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EFFECT OF BEE HONEY AND ROYAL JELLY ADDITION TO EXTENDER ON RABBIT SEMEN FERTILIZING CAPACITY AT ROOM TEMPERATURE

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The proportion of honey, royal jelly (RJ), egg yolk (EY) and tris-citric-glucose (TCG) extender for the extension of New Zealand White (NZW) rabbit semen at room temperature was studied. The effect of bee honey and RJ on the motility, viability, abnormalities and fertilizing capacity of NZW rabbit spermatozoa was performed in the present work. Pooled semen was processed in a TCG extender containing ascending concentrations of bee honey (0, 1, 2, 3, 4, 5, 7.5 and 10%) and descending concentrations (v/v) of egg yolk (20. 19, 18, 17, 16, 15, 12.5 and 10%) for extenders 1, 2, 3, 4, 5, 6, 7 and 8, respectively. RJ was added to tris-buffer extender with 2% bee honey and 18% egg yolk (EY), the concentrations (w/v) of RJ were 10, 20, 30, 40, 50 and 100 mg/ 6mL diluent for extenders 9, 10, 11, 12, 13 and 14, respectively. Dilution rate of semen to extenders was 1:5(v/v) with final concentration of 30×10^6 spermatozoa/ 0.5 ml diluted semen. Semen samples were stored in aliquots at room temperature up to 96 h. Diluted semen was evaluated at 0, 24, 48, 72 and 96 h after storage. Fertilizing capacity (pregnancy rate, PR% and litter size/doe, LS) of semen was examined at 0, 24 and 48 h after storage at room temperature.

***Obtained results showed that** the addition of bee honey and RJ in rabbit semen extenders could maintain both semen quality (progressive motility, viability and abnormalities of spermatozoa) and fertilizing capacity (PR and LS) for at least 2 days when stored at room temperature. In conclusion**,** the results of the present study suggested that the addition of bee honey and RJ in rabbit semen extenders maintained both semen quality and fertilizing capacity when stored at room temperature for 48 h, which would facilitate commercial diluted semen distribution.*

***Conclusively**, these results concluded that NZW rabbit semen diluted with extenders contained bee honey and RJ could be used safely within two*

days for AI when stored at room temperature. The previous results showed a significant positive effect on the quality of stored semen, which would facilitate commercial diluted semen distribution.

Key words: Extender, fertility, honey, rabbit, royal jelly, semen.

Artificial insemination (AI) is a powerful tool for genetic improvement of animals, which also offers better sanitary guarantee. It allows work organization as well as decreased manpower costs. AI of rabbit does appeared on European farms in the late 1980's. (Theau-Clement, 2007). In rabbit industry, a single ejaculate can be divided into 20 – 50 insemination doses for AI (Viudes-de-Castro *et al.*, 1998). For this reason, the ability to detect ejaculates with low *in vivo* fertility potential is important in order to eliminate those ejaculates from artificial insemination programs. Although the true fertilizing potential of an ejaculate can only be determined after using the semen to artificially inseminate females, this practice is time-consuming and accompanied with high economic cost. AI in rabbits is usually performed using fresh diluted semen yielded pregnancy rates similar or less than those would be achieved by natural mating (El-Gaafary and Marai, 1994, Morrel, 1995 and Harkness *et al.*, 2010). AI has been introduced in industrial rabbitries mainly to improve breeding management (Castellini, 1996). The major target for the rabbit breeder is how to preserve the fertilizing capacity of stored rabbit semen. Extenders are certain ingredients added to the ejaculated semen to sustain and protect the spermatozoa thereby preserving its fertility until they are used for insemination (Geoffrey *et al*, 1992).

Bee honey was used to partly replace egg yolk in egg yolk based extender (on goat bucks by Olayemi *et al.* (2011) and on rabbit bucks by El-Sherbiny (2013a). Additionally, honey contains high level of metabolizable energy in the form of glucose and fructose (Molan and Russell, 1988 and Al-Waili, 2004).

Royal jelly is a secretion product of the cephalic glands of nurse bees that has been used for its extraordinary properties and health effects (Pavel *et al*., 2011), RJ has also a multitude of pharmacological activities (Mărghitas, 2008). RJ has immunomodul -atory activities (Gasic *et al*., 2007). El-Sherbiny (2013b) concluded that RJ addition to tris basal extender which contained *dimethysulfoxide (DMSO),* 2% bee honey and 18% egg yolk (EY) improved semen quality and fertility of diluted NZW

rabbit semen after storage at -195ºC. On buffalo, *in vitro* study by Abd-Allah (2012) suggested that treating buffalo sperm with 0.4% RJ in combination with heparin is effective not only to induce sperm acrosome reaction but also is effective for *in vitro* fertilizing capacity of the cryopreserved buffalo spermatozoa. Rabbit semen dilution with Tris-buffer extenders was effective at preserving fertility for two days when stored at 15ºC (Roca *et al*., 2000).

Commercial semen distribution is still of low significance due to semen precipitation and energy expenditure movement during storing. Distribution of diluted rabbit semen is one of the important factors for the success of AI (Zaghloul, 2009), especially in developing countries.

Therefore, the objective of this work was to study the effect of bee honey and RJ addition to tris-buffer extender on the quality and fertilizing capacity of preserved rabbit semen at room temperature.

MATERIALS AND METHODS

The present study was carried out from December, 2013 to April, 2014, at the Intensive Rabbit Production Unit, Faculty of Agriculture, Ain Shams University, Cairo, Egypt and a Private Rabbit farm, Kalioubia, Egypt. Among seasons of the year in Egypt, winter and spring seasons showed good quality semen (El-Sherbiny, 1987).

Experimental animals:

Twenty sexually mature New Zealand White (NZW) rabbit bucks, aged 18 months with average body weight 4.1 kg, were selected for high quality semen (reaction time < 2 minutes, color white only, volume ≥ 0.2 ml, density creamy and milky only, concentration > 60 million/ml semen, dead-sperm percentage < 20%, and sperm abnormalities <20%). For testing the fertilizing capacity of diluted semen conserved at room temperature, 390 hybrid nonparous females at a private rabbit farm, Kalioubia, Egypt, were used in the experiment (10 females per extender, and each time from 0 h to 48 h of storage). All experimental animals were housed individually in flat deck cages and fed a commercial concentrate pelleted diet according to their reproductive condition (NRC, 1977), a free access to fresh water was also provided.

Semen collection and evaluation:

Semen was collected from each buck twice weekly using an artificial vagina (810 ejaculates were used). Two female rabbits were used as a

teaser. Immediately after semen collection, gel plug was removed. Only ejaculates with white color and good mass motility (≥3 on a 0–5 scale) were used for semen processing. After collection, semen from ten bucks was pooled together as described by Safaa *et al.* (2012). The parameters were examined to evaluate semen quality of rabbit bucks (percentages of progressive motility, dead and abnormal spermatozoa) as described by El-Sherbiny (1987) and Madhuri *et al*. (2012).

Semen dilution:

Tris-citric-glucose extender was the basic diluent and had the following composition: 0.25M of Tris buffer (hydroxylmethyl) aminomethan, 0.87M of citric acid monohydrate, 0.47M D (+) glucose, 100000 IU Penicillin and 100 mg streptomycin sulfate (Viudes-de-Castro and Vicente, 1996 and Si *et al.*, 2006), All previous components were dissolved in glass bi-distilled water and completed total volume to 100 ml. Honey was added to the diluent in ascending concentrations (v/v) 0, 1, 2, 3, 4, 5, 7.5 and 10% and descending concentrations of egg yolk 20, 19, 18, 17, 16, 15, 12.5 and 10% (v/v), for extenders 1, 2, 3, 4, 5, 6, 7 and 8, respectively. Fresh egg yolk was added just before semen collection at the percentage (El-Kelawy *et al.*, 2012 and Chen *et al.*, 1989). The honey-included egg yolk percentage was 20% (v/v) of extender according to El-Sherbiny (2013a). RJ was added to tris-buffer extender with 2% bee honey and 18% EY, the concentrations (w/v) of RJ were 10, 20, 30, 40, 50 and 100 mg/6 mL diluent according to El-Sherbiny (2013b), for extenders 9, 10, 11, 12, 13 and 14, respectively. The dilution rate of pooled semen was one volume of semen to five volume extender (v/v) at 37^{o}C. Semen samples were stored in aliquots on a laboratory shelf at room temperature up to 96 h.

Semen storage:

Total sperm-cell concentration (Conc.) was 363.08 million spermatozoa / mL. So, Conc. after dilution was 60.51 million spermatozoa / mL. The diluted semen was packed into aliquots (1 mL), then dipped in a glass of water at room temperature on a laboratory shelf. The period between semen extension and the beginning of storage did not exceed 30 minute.

Examination of diluted semen:

Semen in stored aliquots was examined for percentage of progressive motility, dead and total abnormal spermatozoa. Diluted semen was evaluated at 0, 24, 48, 72, and 96 h after storage at room temperature**.**

Artificial insemination:

Three hundred and ninety nonparous female rabbits were chosen for insemination and were thought to be sexually receptive (had red color of vulva lips). Females were injected intramuscularly with 0.3 mL receptal (GnRH analogue, 1.26 µg of busereline acetate; Intervet, Cairo, Egypt) to induce ovulation. Diluted semen showed progressive motility less than 50% did not use for AI. Then, each doe was inseminated artificially with 0.5 mL diluted semen (containing approximately 30 x 10^6 sperms) just after GnRH injection (Chen *et al.*, 1989). Pregnancy was detected by trans-abdominal palpation 14 days post-insemination (El-Kelawy *et al.*, 2012; EL-Sherbieny *et al.*, 2012 and Safaa *et al.*, 2012) to determine pregnancy rate%. Litter size was determined for each doe directly after kindling. Fertilizing capacity of stored semen was examined at 0, 24 and 48 h after storage at room temperature.

Statistical analysis:

Data of percentages of progressive motility, dead and abnormal spermatozoa of NZW rabbit semen were analyzed using Two Way Analysis of Variance (ANOVA). Litter size data were analyzed using One Way ANOVA. Whereas, for pregnancy rate trait, Catmod procedure and Chai-square test for homogeneity of variance were performed. All statistical analysis for the different traits was realized using SAS program (SAS, 2011).Differences among experimental groups were tested by Duncan's Multiple Range test (Duncan, 1955).

Two way ANOVA was according to the following model:

$$y_{ijk} = \mu + trt_i + time_j + (trt * time)_{ij} + e_{ijk}$$

Where: µ is the overall mean, y_{ijk} is the observation of the studied trait of k^{th} animal of i^{th} trt and j^{th} time, trt_i is the effect of i^{th}treatment (i = 1, 2, ……14), Tim_{ej} is the effect of j^{th} time (j = 1, 2, 3 and 4), $(trt*time)_{ij}$ is the effect of the interaction between trt and time, e_{ijk} is the individual error.

One way ANOVA was according to the following model:

$$y_{ij} = \mu + t_i + e_{ij}$$

Where: µ is the overall mean, y_{ij} is the observation of the studied trait of j^{th} animal of i^{th} treatment, t_i is the fixed effect of treatment (i = 1, 2 ., 14), e_{ij} is the individual error.

RESULTS AND DISCUSSION

Physical characteristics of diluted NZW rabbit semen:

The effect of bee honey and royal jelly on sperm progressive motility (PM) of diluted NZW rabbit semen stored at room temperature is presented in Table 1. Results showed that the effects of either extender or storage time on percentage of PM were significant ($P<0.01$). On the other hand, PM was decreased with the increasing of storage time for all extenders used in the present study. Addition of honey to extender (extenders 2, 3, 4, 5 and 6) improved PM than TCG only, but this improvement was concentration dependent. The lowest value of PM was for extender 8 (10% honey). This finding is partly in agreement with those obtained by El-Sherbiny (2013a) for frozen diluted semen. A sharp decrease in PM was found for TCG extender after 24 h of storage, whereas, extenders included honey at concentrations of 1, 2, 3, 4, 5 and 7.5% honey showed a sharp decrease in PM after 48 h of storage. The inclusion of honey as a source of glucose (Molan and Russell, 1988; Al-Waili, 2004) in egg yolk buffer diluting medium used in the present study supported by the report of Smith *et al.* (1954) that the addition of small amounts of glucose to egg yolk buffer increases and prolongs active motility of spermatozoa. This effect could be due to the antibacterial activity of honey against some microorganisms resistant to the common antibiotics used in the extender, additionally, honey is a good source of glucose and fructose (Molan and Russell, 1988). Syazana *et al*. (2011) suggested that honey has the potential to increase the fertility of male rats by increasing sperm count and number of sperm with normal morphology. In the present study, the inclusion of honey in egg yolk based extenders sustained both sperm motility and livability. However, this effect was found to be dependent on the ratio of honey to egg yolk in the extender. This is in agreement with the findings of Olayemi *et al*. (2011) on goat semen.

Tris-citric-glucose extender was used as a basal extender in the present study. Roca *et al.*, (2000) mentioned that Tris-citric-glucose extender is effective for dilution and storage of rabbit semen at 15^{o}C. Obtained results are in agreement with those obtained by El-Gaafary (1994) who showed that storage and incubation time decreased ($P<0.01$) sperm motility. While, the addition of RJ enhanced PM at 0, 24 and 48 h when stored at room temperature. The present results proved that all concentrations studied for RJ addition showed better PM than other extenders used. The best values of PM

Table 1: Effect of bee honey and royal jelly on spermatozoa progressive motility of diluted NZW rabbit semen stored at room temperature

Extender	Spermatozoa Progressive Motility (%) Time (h)					Overall mean ±SE	Sig.
	0	**24**	**48**	**72**	**96**		
1	87.3	78.7	27.7	6.3	0.0	**40.0± 0.80^{F}**	**
2	90.3	89.0	85.6	5.7	0.0	**54.1± 0.80^{C}**	**
3	94.7	92.7	89.0	16.7	0.0	**58.6± 0.80^{B}**	**
4	93.0	90.0	88.0	9.0	0.0	**56.0± 0.80^{C}**	**
5	94.0	88.3	66.7	4.0	0.0	**50.6±0.80^{D}**	**
6	89.7	78.3	65.0	4.7	0.0	**47.5± 0.80^{E}**	**
7	83.3	73.0	50.0	25.0	0.0	**46.2± 0.80^{E}**	**
8	60.3	4.7	1.0	0.0	0.0	**13.2± 0.80^{G}**	**
9	95.0	92.7	83.7	27.7	0.0	**59.8± 0.80^{B}**	**
10	95.0	92.0	84.0	27.3	0.0	**59.7± 0.80^{B}**	**
11	95.0	92.0	89.0	21.7	0.0	**59.5± 0.80^{B}**	**
12	96.7	96.0	92.0	28.3	15.0	**65.6± 0.80^{A}**	**
13	95.7	93.7	89.0	28.3	15.0	**64.3± 0.80^{A}**	**
14	95.0	93.0	92.0	0.0	0.0	**56.1± 0.80^{C}**	**
Overall mean ±SE	**90.4 ± 0.48^{A}**	**82.4 ± 0.48^{B}**	**71.7 ± 0.48^{C}**	**14.6 ± 0.48^{D}**	**2.1 ± 0.48^{E}**		
Sig.	**	**	**	**	**		

Overall means within a column or a row with different letter superscripts, differ significantly (P< 0.05).
Standard error of replicates within the treatments and hours is equal to ± 1.78
Sig.: Significance **P<0.01

was for extender 14 till 48 h storage time, while, extenders 12 and 13 exhibited 28 and 15% of PM at 72 and 96 h of storage at room temperature, respectively. The previous results when compared with those of frozen rabbit semen (El-Sherbiny, 2013b), explained that the effective doses of RJ vary according to conservation temperatures.

Results represented in Table 2 showed the effect of bee honey and RJ on percentage of dead spermatozoa (DS). These results indicated that, percentageof DS increased with increasing storage time for all extenders examined. The impact of either extender or time on DS was significant at P<0.01; while, bee honey addition in concentration of 10% showed higher DS than the other honey-extenders and Tris-basal extender. On the other hand, RJ addition to extender revealed the lowest values of DS all the storage time at room temperature.

Table 2: Effect of bee honey and royal jelly on spermatozoa dead percentage of diluted NZW rabbit semen stored at room temperature.

Extender	Dead Spermatozoa (%) Time (h)					Overall mean ±SE	Sig.
	0 h	24 h	48 h	72 h	96 h		
1	10.7	17.0	34.0	55.3	81.3	**39.7±0.62^{B}**	**
2	9.3	9.3	12.0	45.0	74.0	**30.0±0.62^{DE}**	**
3	6.0	6.0	9.3	41.0	69.0	**26.3±0.62^{F}**	**
4	7.3	8.0	9.3	43.3	68.3	**27.3±0.62^{F}**	**
5	6.7	9.3	16.7	41.7	71.7	**29.2±0.62^{E}**	**
6	8.3	13.0	16.7	45.0	73.0	**31.2±0.62^{D}**	**
7	13.0	17.3	25.0	48.3	84.3	**37.6±0.62^{C}**	**
8	26.3	41.7	54.3	67.3	85.3	**55.0±0.62^{A}**	**
9	3.7	5.0	9.0	21.0	59.7	**19.7±0.62^{I}**	**
10	4.7	6.0	9.0	20.0	64.0	**20.7±0.62^{HI}**	**
11	5.3	6.3	7.7	29.0	60.3	**21.7±0.62^{H}**	**
12	3.0	3.3	7.0	20.3	39.0	**14.4±0.62^{J}**	**
13	4.0	5.0	8.0	17.3	38.7	**14.6±0.62^{J}**	**
14	4.7	6.0	6.0	41.0	63.0	**21.1±0.62^{G}**	**
Over all mean ±SE	**8.1 ±0.37^{E}**	**11.0 ±0.37^{D}**	**16.0 ±0.37^{C}**	**38.3 ±0.37^{B}**	**66.6 ±0.37^{A}**		
Sig.	**	**	**	**	**		

Overall means within a column or a row with different letter superscripts differ significantly (P< 0.05)
Standard error of replicates within the treatments and hours is equal to ±1.38
Sig.: Significance **P<0.01

These findings may demonstrate that the effect of RJ on DS when compared with those obtained in frozen semen (El-Sherbiny, 2013b) is dependent on conservation conditions and temperature of diluted semen.

The same trend of bee honey and RJ effects was found for abnormal spermatozoa (AS) when stored at room temperature (Table 3). The results indicated that both extender and storage time had significant effect on AS (P<0.01). The lower values obtained for AS were for concentrations of 1, 2, 3, 4 and 5% bee honey, but the lowest values were for all RJ concentrations.

Dead and abnormal percentages of spermatozoa were increased with the increase of storage time, while progressive motility decreased. That may be due to the concentration of toxic substances in the extender during storage, stress from continuous motility of sperm and low energy sources available.

Therefore, both bee honey and RJ extenders are suitable for rabbit semen dilution within the first two days of storage at room temperature. This is near with the observations of Roca *et al.* (2000) and Echegaray-Torres *et al.* (2004).

Table 3: Effect of bee honey and royal jelly on spermatozoa abnormalities percentage of diluted NZW rabbit semen stored at room temperature.

Extender	Abnormal Spermatozoa (%) Time (h)					Overall mean ±SE	Sig.
	0	24	48	72	96		
1	8.7	14.3	20.0	25.0	31.0	**19.8±0.33^{B}**	**
2	6.7	8.0	22.7	22.7	26.3	**14.5±0.33^{ED}**	**
3	4.0	5.0	7.0	22.0	32.0	**14.0±0.33^{EDF}**	**
4	6.0	6.3	8.3	21.0	31.7	**14.7±0.33^{D}**	**
5	4.3	8.3	14.0	23.3	32.0	**16.4±0.33^{C}**	**
6	7.0	11.3	13.7	22.0	31.0	**17.0±0.33^{C}**	**
7	12.3	13.7	15.0	21.7	36.0	**19.7±0.33^{B}**	**
8	12.3	18.3	22.7	24.7	35.0	**22.6±0.33^{A}**	**
9	3.0	4.3	8.0	13.3	31.7	**12.1±0.33^{H}**	**
10	3.7	5.0	7.7	14.0	35.7	**13.2±0.33^{FG}**	**
11	3.7	5.7	6.7	18.0	33.7	**13.5±0.33^{EFG}**	**
12	2.3	3.7	6.7	14.0	37.0	**12.7±0.33^{GH}**	**
13	3.7	4.3	7.3	15.0	39.7	**14.0±0.33^{EDF}**	**
14	5.0	5.3	6.0	19.7	33.7	**13.9±0.33^{EDF}**	**
Over all mean ±SE	**6.1 ±0.20^{E}**	**8.0 ±0.20^{D}**	**10.8 ±0.20^{C}**	**19.7 ±0.20^{B}**	**33.3 ±0.20^{A}**		
Sig.	**	**	**	**	**		

Overall means within a column or a row with different letter superscripts differ significantly (P< 0.05)
Standard error of replicates within the treatments and hours is equal to ± 0.75
Sig.: Significance **P<0.01

Fertilizing capacity of diluted NZW rabbit semen:

Table 4 represents pregnancy rate (PR) and litter size (LS) of the 14 extenders among storage time at room temperature. There were no significant differences between experimental groups in both PR and LS at 0, 24 or 48 h after storage of diluted rabbit at room temperature. On the other hand, PR decreased significantly (P<0.05) with increased storage time. While, differences between extenders used in LS among the storage periods were significant at P<0.05.

The results showed that insemination of nonparous rabbit does artificially with diluted semen of all extenders contained honey till 48 h of storage at room temperature, otherwise, addition of 10% honey to extender showed 70% PR at 0 h, after that, spermatozoa progressive motility was not capable (4.7%) to be used for AI. Extenders contained 1, 2, 3, 4, 5 and 7.5% bee honey showed normal fertilizing capacity at 0, 24 and 48 h of storage, which may be due to that honey has the potential to increase the

Table 4: Effect of bee honey and royal jelly on fertilizing capacity of diluted NZW rabbit semen stored at room temperature.

Extender	Pregnancy rate (%) / 10 does				Litter Size / Doe				
	0h	24h	48h	Sig.	0h	24h	48h	Sig.	
1	80.0±18.6	70.0±18.6	-		7.6±0.24^{ABC}	6.9±0.26^{BC}	-		BCD**
2	80.0±18.6	80.0±18.6	70.0±18.6	*	7.9±0.24^{AB}	7.5±0.24^{AB}	6.9±0.25^{BC}		ABCD**
3	90.0±18.6	80.0±18.6	70.0±18.6	*	8.0±0.22^{A}	7.5±0.24^{AB}	6.7±0.29^{BC}		ABCD**
4	90.0±18.6	80.0±18.6	70.0±18.6	*	7.9±0.22^{AB}	7.5±0.24^{AB}	6.7±0.27^{BC}		ABCD**
5	90.0±18.6	70.0±18.6	70.0±18.6	*	7.9±0.22^{AB}	6.7±0.26^{C}	6.1±0.30^{C}		D**
6	80.0±18.6	70.0±18.6	70.0±18.6	*	7.8±0.24^{ABC}	7.0±0.24^{BC}	6.4±0.30^{BC}		D**
7	80.0±18.6	80.0±18.6	70.0±18.6	*	7.0±0.27^{C}	6.7±0.26^{C}	4.6±0.35^{D}		E**
8	70.0±18.6	-	-		7.2±0.30^{BC}	-	-		CD**
9	90.0±18.6	90.0±18.6	70.0±18.6	*	8.2±0.22^{A}	8.0±0.23^{A}	7.1±0.30^{AB}		A**
10	90.0±18.6	90.0±18.6	60.0±18.6	*	8.1±0.22^{A}	8.0±0.23^{A}	7.0±0.32^{BC}		AB**
11	90.0±18.6	90.0±18.6	70.0±18.6	*	7.7±0.22^{ABC}	8.0±0.23^{A}	7.1±0.30^{AB}		ABC**
12	90.0±18.6	90.0±18.6	90.0±18.6	*	7.4±0.22^{ABC}	8.1± 0.23^{A}	8.0±0.26^{A}		A**
13	90.0±18.6	90.0±18.6	80.0±18.6	*	7.8±0.22^{AB}	8.0±0.23^{A}	7.3±0.28^{AB}		ABC**
14	90.0±18.6	80.0±18.6	70.0±18.6	*	7.9±0.22^{AB}	7.9±0.23^{A}	7.1±0.30^{AB}		ABC**
Sig.	**NS**	**NS**	**NS**		**A****	**B****	**C****		

Overall means within a column or a row with different letter superscripts differ significantly (P< 0.05)

NS = Non significant Sig.: Significance *P<0.05 **P<0.01

fertility by increasing sperm count and number of sperm with normal morphology as mentioned by Syazana *et al*. (2011).

Addition of RJ to extenders improved fertilizing capacity (PR and LS) of diluted rabbit semen stored at room temperature till the second day of storage. Many investigators studied the effect of royal jelly on male fertility. Royal jelly is a beneficial treatment of male rats especially on sperm count and livability (Hassan, 2009).

In the present study, nonparous female rabbits were used for AI. The reproductive performance of multiparous does is lower, because of the intensive reproductive rhythms (Castellini et al., 2005). In this work, diluted semen showed more than 50% PM was used for AI, which was in agreement with Brun *et al*. (2002) who observed that mass motility and the total number of motile spermatozoa per insemination dose were highly correlated with kindling rate in rabbits. In this study, insemination dose had 30 million spermatozoa. Fertility test also, showed that rabbit semen fertilizing capacity deteriorated over storage time more quickly (Echegaray-Torres *et al*., 2004). Roca

et al. (2000) obtained 75.1 % fertility and 7.68 kids/ kindling doe when does inseminated at zero time with rabbit semen diluted in TCG extender. The results of the present study are close to that obtained by Viudes-de-Castro and Vicente (1997) who reported 91% fertility which is close to that obtained in the present study, and 9.4 kids/ kindling doe for the same time and extender type. Roca *et al.* (2000) suggested that sperm concentration in rabbit insemination dose could be slightly less than 15 million.

The present work showed that most of honey concentrations and all RJ concentrations maintained diluted semen quality and fertilizing capacity.

El-Sherbiny (2013 a & b) revealed that addition of bee honey in concentrations of 2 and 3% to TCG extender showed higher semen quality and fertilizing ability compared with other honey-extenders when diluted NZW rabbit semen stored frozen and showed that the addition of RJ at 10 mg / 6 mL extender had the best result of diluted rabbit semen quality and fertilizing ability after freezing and thawing. Future studies are needed to clarify the effect of bee honey and RJ on stored diluted rabbit semen among the four seasons of the year in Egypt, especially at summer season, where semen has lower quality (El-Sherbiny, 1987).

The results of the present study suggested that the addition of bee honey and RJ in rabbit semen could maintain both semen quality and fertilizing capacity for 2 days when stored at room temperature. Further studies are needed to clarify the mechanism of action of both bee honey and RJ on diluted rabbit semen quality and fertilizing capacity among different conservation conditions and temperature.

Conclusively, these results concluded that NZW rabbit semen diluted with extenders contained bee honey and RJ could be used safely within two days for AI when stored at room temperature. The previous results showed a significant positive effect on the quality of stored semen, which would facilitate commercial diluted semen distribution.

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تأثير إضافة عسل النحل و غذاء الملكات إلى المخفف على القدرة الإخصابية للسائل المنوى للأرانب على درجة حرارة الغرفة

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تم دراسة إضافة عسل النحل و الغذاء الملكي و صفار البيض و مخفف التريس-ستريك- جلوكوز لتخفيف السائل المنوى للأرانب النيوزيلندى الأبيض و حفظها على درجة حرارة الغرفة. و تم دراسة تأثير عسل النحل و الغذاء الملكى على حركة و حيوية و نسبة الشواذ و القدرة الإخصابية للسائل المنوى. تم تجميع السائل المنوى و إضافته للمخففات التى أساسها مخفف تريس-ستريك- جلوكوز و التى تحتوى على تركيزات تصاعدية من عسل النحل (٠ و ١ و ٢ و ٣ و ٤ و ٥ و ٧,٥ و ١٠ %) و تركيزات تنازلية (حجم/حجم) من صفار البيض (٢٠ و ١٩ و ١٨ و ١٧ و ١٦ و ١٥ و ١٢,٥ و ١٠%). تم إضافة الغذاء الملكى لمخفف التريس المحتوى على ٢% عسل نحل و ١٨% صفار بيض، و قد كانت تركيزات الغذاء الملكى (وزن/حجم) ١٠ و ٢٠ و ٣٠ و ٤٠ و ٥٠ و ١٠٠ مجم / ٦ ملليلتر من المخفف. نسبة التخفيف كانت ١ سائل منوى إلي ٥ مخفف بحيث يحتوى كل ٠,٥ ملليلتر من السائل المنوى المخفف على ٣٠ × $١٠^{٦}$ حيوان منوى. تم تخزين عينات السائل المنوى المخفف فى عبوات محكمة على درجة حرارةالغرفةلفترات تصل إلى ٩٦ ساعة. تم تقييم السائل المنوى المخفف بعد الحفظ على درجة حارة الغرفة بفترات ٠ و ٢٤ و ٤٨ و ٧٢ و ٩٦ ساعة. تم تقدير القدرة الإخصابية (معدل الحمل% و عدد الخلفة لكل أم) بعد ٠ و ٢٤ و ٤٨ ساعة من التخزين على درجة حرارة الغرفة.

أظهرت النتائج أن إضافة عسل النحل و الغذاء الملكى فى مخففات السائل المنوى للأرانب النيوزيلندى الأبيض من الممكن أن تحافظ على الخصائص النوعية (النسبة المئوية للحركة التقدمية و الحيوية و الشواذ للحيوانات المنوية) و القدرة الإخصابية (نسبة الحمل و حجم الخلفة/أم) له لمدة لا تقل عن ٤٨ ساعة من الحفظ على درجة حرارة الغرفة.

التوصية: يتضح من نتائج هذه الدراسة أن إضافة عسل النحل و الغذاء الملكى إلى مخففات السائل المنوي للأرانب قد حافظت على كلا من الخصائص النوعية للسائل المنوى و القدرة الإخصابية له عند حفظه و تخزينه على درجة حرارة الغرفة و الذى قد يسهل عملية النقل التجاري للسائل المنوي المخفف.