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Royal jelly: A bioactive treasure for health, fertility, and cryopreservation research

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Abstract

Royal jelly (RJ), a nutrient-dense secretion produced by nurse honey bees, is one of the most valuable curative products recognized for its promising medical and nutritional applications. Predominantly composed of water, proteins, sugars, and lipids. It exhibits a wide range of beneficial properties (antioxidant, antimicrobial, antitumor, anti-inflammatory, and immunomodulatory effects). These functions enhance health and welfare in humans and animals, contributing to its widespread use in medical & commercial applications, and health foods. Additionally, RJ plays a vital role in honeybee development, serving as essential nourishment for larvae and the queen bee. RJ demonstrates estrogenic activity, influencing reproductive health by enhancing oviduct morphology, improving fertility, and alleviating menopausal symptoms. Studies on farm animals highlight its role in boosting pregnancy rates, progesterone levels, and reproductive performance while reducing oxidative stress. Recently, its favorable effect on male fertility have been reported in various animals. It has gained attention as a semen extender additive due to its potent antioxidant properties, which enhance sperm motility, viability, and membrane integrity. This review explores RJ's production, biochemical composition, and mechanisms, emphasizing its applications in reproductive sciences. RJ's unique properties position it as a promising tool in human and veterinary medicine.

Keywords: Estrogenic effect, Fertility, Royal jelly, Reproduction, Semen

Introduction

Royal jelly (RJ), a nutrient-rich "superfood" secreted by nurse honey bees through their hypopharyngeal and mandibular glands, is used to nourish larvae and the queen bee (Ahmad *et al.*, 2020; Li *et al.*, 2010)^[1, 2]. Its chemical composition was first analysed in 1888 (Khalfan *et al.*, 2024)^[3], revealing it is mainly composed of water (60–70%), proteins (9–18%), sugars (7–18%), and lipids (3–8%) (Ramadan and Ghamdi, 2012)^[4]. Its health benefits and pharmaceutical properties have been extensively studied in both animal models and humans. As a nutritional derivative of honey and bee bread, RJ is widely marketed in Asia, particularly in China and Japan, for health foods and cosmetics (Clarke and McDonald, 2017; Fratini *et al.*, 2016)^[5, 6]. It is also explored for therapeutic applications and traditional remedies for humans and animals (Pasupuleti *et al.*, 2017)^[7]. The global interest in RJ is evident from the increasing number of studies and citations in scientific literature (Ahmad *et al.*, 2020)^[1].

It exhibits estrogenic activity both *in vivo* and *in vitro*, resembling *estradiol-17β*, due to its fatty acids and sterols (Mishima *et al.*, 2005; Suzuki *et al.*, 2008)^[8, 9]. This activity can enhance oviduct morphology (El-Tarabany *et al.*, 2021)^[10]. Rodent studies highlight RJ's mechanisms on fertility, including antioxidant, hormonal, and anti-inflammatory effects (Gáspár and Seres, 2022)^[11]. In farm animals such as chickens, rabbits, buffaloes, and sheep, RJ improves pregnancy and birth rates and enhances *in vitro* fertilization success (Gáspár and Seres, 2022). It alleviates menopausal symptoms (Sharif and Darsareh, 2019)^[12], boosts fertility (Elnagar, 2010)^[13], improves reproductive performance, and reduces oxidative stress in male rabbits (El-Hanoun *et al.*, 2014)^[14]. In female sheep, RJ increases progesterone levels (Husein and Kridli, 2002)^[15] and pregnancy rates (Kridli and Husein, 2003)^[16]. RJ also interacts with estrogen receptors, modulates estrogenic activity, and enhances the expression of estrogen-responsive genes (Mishima *et al.*, 2005)^[8]. Artificial insemination enables the widespread use of genetically superior sires, creating desirable herds, reducing inbreeding, and minimizing animal movement, which helps prevent disease

spread (Youngquist and Threlfall, 2006)^[17]. Cryopreservation extends sperm longevity, with substances like egg yolk, lipoproteins, lecithin, and proteins aiding in storage (Cetin and Karaca, 2023)^[18]. Egg yolk protects sperm from cold shock during freezing and thawing but has drawbacks, including variability, sterilization challenges, and contamination risks (Bergeron and Manjunath, 2006; Gamal *et al.*, 2016)^[19, 20]. Soy lecithin has emerged as an alternative, stabilizing sperm cells through phosphatidylcholine and specific fatty acids, and resisting egg yolk coagulating enzymes (Bhai *et al.*, 2015)^[21]. Additionally, antioxidants in semen extenders improve sperm quality by mitigating oxidative stress and damage during freezing and thawing, protecting membranes, lipids, proteins, and cell organelles (Bansal and Bilaspuri, 2011; Bucak and Tekin, 2007)^[22, 23]. RJ, a potent antioxidant, enhances sperm motility, viability, and membrane integrity in extenders. Studies show low RJ concentrations (0.05–0.1%) improve sperm quality in bulls and buffaloes, although higher doses post-thawing show limited benefits (Arboud *et al.*, 2021; Shahzad *et al.*, 2016)^[24, 25]. Its unique composition and biological properties have garnered significant attention in human and veterinary sciences. This review discusses royal jelly's production process, biochemical components, and mechanisms underlying its beneficial roles in health and reproduction.

What is Royal Jelly (RJ)

The head region of honey bees contains specialized secretory structures, including the hypopharyngeal glands (HGs) and mandibular glands, situated in the anterior part of the worker bee's head. These glands secrete substances through the mouthparts and serve three primary functions: producing saliva for food processing, secreting brood food for larval nourishment, and releasing chemical signals for intra-colony communication, including anesthetic compounds (Hassanyar *et al.*, 2025)^[26]. RJ a secretion produced by worker bees (*Apis mellifera*), is a “superfood” nutrient-dense, milky-white secretion synthesized and secreted by nurse honey bees through their hypopharyngeal and mandibular glands (Hassanyar *et al.*, 2025)^[26]. This nutrient-rich substance is provided to all bee larvae during the initial stages of development. It is secreted into specialized queen cells within the hive to exclusively feed queen larvae throughout their development (Shorter *et al.*, 2015; Yang *et al.*, 2017)^[27, 28]. This diet triggers the morphological and physiological differentiation of queen bees, setting them apart from worker bees and drones. Honeybees are social bees and lives in hives or colonies which consists of three types of adult bees. Each colony typically contains thousands of worker bees, a single queen bee, and hundreds of drones. The harmonious functioning of all three bee types is essential for the colony's survival and stability.

1. **Worker Bees:** Worker bees are females that perform a wide range of essential tasks within the colony which are not used for breeding purpose. These include caring for the queen and her eggs, foraging for nectar and pollen, constructing and maintaining the honeycomb, guarding the hive and stored honey, cleaning the hive, and producing honey.
2. **Queen Bee:** The queen bee is the sole sexually mature female in the colony. Her primary role is reproduction, laying fertilized eggs that develop into worker bees and unfertilized eggs that become drones. Her reproductive activity ensures the colony's continuity and growth.

3. **Drones:** Drones are the male bees of the colony, and their primary function is to mate with a virgin queen. Once successful in mating, drones typically die, as their role concludes with fertilization.

Life Cycle of Honeybees

In all three honeybee forms, eggs hatch within three days, developing into larvae known as grubs. Initially, all grubs are nourished with royal jelly; only future queens continue on this diet. The primary compound in royal jelly is royalactin, facilitates the transformation of larvae into queen bees (Kamakura, 2011)^[29]. Once fully developed, the grubs enter the pupal stage. Queens emerge in 16 days, workers in approximately 21 days, and drones in 24 days. Upon emergence, the queens engage in combat until one remains, while the previous queen and most of her workers typically leave the hive. During this swarming event, new colonies may form at separate nesting sites.

Harvesting Royal Jelly

Royal jelly is harvested from queen cells, typically when larvae are three days old, as this maximizes yield and nutrient concentration. Careful techniques, including removing larvae and collecting the secretion with specialized tools, ensure the product's purity.

Part 1: Removing Cells from the Beehive

1. **Wear Protective Gear:** Equip yourself with long sleeves, pants, gloves, and a beekeeper's mask attached to a jacket to avoid gaps and ensure safety and comfort, especially in warm weather.
2. **Wait Until Larvae are 3 Days Old:** Harvest RJ when larvae reach three days of age for maximum yield. If handled carefully, larvae can be returned to the cells after extraction.
3. **Remove Frames and Bees:** Gently extract the frame containing royal jelly cells, sweeping off bees without agitating them.
4. **Relocate the Frame:** Move the frame indoors or away from the hive to store RJ and prevent bees from returning to the cells.

Part 2: Extracting Royal Jelly

1. **Cut Cell Openings:** Use a sharp knife to trim the narrow ends of the cells containing larvae and jelly. Cut individually or several at once by running the knife along the edges.
2. **Remove Larvae:** Carefully extract larvae using tweezers or forceps, ensuring jelly purity. Place larvae in a separate container.
3. **Collect RJ:** Use a small spatula or pipette to extract the remaining jelly from each cell.
4. **Store Jelly:** Place the extracted RJ into a glass jar, keeping it on ice to prevent spoilage. Refrigerate or freeze promptly, as jelly spoils within hours at room temperature.

Part 3: Storing Royal Jelly

1. **Transfer to Dark Glass Vials:** To protect RJ from light, move it to dark glass containers with secure lids. Leave a small space at the top of the freezing to accommodate expansion.
2. **Refrigeration:** Store at 0-5 °C (32–41 °F) for up to 18 months.

- Freezing: For extended preservation, freeze jelly at -17 °C (1 °F) or below, extending shelf life to 24 months. After thawing, refrigerate and use within 12 months.

Biochemical Composition

RJ is a complex mixture of proteins, lipids, carbohydrates, vitamins, and minerals, with bioactive components responsible for its therapeutic effects are shown in the Figure 1 (Ahmad *et al.*, 2020) ^[1].

- Proteins: Major royal jelly proteins (MRJPs) constitute 50-60% of their dry weight. MRJP^[30] is the most abundant, playing a key role in queen development.

- Lipids: Fatty acids like *10-hydroxy-2-decenoic acid* (10-HDA) contribute to its antibacterial and anti-inflammatory properties.
- Carbohydrates: Glucose and fructose are the primary sugars, providing energy.
- Vitamins and Minerals: Rich in B-complex vitamins (e.g., B5 and B6), RJ also contains trace elements such as calcium, magnesium, and iron.
- Bioactive Compounds: Royalactin, a glycoprotein, drives epigenetic modifications leading to queen differentiation.

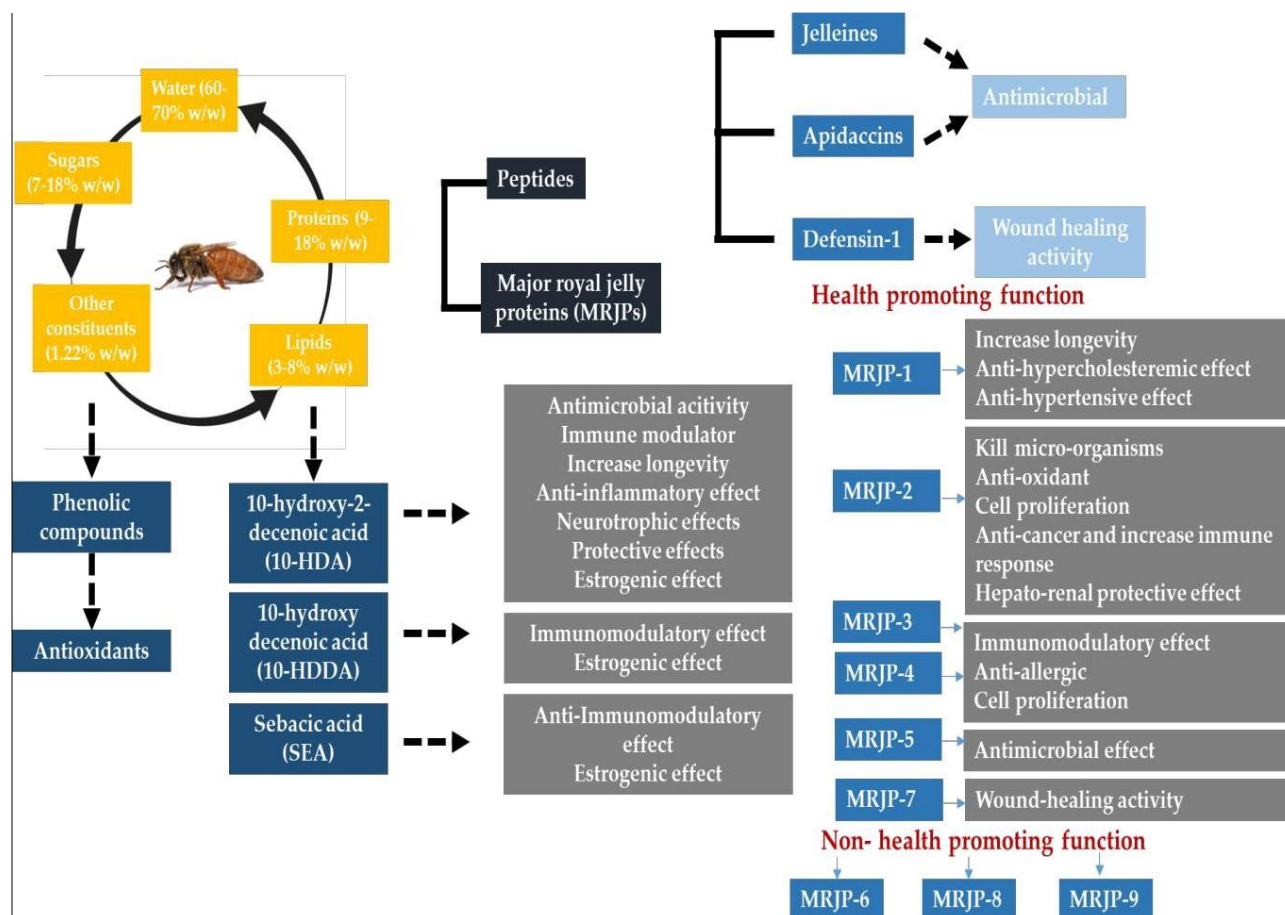


Fig 1: Primary bioactive components of Royal Jelly and their associated functional roles (Ahmad *et al.*, 2020) ^[1].

Major External Factors Influencing the Quality of Royal Jelly

1. Environmental Conditions

Environmental factors such as seasonal shifts, climate extremes, and changing feeding patterns significantly influence the protein content and amino acid composition of bee products. Geographical and temporal variations in nectar availability are crucial for understanding these differences. Temperature-dependent changes in RJ's microstructure and rheological properties show an increase in the Brix value (sugar concentration) with rising temperature due to particle swelling and yield stress (Saricaoglu *et al.*, 2019) ^[31]. Additionally, anthropogenic activities like pesticide use threaten honeybee foraging, reproduction, and food sourcing. Neonicotinoids, even when properly applied, impair honeybees motor, sensory, and cognitive functions, reducing foraging efficiency and potentially harming colony health (Ohlinger *et al.*, 2022) ^[32].

2. Source of Nectar

Floral nectar contains 2.90% to 33.51% protein, with over 16 amino acids (Nicolson & Human, 2013) ^[33]. The crude protein content in nectar and pollen plays a significant role in determining the properties of bee products like honey and RJ. Pollen from diverse floral sources across different blooming seasons contributes to variations in RJ protein ratios, reflecting its botanical origin. Lipid composition also influences RJ quality. Phospholipids range from 4.34–6.62 mg/100 g, and 10-HDA, a monounsaturated fatty acid, constitutes over half of RJ's total fatty acids. Polyunsaturated fatty acids range between 96.38 and 125.47 mg/100 g. The forage plant source notably affects lipid profiles, including phospholipids and MUFAs, impacting RJ's bioactivity and quality (Martin *et al.*, 2019; Yan *et al.*, 2022) ^[34, 35].

3. Harvesting Conditions

Harvesting RJ does not require advanced technical skills but must be performed without disturbing the hive. A colony produces approximately 900 grams of RJ during the 5–6 month blooming season. RJ is mainly harvested from queen cells when larvae are five days old, as worker bee RJ production exceeds consumption at this stage. The process involves slicing the cells thin end, extracting RJ with a micro spatula without harming the larva, and ensuring proper storage (Gemedá *et al.*, 2020) [36]. RJ can be strained, frozen for long-term storage, or processed into powders and other products (Krell, 1996) [37]. The timing of RJ harvesting significantly impacts its yield and composition. RJ harvested 72 hours post-larva grafting demonstrates the highest yield, crude protein, ash, fructose, and glucose levels, alongside the lowest water content, whereas RJ harvested 24 hours post-grafting has the highest lipid content (Al-Kahtani & Taha, 2021) [38]. Contrarily, another study found increased protein, polyphenol, and beneficial compound levels in RJ collected 24 hours post-grafting (Liu *et al.*, 2008) [39]. Such discrepancies may arise from differences in experimental conditions, beekeeping practices, or regional factors. Proper postharvest storage is essential to preserve RJ quality, particularly proteins and vitamins. Mineral content, including Mg, Ca, K, Na, Fe, Cu, and Mn, peaks in RJ harvested 48–72 hours postgrafting (Al-Kahtani & Taha, 2020) [40].

4. Honeybee Species

RJ production is influenced by genetics, colony conditions, food availability, and the queen's egg-laying activity. Beekeepers and researchers have developed methods to enhance RJ production, including breeding and genetic selection. In the 1980s, China introduced the Italian bee strain (*Apis mellifera ligustica*), which produces RJ at rates 10 times higher than native Italian bees (Li *et al.*, 2010; Han *et al.*, 2015) [2, 41]. Different honeybee races exhibit unique productivity and resistance traits shaped by environmental conditions (Khan *et al.*, 2021) [42]. Some races are more resistant to mites, while others excel in pollination (Le Conte & Navajas, 2008) [43]. Crossbreeding combines desirable traits, improving colony productivity and supporting wild bee populations, which benefits the environment (Ellis & Ellis, 2009) [44].

Effect of RJ on reproductive system

Puberty is the fundamental reproductive process during which the body undergoes several maturation changes to achieve the adult phenotype. The onset of puberty is highly sensitive to the organism's energy reserves and nutritional status. It involves a dynamic interplay between environmental factors and genetic regulation (Castellano *et al.*, 2016) [45]. In females, estrogens play a pivotal role in the differentiation, growth, and function of the reproductive organs (Shirwalkar *et al.*, 2007) [46]. Increased oxidative stress and continuous ovulation contribute

to the depletion of antioxidants such as superoxide dismutase (SOD), catalase, and glutathione S-transferase (GST). This depletion leads to a reduction in the follicle pool and a decline in oocyte quality, resulting in hormonal dysfunction. Estrogen (E_2) and inhibin from ovarian cells regulate follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels. A reduction in the follicle pool size causes inadequate secretion of E_2 and inhibin, leading to an increase in FSH levels. This feedback mechanism further depletes the follicle pool, compromising oocyte quality and accelerating ovarian aging. In ovarian cells, higher concentrations of E_2 and inhibin are required to suppress FSH and LH levels effectively. Supplementation with RJ can counteract these age-related changes. The major active compound in RJ, 10-hydroxy-2-decenoic acid (10H2DA), enhances the synthesis of ovulation-related hormones, maintaining lower levels of FSH and LH in young ovarian cells. Additionally, RJ prevents follicle pool depletion, enhances hormonal regulation, and preserves oocyte quality, thereby acting as an anti-aging agent. As ovarian aging progresses, the reduced follicle pool further exacerbates hormonal dysregulation, affecting both follicular size and oocyte quality. FSH and LH are key contributors to this aging process. RJ supplementation not only mitigates these effects but also promotes ovarian health. The most common fatty acids in RJ, including 10-hydroxy-2-decenoic acid (10H2DA), sebacic acid (SA), and 10-hydroxydecanoic acid (10-HDA), mediate E_2 signaling by enhancing the activity of estrogen receptors (ER). Suzuki *et al.* (2008) [9] demonstrated that lipidic compounds from RJ (e.g., trans-2-decenoic acid, 10-hydroxy-trans-2-decenoic acid, 10-HDA, and 24-methylenecholesterol) inhibit the binding of 17 β -estradiol to ER β , providing evidence of the estrogenic effects of RJ. In a study on ovariectomized rats, RJ competed with E_2 for binding to estrogen receptors ER α and ER β , demonstrating its estrogenic activity (Mishima *et al.*, 2005) [8]. Daily administration of RJ promoted ovarian hormone secretion and follicular development in rats, improving fertility parameters. Furthermore, RJ enhanced oocyte maturation, ameliorated redox balance, and activated glucose pathways in cumulus oophorus cells through interaction with ER. (Eshtiyaghi *et al.*, 2016) [47]. In addition, Husein *et al.* (2002) [15] reported that RJ administration in conjunction with exogenous progesterone increased pregnancy rates and enhanced the estrogenic response in ewes. Several studies have reported that dietary supplementation with royal jelly at levels ranging from 50 to 200 mg/kg in poultry significantly enhances body weight gain, egg production, semen quality, and immune function, in addition to contributing to the production of healthier animal-derived products (Abd *et al.*, 2023; El-Tarabany *et al.*, 2018; Ezzat *et al.*, 2020; Saeed *et al.*, 2018; Seven *et al.*, 2016) [30, 48–51]. These findings underscore RJ's ability to improve reproductive function across species, making it a valuable tool in addressing fertility issues (Table and Figure 2).

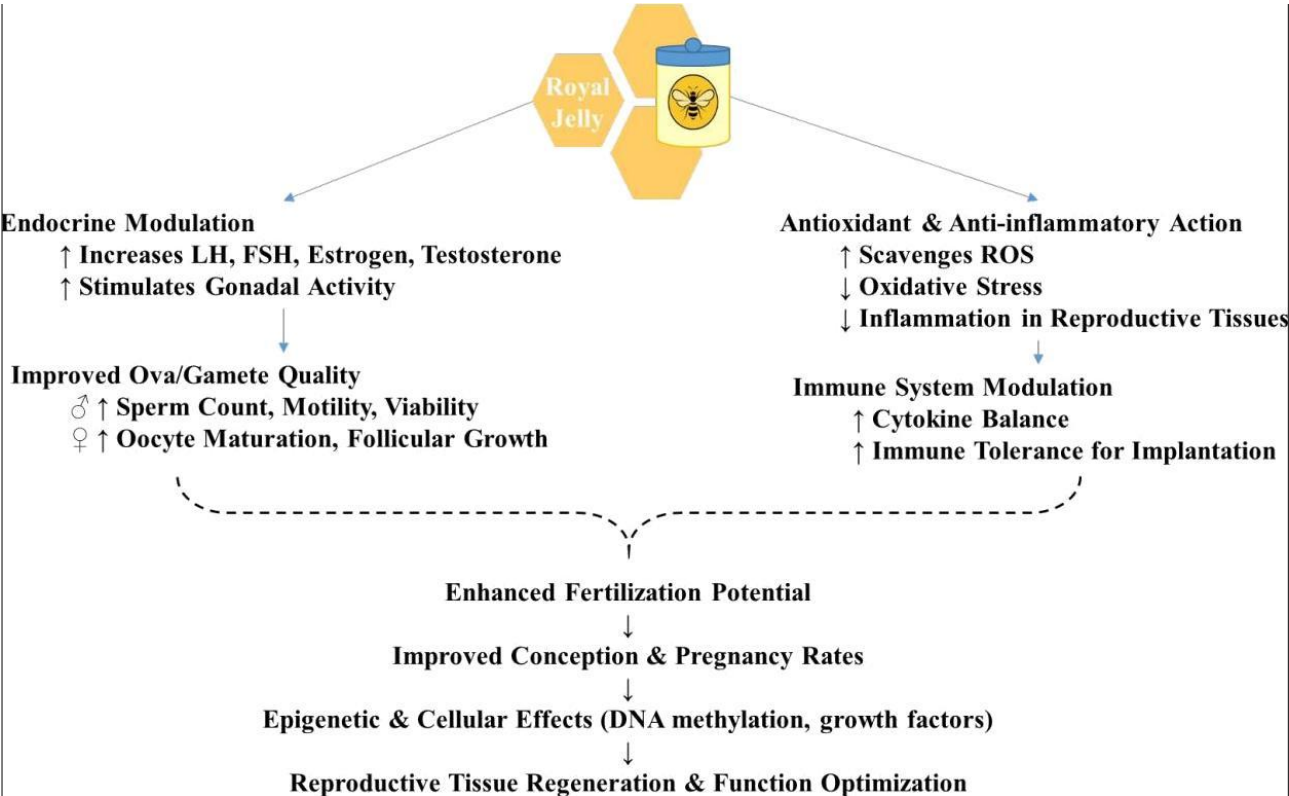


Fig 2: Role of RJ in enhancing fertility

Testosterone is essential for spermatogenesis, driving the differentiation of spermatogonia into spermatids. During puberty, gonocytes migrate to the periphery of the tubules, and sertoli cells develop in response to elevated pre-pubertal gonadotropins (Hafez and Hafez, 2000) [41]. RJ possesses strong antioxidant properties and radical scavenging capabilities, making it a valuable health product (Ghanbari et al., 2015; Silici et al., 2009) [52]. It exhibits diverse biological activities, including potential benefits for treating sexual impairments (Azad et al., 2018) [54]. RJ contains critical compounds such as testosterone, estradiol, prolactin, progesterone, and *insulin-like growth factor-1* (Moghaddam et al., 2013) [55]. RJ supports reproductive health by enhancing seminal fluid production from secondary sex organs, which is vital for sperm motility and viability. Its zinc content further improves motility (Abdelnour et al., 2019) [56]. Antioxidant, proteins and micronutrients in RJ enhance metabolism and sperm health, especially under heat stress, by reducing reactive oxygen species (ROS) and sperm abnormalities (Asadi et al., 2019; El-Hanoun et al., 2014) [14, 57]. RJ protects sperm membranes during cooling and freezing through bioactive compounds such as proline, cysteine, and 10HDA, which stabilize membrane structures, neutralize ROS, and promote glutathione production, Vitamins C and E further reinforce membrane integrity (Toker et al., 2016) [58]. Optimal

RJ supplementation (e.g., 3%) significantly improves sperm membrane and acrosome integrity in chilled and frozen-thawed samples, though excessive concentrations can have detrimental effects (Amini et al., 2019; Atalla et al., 2019) [59, 60]. RJ supplementation is critical for enhancing sperm viability, motility, and fertility (Abdelnour et al., 2020; Khazaei et al., 2018; Mahdivand et al., 2019) [61-63]. Low concentrations of RJ at 5 °C effectively reduce morphological abnormalities and dead spermatozoa (El-Sherbiny, 2013) [64]. Low levels of RJ improve sperm motility during liquid storage in Arabi rams (Raeesi Dehkohne et al., 2017) [65], while adding 1% RJ to extenders enhances motility, viability, and plasma membrane integrity in cryopreserved buck sperm (Kaleem et al., 2017) [66]. Mohammadian et al. (2016) [67] found that 1% RJ in extenders improved motility and viability in Mahabadi bucks after freezing. RJ's antioxidative activity and hydroxyl radical scavenging properties are also crucial for maintaining cell membrane integrity (Bansal & Bilaspuri, 2011; Guo et al., 2008) [22, 68]. However, excessive RJ concentrations in extenders can negatively impact sperm quality, causing residue formation, increased viscosity, and decreased osmotic pressure. Overuse may disrupt the redox balance, leading to oxidative stress and spermatozoa damage (Gawish et al., 2016; Moradi et al., 2013) [69, 70].

Table 1: Effect of Royal Jelly supplementation in different species

Species	Effect of Royal Jelly supplementation	References
Rat	Chemoprotective effects on liver and kidneys, nephroprotection, anti-fibrotic activity in pulmonary fibrosis, stimulation of folliculogenesis and steroid hormone secretion, bone strengthening and osteoporosis prevention post-ovariectomy, and enhancement of male fertility and reproductive success.	Stevanović et al., 2024 [77]
Mouse	Immunomodulatory, antioxidant and anticancer effects Antioxidant, anti-inflammatory, apoptotic and anticancer effects Anti-aging effect Improving male fertility and reproductive success	Stevanović et al., 2024 [77]
Mice	Act as potent and safe antioxidant against oxidative stress-related toxicities. It has vitamin C, vitamin E, and arginine which improves sperm quality. RJ inhibits the production of pro-inflammatory cytokines by activated macrophages.	Najafi et al., 2014[71]

Female Lab Animals	Antioxidants; Hormone balance; Antioxidative agents	Imai et al. 2012 ^[72] ; Najafi et al. 2014 ^[71]
Male hamsters	Increases the testosterone levels and spermatogenesis by stopping the age-associated decline in testicular function	Kohguchi et al., 2007 ^[73]
Rabbit	Relieving neurological problems after ovariectomy Improving male fertility and reproductive success	Stevanović et al., 2024 ^[77]
Rabbit	Improved sperm concentration, sperm motility, live sperm, total sperm output.	EL-Hanoun et al., 2014 ^[14]
Hens	Improved follicular growth and oviduct morphology	El-Tarabany et al., 2021 ^[10]
Ewes	Ameliorates the estrous effect and pregnancy rate	Husein and Kridli, 2002 ^[15]
Men	Increases sperm active motility, luteinizing hormones, and testosterone levels	Al-Sanafi et al., 2007 ^[74]
Women	Reduce premenstrual syndrome; Postmenopausal treatments	Seyyedi et al. 2016 ^[75] ; Taavoni et al. 2014 ^[76]

Conclusion

Royal jelly emerges as a multifunctional bioactive substance with far-reaching implications for both human and animal health. Its rich biochemical profile underlies a diverse spectrum of therapeutic benefits, ranging from antioxidant and immunomodulatory effects to profound impacts on reproductive physiology. The evidence underscores RJ's efficacy in enhancing fertility, improving reproductive outcomes, and protecting gametes during cryopreservation, especially at optimal low concentrations. Given its estrogenic activity, role in hormonal regulation, and ability to support sperm quality and ovarian function, RJ stands out as a promising natural agent in reproductive biomedicine. As research continues to uncover its mechanisms and refine its applications, royal jelly holds significant potential as a valuable adjunct in both clinical and veterinary reproductive technologies.

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Conflicts of interest

No potential conflicts of interest were reported by the authors.

Data availability

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