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Effect of Oral Administration of Royal Jelly towards Fertility Performance of Rabbit through Artificial Insemination

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ABSTRACT

The rabbit livestock industry is rising rapidly as an alternative protein source in Malaysia. As the global population increases, alternative food sources such as rabbit-based products are important for meeting human demand for livestock products. Optimization of rabbit production relies on reproductive technology. Assisted reproduction technologies (ART) like artificial insemination (AI) are being introduced to the farmer to maximize the production of the rabbit industry. However, local livestock farmers still need to improve their knowledge and technique. Thus, this study aims to investigate the fertility rate of does using artificial insemination compared to natural mating (NM) and to determine the effect of different concentrations of oral administration of royal jelly (RJ) on semen fertility in rabbits. A total of 12 bucks and 20 does of American White rabbits (Oryctolagus cuniculus) were used, with ages between 7 to 12 months and average body weights of 2.5 to 3 kg. The bucks were assigned to 4 experimental groups for oral administration: (a) control A, 0 mg/kg of royal jelly; (b) control B, 0 mg/kg of royal jelly; (c) treatment 1, 100 mg/kg of royal jelly; and (d) treatment 2, 50 mg/kg of royal jelly. Control A rabbit was mated naturally, while the other groups were mated using AI. Sperm quality was evaluated through physical criteria: volume, colour, pH, odour, and viscosity. Pregnancy diagnosis (manual abdominal palpation, real-time ultrasound) was performed to evaluate the conception rate of the doe. The result that the conception rate of natural mating (83.33%) was higher compared to artificial insemination (16.13%). Moreover, oral administration of royal jelly showed a negative effect on the conception rate of does, where 0 mg/kg of royal jelly showed 36.36%, 100 mg/kg showed 0%, and 50 mg/kg showed 10% of conception rate.

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Introduction

In recent times, the rabbit industry is growing fast and is in demand. In comparison to significant livestock industries such as ruminants, poultry, equines, and aquaculture, the domestication of rabbits remains insufficient. The rabbit can accommodate and convert around 20% of the cellulose-rich forages and feeds into edible meat through its digestive system (Cullere and Zotte, 2018). This feature is highly profitable for the farmer due to its high feed conversion ratio (FCR). Therefore, farmers are increasingly focusing on rabbit reproduction to produce a larger number of commercial rabbits through either natural mating (NM) or artificial insemination (AI). Despite the widespread application of AI in other livestock industries, the rabbit industry still has the potential to expand the use of this technique. Viudes-de-Castro and Vicente (1996) reported that insemination of does with fresh and frozen semen showed no differences in fertility rate and prolificity between fresh and frozen semen. Hence, a suitable oral supplement becomes even more important in improving the fertility and conception rate.

Royal jelly (RJ) has several favourable functions for male fertility in animals. Researchers have reported that RJ possesses testosterone and steroid hormone-type activities (Hidaka et al., 2006). Furthermore, the consumption of RJ can increase the seminal parameters, especially motility and sperm count, resulting in excellent reproductive function and a longer lifespan for the queen bee compared with other bee workers (Ahmadnia et al., 2015). Especially in Malaysia, the exposure of bucks to heat stress may adversely influence bucks' fertility and cause production losses. The oral administration of royal jelly counteracts summer infertility in bucks and enhances their' physiological status (Abdelnour et al., 2020). However, compared to other livestock species, only a limited number of researchers are focused on effective oral supplements for artificial insemination in rabbits. Therefore, the objective of this study was to investigate the fertility rate of does using artificial insemination (AI) compared to natural mating (NM) and to determine the effect of different concentrations of oral administration of royal jelly (RJ) on semen fertility performance.

Material and method

Experimental animal

A total of 12 bucks and 20 does of American White rabbits (*Oryctolagus cuniculus*) with ages between 7 to 12 months and average body weights of 2.5 to 3.0 kg were involved. Four experimental groups (control A, control B, treatment 1, and treatment 2) were involved in this study. 3 bucks and 5 does were assigned to each group.

Feeding trial and semen collection

In a completely randomized design, 12 bucks were allotted to four experimental groups for control A, control B, and two different dietary treatment groups (treatment 1 and treatment 2). Every experimental group had three replicates, 1 buck per replicate (n = 3/group). The volume of dietary treatment given was based on the buck's body weight (BW). According to Abdelnour et al. (2020), the dietary treatments were randomly given to the experimental groups for 60 days, with control groups A and B receiving no royal jelly, treatment group 1 getting 100 mg of royal jelly per kg of body weight, and treatment group 2 getting 50 mg of royal jelly per kg of body weight. The bucks (n = 3/group) were treated 3 days per week during the early morning.

The semen collection was carried out in the 4th week (day 30) and 8th week (day 60) from sexually mature bucks for semen evaluation and artificial insemination. An artificial vagina (AV) was used to collect the ejaculation in the early morning. During the collection period, the temperature of AV was maintained at 48°C to 49°C. The physical characteristics of ejaculated semen were evaluated through their volume, colour, odor, pH level, and viscosity using freshly collected semen. The evaluation was measured within 5–10 min after ejaculation.

Artificial insemination and natural mating

We used an insemination pipette or a rabbit AI gun to carry out artificial insemination (AI). Fifteen does participated in AI for reproduction, with five does assigned to each of the three experimental groups: control B, Treatment 1, and Treatment 2. There were five replicates (1 doe per replicate) for every experimental group (n = 5/group). Fresh semen collected from three experimental groups of bucks (control B, treatment 1, and treatment 2) was placed inside the tris-citrate-fructose-yolk (TCFY) semen extender before being used for AI. The insemination gun was inserted into the vagina, 0.5 mL of diluted semen (1:9 dilution ratio) was released into the upper vagina, and the AI gun was removed (Morrell, 1995). The AI process was performed once for each does during the same day at night.

Control A bucks (n = 3) were mated naturally with 5 does (n = 5). The buck and doe were allowed to stay in the same cage until two or three successful ejaculations had been completed. Following the successful NM and AI, we separated the does and established a suitable management regime until parturition (McNitt et al., 2013).

Pregnancy diagnosis

The day of AI and NM is regarded as the first day of pregnancy. Referring to Mazandarani et al. (2021), the pregnancy diagnosis was performed according to the tertiles of rabbit pregnancy (TOPs). The first TOP is 0 to 10 days post-mating (dpm), the second TOP is 11 to 20 dpm, and the third TOP is 21 dpm for kindling. Pregnancy diagnosis was performed using manual abdominal palpation and real-time ultrasound. We used a digital diagnostic ultrasound system for ultrasonography. The maternal behaviours of the does were observed along the gestation period, especially a few days before the parturition.

Statistical analysis

Data were analysed using an Excel spreadsheet and presented as histogram figures and tables in percentage. The statistical data was not able to be analysed due to the limitation in data collected and sample size. The conception rates of the does mated using natural mating and artificial insemination methods were compared. The fertility performance of bucks treated with different treatment groups was analysed by calculating the conception rate.

Result and discussion

Table 1. Effect of different reproductive techniques to the conception rate of doe.

Reproductive Techniques	Natural Mating	Artificial Insemination
Conception rate (%)	83.33	16.13

Table 1 displays the conception rates of does mate using natural mating (NM) and artificial insemination (AI) methods. The result revealed that a conception rate of 83.33% was observed in does that were mated using the NM method compared to a 16.13% conception rate of does that were mated using the AI method. As a result, the conception rate of naturally mated does was higher than that of AI-mated does. These results somewhat matched what Baruselli et al. (2018), in which they used estrus detection (ED) before AI led to a lower pregnancy rate than NM, probably due to mistakes made by the operators in detecting estrus. There was, in line with the findings of Ndors et al. (2015), that conception rate and litter size were significantly affected by the artificial mating frequency. Hence, the frequency of performing AI per day may be the reason for the low conception rate in this study.

The factors that directly or indirectly influence the success rate of the AI process were discussed in the research of Soliman and El-Sabrout (2020), who stated that based on the genetic makeup of the rabbit, the success rate of doing AI depends on the semen quality, percentage of live and normal spermatozoa, the duration between semen collection and AI, the doe's condition during insemination, the depth of semen deposition in the doe's reproductive tract, the receptiveness of the doe, and the insemination operator. Furthermore, Rriad et al. (2016) mentioned that the conception rate of does that are inseminated artificially is affected by the sperm cell concentration and the insemination site. The increase in the number of spermatozoa (45.04×10^6 spermatozoa in 0.5 ml) in dilution rate 1:5 and deposit in vagina at 12 cm showed an improvement in the doe conception and kindling rates with no effect on litter size (Rriad et al., 2016). Nizza *et al.* (2003) mentioned that the high frequency of ejaculation in bucks supplies a lower number of useful ejaculations, semen volume, and the number of spermatozoa without affecting the semen quality traits of ejaculate.

Table 2. Effect of different concentrations of royal jelly on the conception rate.

Variable	Control A & B (0 mg/kg BW)	Treatment 1 (100 mg/kg BW)	Treatment 2 (50 mg/kg BW)
Conception Rate (%)	36.36	0.00	10.00

Table 2 shows that the conception rate of the buck in the control groups is 36.36%, Treatment 1 is 0%, and Treatment 2 is 10%. The buck conception rate decreased when the RJ diet was administered orally. The higher the RJ diet volume, the lower the conception rate observed in this study. The conception rate of the bucks revealed the adverse effects of oral RJ administration. Although the effect of different royal jelly concentrations on rat conception rate was never discussed in this study, the results are consistent with the findings of Abdelnour et al. (2020), who stated that the high administration of RJ (800 g/kg) to pubescent male rats for one month had negative effects on their reproductive system. The discontinuation of the administration mitigated these undesired effects. This statement explains the 0% conception rate of the bucks from Treatment 1 (100 mg/kg) in this study. However, according to Khadr et al. (2015), the intake of RJ orally improves reproductive aspects, semen quality, and in vitro fertilization (IVF) outcomes. The bucks that were treated with RJ or bee honey at any level positively influenced the bucks' semen quality and fertility and triggered the puberty age of the bucks. The administration of RJ increased

the body weight, testis weight, testis index, and epididymis weight of the bucks. The early puberty of bucks led to better reproductive performance, including higher testosterone levels, more ejaculate volume, better sperm movement, and fewer abnormal and dead sperm (Khadr et al., 2015). Similarly, Xu et al. (2011) stated that the bucks that were treated with RJ at 0.1% of body weight showed an increase in sperm intensity, density, and motility.

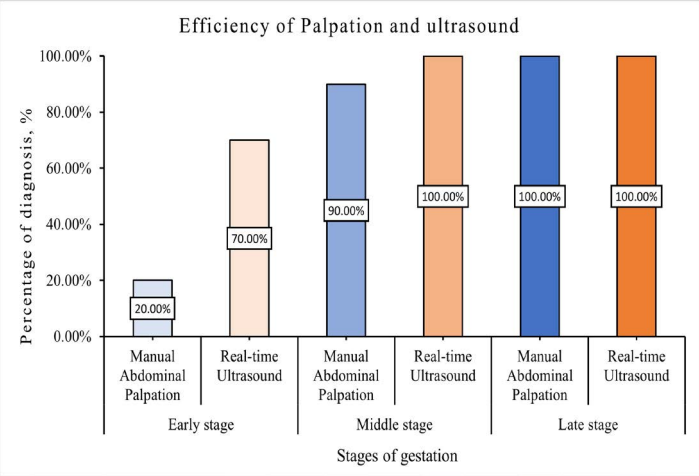

Figure 1. Percentage of pregnancy detected using manual abdominal palpation and real time ultrasound for three stages of gestation.

As shown in Figure 1, most of the conclusive establishment of pregnancy was observed during the middle stage of gestation (day 11–20 of gestation), with 90% of accurate diagnoses and 100% of reliable pregnancy diagnoses in the late stage using manual abdominal palpation. This result agrees with McNitt et al. (2013), who stated that after the tenth day of gestation, especially day 12 of gestation, is the best and most reliable stage to perform palpation. El-Gayar et al. (2014) also reported that manual abdominal palpation provided a reasonably reliable diagnosis by day 10.9 ± 0.3 of pregnancy. Figure 1 also shows that, starting from the early stage of gestation (days 1–10), a fairly accurate pregnancy diagnosis has been detected using real-time ultrasound, where 70% of the positive diagnoses were detected. The 100% reliability of real-time ultrasound pregnancy detection was recorded from the middle stage of gestation (day 11 to day 20) until the late stage (day 21 to day 30). As a result, this study agrees with El-Gayar et al. (2014), who reported that the first observation of uterine fluid was made using real-time ultrasonography between the 5th and 9th day of gestation. The fluid-filled vesicles were recognized as a sign of pregnancy because of their spherical structure. The fetuses were visible as having a high echogenicity inside vesicles beginning on the seventh day (El-Gayar et al., 2014). According to Idris et al. (2016), using ultrasound in pregnancy diagnosis could be effective as early as day 7 of gestation.

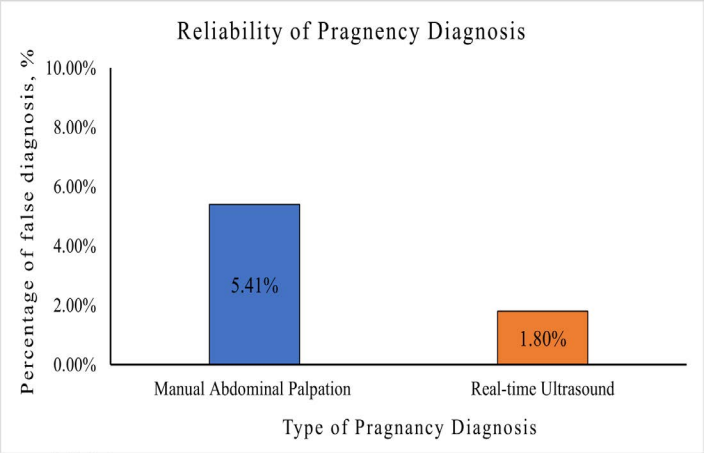

Figure 2. Percentage of false positive diagnosis of pregnancy using manual abdominal palpation and real-time ultrasound

Figure 2 shows that the false positive diagnosis of pregnancy using manual abdominal palpation is 5.41%, which is higher than using real-time ultrasound, with only 1.80% false positive diagnoses. In a pregnancy diagnosis using manual abdominal palpation, the percentage of false-positive diagnoses was higher than in a real-time ultrasound. A similar result was reported by Toal et al. (1986), who diagnosed the pregnancy of female dogs using both real-time ultrasound and manual abdominal palpation in trimester phases starting the 3rd week post-coitus. The result indicated that real-time ultrasound had a 94% accuracy rate with no false-positive diagnoses, and manual abdominal palpation had an 88% accuracy rate with one false-positive diagnosis. Similarly, real-time ultrasound recorded 36% of the accurate fetal count, and manual abdominal palpation recorded 12% (Toal et al., 1986).

Akar and Canooğlu (2009) reported that there was no difference between manual abdominal palpation and real-time ultrasound in general. On the other hand, diagnoses of doe pregnancy using ultrasonography were more successful in the early stage of gestation. Additionally, using ultrasonography with adequate equipment yielded more effective and satisfactory results compared to manual abdominal palpation (Akar and Canooğlu, 2009). Furthermore, McNitt et al. (2013) reported that diagnosing doe pregnancy using manual abdominal palpation may be difficult due to the difficult distinction between small embryos in the uterus and the pellet-shaped fecal material in the large intestine during the early stage of gestation (days 1–10). Furthermore, after the 14th day of gestation, it is more difficult to distinguish between the developing young and the digestive organs when palpating (McNitt et al., 2013). A similar statement was reported in the research of El-Gayar et al. (2014), who stated that the food and gas contents in the doe bowels might cause the erroneous diagnosis.

Conclusion

In conclusion, a higher conception rate was observed from the does mated with the natural mating method and a lower conception rate from the does mated using artificial insemination. The oral administration of royal jelly had a negative effect on the conception rate and fertility performance of bucks compared to the control group. The higher the volume of royal jelly treated, the lower the conception rate was recorded. Additionally, the effectiveness and accuracy of pregnancy testing methods were assessed, showing that real-time ultrasound can correctly identify pregnancies early on, while manual abdominal palpation can only provide reliable results from the middle of the pregnancy. Manual abdominal palpation performed a higher percentage of false positive diagnoses compared to real-time ultrasound.

We suggest doing more research with a larger group of both male and female animals, which would give us better information about how royal jelly affects the fertility of the males and how the pregnancy rates compare between natural mating and artificial insemination for the females. Further study about vaginal cytology is also recommended for the confirmation of the standing estrus time of the doe.

Competing Interest

The authors declare that there is no any conflict of interest.

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