doi.org/10.21608/ejrs.2014.47477

Ghanbari, E., Khazaei, M. R., Khazaei, M., & Nejati, V. (2018). Royal jelly promotes ovarian follicles growth and increases steroid hormones in immature rats. *International Journal of Fertility and Sterility*, *11*(4), 263–269. Retrieved from https://doi.org/10.22074/ijfs.2018.5156

Ghanbari, E., Nejati, V., Najafi, G., Khazaei, M., & Babaei, M. (2015). Study on the effect of royal jelly on reproductive parameters in streptozotocin-induced diabetic rats. *International Journal of Fertility and Sterility*, *9*(1), 113–120. Retrieved from https://doi.org/10.22074/ijfs.2015.4215

Hashim, R., Mohd Aminuddin, Z., Idris, S. H., & Prihandono, I. (2023). To farm or not to farm : cuniculture issues towards green marketing. *11th ASIAN Conference on Environment-Behaviour Studies*, 1–7.

Hassan, A. A. (2009). Effect of royal jelly on sexual efficiency in adult male rats. *Iraqi Journal of Veterinary Sciences*, *23*(Supplement II), 155–160. Retrieved from https://doi.org/10.33899/ijvs.2012.168705

Inoue, S., Koya-Miyata, S., Ushio, S., Iwaki, K., Ikeda, M., & Kurimoto, M. (2003). Royal jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage. *Experimental Gerontology*, *38*(9), 965–969. Retrieved from https://doi.org/10.1016/S0531-5565(03)00165-7

Kanbur, M., Eraslan, G., Beyaz, L., Silici, S., Liman, B. C., Altinordulu, Ş., & Atasever, A. (2009). The effects of royal jelly on liver damage induced by paracetamol in mice. *Experimental and Toxicologic Pathology*, *61*(2), 123–132. Retrieved from https://doi.org/10.1016/J.ETP.2008.06.003

Karaca, T., Demirtaş, S., Karaboğa, İ., & Ayvaz, S. (2015). Protective effects of royal jelly against testicular damage in streptozotocin-induced diabetic rats. *Turkish Journal of Medical Sciences*, *45*(1), 27–32. Retrieved from https://doi.org/10.3906/sag-1311-103

Khadr, A. H., Abdou, A., & El-Sherbiny, A. M. (2015). Age of puberty and fertility of male new zealand white rabbits orally administered with royal jelly or/ and bee honey. *Journal of Animal and Poultry Production*, *6*(4), 201–217. Retrieved from https://doi.org/10.21608/jappmu.2016.52748

Kohguchi, M., Inoue, S. I., Ushio, S., Iwaki, K., Ikeda, M., & Kurimoto, M. (2004). Effect of royal jelly diet on the testicular function of hamsters. *Food Science and Technology Research*, *10*(4), 420–423. Retrieved from https://doi.org/10.3136/fstr.10.420

Lendvai, M. D. D. (2019). *Morphology and histology of the testicle. Spermiogenesis.* Retrieved from https://semmelweis.hu/anatomia/files/2019/01/histology-of-the-testicle-spermatogenesis_2019_LD.pdf

Liu, H., Liu, X., Zhang, C., Zhu, H., Xu, Q., Bu, Y., & Lei, Y. (2017). Redox imbalance in the development of colorectal cancer. *Journal of Cancer*, *8*(9), 1586–1597. Retrieved from https://doi.org/10.7150/jca.18735

Nagai, T., & Inoue, R. (2004). Preparation and the functional properties of water extract and alkaline extract of royal jelly. *Food Chemistry*, *84*(2), 181–186. Retrieved from https://doi.org/https://doi.org/10.1016/S0308-8146(03)00198-5

Nawar, M. H., Al-dujaily, S. S., Alwachi, S. N., & Hatem, M. W. (2015). Effect of royal jelly on epididymal sperms characters in vasectomized mice. *Online International Interdisciplinary Research Journal*, *5*(4).

Ng, P. S., Mohd Sabri, F. A., Mohd Azman, M. N. I., Rahman, M. M., & Raja Khalif, R. I. A. (2022). Effect of extender supplemented with date palm pollen grain on caprine semen qualities. *IOP Conference Series: Earth and Environmental Science*, *1102*(1), 1–8. Retrieved from https://doi.org/10.1088/1755-1315/1102/1/012050

Park, M. J., Kim, B. Y., Park, H. G., Deng, Y., Yoon, H. J., Choi, Y. S., . . . Jin, B. r. (2019). major royal jelly protein 2 acts as an antimicrobial agent and antioxidant in royal jelly. *Journal of Asia-Pacific Entomology*, *22*(3), 684–689. Retrieved from https://doi.org/10.1016/j.aspen.2019.05.003

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., . . . Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, *2017*, 1–13. Retrieved from https://doi.org/10.1155/2017/8416763

Raafat, M. H., & Hamam, G. G. (2012). The possible protective role of royal jelly against cisplatin-induced testicular lesions in adult albino rats: A histological and immunohistochemical study. *Egyptian Journal of Histology*, *35*(2), 353–365. Retrieved from https://doi.org/10.1097/01.EHX.0000414840.79069.9d

Sadia, K., Leonel Javeres, M. N., Tahir, F., Shah, S. T. A., Habib, R., Muneer, Z., . . . Nurulain, S. M. (2023). Acetylcholinesterase, pro-inflammatory cytokines, and association of ACHE SNP rs 17228602 with male infertility. *PLOS ONE*, *18*(4), e0282579. Retrieved from https://doi.org/10.1371/journal.pone.0282579

Saidon, J., Hashim, R., Ismail, H. N., Aminuddin, Z. M., Mohd Yusof, N., Idris, S. H., . . . Apandi, B. M. (2023). Sustainable entrepreneurship, technology usage, and marketing strategies in malaysia's cuniculture industry toward food security. *International Journal of Academic Research in Business and Social Sciences*, *13*(3), 430–438. Retrieved from https://doi.org/10.6007/ijarbss/v13-i3/16637

Shi, Z., Enayatullah, H., Lv, Z., Dai, H., Wei, Q., Shen, L., . . . Shi, F. (2019). Freeze-dried royal jelly proteins enhanced the testicular development and spermatogenesis in pubescent male mice. *Animals*, *9*(977), 1–19. Retrieved from https://doi.org/10.3390/ani9110977

Silici, S., Ekmekcioglu, O., Eraslan, G., & Demirtas, A. (2009). Antioxidative effect of royal jelly in cisplatin-induced testes damage. *Urology*, *74*(3), 545–551. Retrieved from https://doi.org/10.1016/J.UROLOGY.2009.05.024

Tasdogan, A. M., Pancar, Z., Özdal, M., Vural, M., Pancar, S., & Birinci, Y. Z. (2020). The effect of short-term royal jelly supplement on testosterone levels in sedentary and healthy individuals. *Progress in Nutrition*, *22*(1), 275–280. Retrieved from https://doi.org/10.23751/pn.v22i1.8746

Watanabe, S., Suemaru, K., Takechi, K., Kaji, H., Imai, K., & Araki, H. (2013). Oral mucosal adhesive films containing royal jelly accelerate recovery from 5-fluorouracil-induced oral mucositis. *Journal of Pharmacological Sciences*, *121*(2), 110–118. Retrieved from https://doi.org/10.1254/jphs.12181FP

Waykar, B. B., & Alqadhi, Y. A. (2020). Protective role of royal jelly and honey against cisplatin induced sperm function parameters in male rats. *Indian Journal of Public Health Research & Development*, *11*(7), 153–158. Retrieved from https://doi.org/10.37506/ijphrd.v11i7.10072