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Full Length Article

The Role of Artificial Insemination and the Type of Semen Extender in Improving the Reproductively of Female Rabbits during the Hot Summer Season

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Abstract

The study aimed to improve the reproductive performance of female rabbits under the high environmental temperature of the summer season using artificial insemination (AI) techniques as compared with natural mating (NM) and defining the better dilution extender that may be used in AI. 45 virgin female New Zealand white (NZW) rabbits were employed in this study. Female rabbits were distributed to three groups. First, rabbits were mated by natural mating (NM). Groups two and three, rabbits were mated using AI with tris-citrate-glucose extender and citrate-egg yolk extender, respectively. The experiment lasted July and August months beginning from mating directly and continuing during pregnancy and suckling their bunnies till completion the weaning of offspring. Results showed that significant improvement in conception rate (CR), litter number, bunny weight, and litter weight at birth and weaning were observed in the two groups of AI compared with the first group of NM. P_4 levels in the two groups of AI were higher significantly than NM at days 15 and 28 after mating, respectively. AI technique with tris-citrate-glucose extender is better than AI technique with citrate-egg yolk extender in the reproductive performance of female rabbits, especially, under the high environmental temperature of the summer season in Egypt.

Keywords: Artificial Insemination; Natural Mating; Heat Stress; Rabbits; Reproductive Traits.

Introduction

In hot countries, climatic factors are the highest constraint on the rabbit's performance. The reproductively of female rabbits in Egypt is reduced by more than 50% as a consequence of contact with the rabbits to high environmental temperature (Marai *et al.* 2002; Habeeb *et al.* 2018a). Exposure rabbits to 30 THI units or more during the hot summer season adversely affect rabbit's performance due to some harsh alterations that happen in biological purposes of animals which is accountable for the despair of productive and reproductive efficiency of heat-stressed rabbits (Askar and Ismail 2012; Habeeb *et al.* 2018b). Ameliorations of the stressful conditions of the hot summer season can be declined or eliminated that adverse effect rabbit's performance (Habeeb *et al.* 2018c). A further reproductive plan is obligatory to diminish the harmful effects of the hot summer season on rabbits reproductively. The most important plan may be the procedure of artificial insemination (AI). These protocols contain prepared the time of gonadotropin-releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) injections to regulator the synchronization of ovulation time that is exact adequate to attain standard pregnancy rates (Pursley *et al.* 1995; Zhang and Shang 2009). Besides, AI is a great technique for improving the animal's genetic which also proposals well healthy quarantine and AI allows better work organization as well as decreased manpower costs (Theau-Clement 2007; Ndors *et al.* 2015). AI may be considering one of the important methods for improving the reproductive performance of rabbits during the hot conditions due to a decrease in the interval to the first service and increased pregnancy rates (Szendro and Biro-Nemeth 1991; Szendro *et al.* 1999; Dimitrova *et al.* 2009). The timed AI program may be improving the animal fertility during the hot summer when concomitant with an injection of GnRH to induce the ovulatory follicle (Arechiga *et al.* 1998). It is well established that ovulation in the female rabbit does not happen naturally but has to be prompted through a neuro-hormonal response started during coupling (Castellini 1996; Mobarak *et al.* 2015). Ovulation in female rabbits has to be convinced by artificial hormonal stimulation when using AI

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(Ajuogu and Ajayi 2010). The ovulation-inducing method used is an intramuscular injection by GnRH and the timed AI is done by administering a sequence of GnRH and $PGF_{2\alpha}$ injection and insemination are performed at the time following the GnRH injection (Quintela *et al.* 2004; Marco-Jimenez *et al.* 2017). The effect of the AI using the fresh semen diluted in two extenders in female heat-stressed rabbits to eliminate the adversely affect rabbit's performance for improving the reproductive efficiency of rabbits and defining the best dilution extender may be used in AI under hot summer conditions.

Materials and Methods

Experimental area

The practical work was conducted in the Farm of Rabbis of the Experimental Farms Project, Atomic Energy Authority, at Inshas area, Sharkia governorate, Egypt (latitude 31º12' N to 22 º2' N, longitude 25º53' E to 35º53' E) during the July and August months of the hot summer season.

Experimental ethics

The study was permitted by the Animal Care and Welfare Committee of the Egyptian Atomic Energy Authority, Egypt. These consciences cover applicable evidence on the effort to diminish animal distress and observance to greatest notices in veterinary care allowing to the International Council for Laboratory Animal Science procedures.

Animal feeding and management

Rabbits in the three trial groups were provided with a similar food throughout the experimental period. The components of the marketable food are 40% clover hay, 25% wheat bran, 15% yellow corn, 10 soybeans, 5% molasses, 2% bone meal, 1.0% calcium carbonate, 1.0% sodium chloride. 0.5% Vitamins & minerals premix and 0.5% DL-Methionine. Chemical examination of the food as DM percentage are 18.5% crude protein, 12.5% crude fiber, 3.5% ether extract, 56% nitrogen-free extract and 9.5% ash. Digestible energy is 2600 kcal/kg DM.

The animal house was certainly aired by underwired windows. The animals were separately kept in galvanized battery pens (50 × 55 × 39 cm). Each pen was provided with a feeder, reflex nipple for drinking water and a black crock. The crock was used to amount the water intake after setting off the spontaneous nipple drinker. Urine and feces let fall from pens and cleaned daily. The experimental female rabbits before the experiment were protected with clostridia enterotoxaemia bloat.

Experimental design

Forty-five mature female healthy New Zealand White rabbits in the 1st parity were used in the research which lasted two months (from the first of July to the end of August) during the hot summer season of Egypt. The rabbits were haphazardly separated into three experimental groups, with fifteen animals in each one. Rabbits in group one were mated by natural mating (NM) with good bucks at the rate of one ♂ for five ♀. In groups two and three, the artificial Insemination technique (AI) was carried out by inseminating with tris-citrate-glucose extender and citrate-egg yolk extender, respectively. Semen was collected from the same bucks which were used in natural mating by an artificial vagina. The heat of the water in the internal elastic cover of the artificial vagina was adjusted to 40–42°C and the lubrication of the internal envelope was achieved using white Vaseline. Induction of ovulation was done by administration of a Gonadotropin-releasing hormone (GnRH) analog, as Buserelin (Receptal) 0.2 mL subcutaneous injection according to Heba-T-Allah *et al*. (2016). The pattern of hormonal stimulation using only GnRH request 0.2 mL intramuscularly at the moment of insemination was more effective for insemination of rabbit does (Dimitrova *et al.* 2009). Female rabbits were inseminated using fresh semen at the rate of 20 million spermatozoa diluted in 0.5 mL of Tris-citrate-glucose extender or citrate-egg yolk extender. During the time of insemination, each female rabbit has managed an intramuscular injection of buserelin acetate (1 μg/doe) to encourage the ovulation and oestrus synchronization according to Abd El-Nour *et al*. (2017). The abdominal palpation on the 12th day after the mating was carried out to diagnostic of the pregnancy.

Importance of extenders and the two types of extenders used in the experiment

Extenders offer the nutrients desirable for the metabolic conservation of sperm cells and regulator the pH and the osmotic density of the intermediate (López and Alvariño 1998). The composition of extenders plays a very important role in sperm cells viability. Different extenders have been used to protect the sperm during processing and storage in chilled and frozen semen (Di-Iorio *et al.* 2014). Tris-citrate-glucose (TCG) and Citrate-egg yolk (CEY) extenders are generally the most applied for liquid rabbit semen storage and the best extenders identified and suitable extenders for the sawing of rabbit spermatozoa up to72 h (Aurich *et al.* 2007). The two extenders used in this research are Tris-citrate-glucose (TCG) and sodium citrate-egg yolk extender (CEY).

Tris-citrate-glucose (TCG) extender contained 88 m*M* of citric acid anhydrous, 250 m*M* of tris-hydroxy methyl-amino methane, 47 m*M* of glucose and 80 mg/L of kanamycin sulfate. Distilled water was added to these components to have 100 mL final volume (Di-Iorio *et al.* 2014).

The sodium citrate-egg yolk extender (CEY)

contained 100 mL of distilled water, 58 mg of glucose, 5 g of sodium citrate dehydrate, 20 mL of egg yolk, 1 mg of penicillin and 1 mg of streptomycin as was reported by Ewuola *et al*. (2014).

Conception rate (CR) was estimated by the abdominal palpation achieved in each doe after 12 days from AI according to Roca *et al*. (2000) under the two techniques as follows:

CR = No. of pregnant doe delivered / No. of doe joined to the buck ×100 in NM

CR =No of pregnant doe delivered/ /No of doe inseminated × 100 in AI

Blood samples and estimation of progesterone level

Three blood samples from each doe of the three groups were withdrawn from the ear vein into new tubes at 0, 15 and 28 days from mating. Serum was separated from the blood samples by centrifugation at the rate of 3,000 rounds per minute for 15 min. Serum was kept at -20ºC until the time of the assessment of progesterone level (P_4). P_4 hormonal level was assessed using the Radioimmunoassay technique (RIA) by marketable kit (Diagnostic Product Corporation, Los Angeles, USA). The tracer in the P_4 hormone was labeled with iodine-125 (I^{125}). After the incubation period, the fluid substances of the tubes are removed, and the radioactivity of labeled iodine is counted in a computerized gamma counter at the Biological Applications Department of Egyptian Atomic Energy Authority.

Estimation of the environmental conditions

Ambient temperature (AT,°C) and percentage of humidity (RH%) were recorded inter the Farm housing by digital Thermo- hygrometer at 13.00 a.m. hours daily once a week. The average of the three measurements was presented as the weekly value. Averages of AT and RH% throughout the experimental period were 34.5 and 81.2, respectively. The collective influence of the ambient air temperature and relative humidity as the temperature-humidity index (THI) was calculated according to the equation of Marai *et al*. (2001). The average THI value throughout the experiment was 31.5 units. Documents presented those female experimental rabbits during the AI process were exposed to very severe heat stress of the hot summer season.

Statistical analysis

Statistics were examined by process of SPSS (2012) version19 conferring to the subsequent model: $Y_{ij} = \mu + T_i + e_{ij}$ Where: Y = dependent variable, μ= the overall mean, T_i = the effect of treatments (1= Natural matting, 2= AI using Tris-citrate-glucose extender, 3= AI using citrate-egg yolk extender, e_{ij} = remaining error.

The differences among means were matched by Duncan's new multiple ranges test (Duncan 1955). The possibility of the CR and percentage of mortality were tested by the Chi-square test and important outcomes were estimated using the multiple Z-tests to parallel equivalent amounts.

Results

Effect of artificial insemination on conception rate

The number of pregnant does increase significantly from 8.0 in NM to 11.0 and 10.0 in AI using Tris-citrate-glucose extender and AI using citrate-egg yolk extender, respectively. The overall mean of the number of pregnant does in the two AI groups was significantly ($P < 0.01$) higher than in the NM group by 31.3%. The conception rate (CR) in the NM group of female rabbits was 53.3% while in the AI using Tris-citrate-glucose extender CR improved ($P \leq 0.01$) to 73.3% (+37.52%) and improved ($P \leq 0.01$) to 66.7% (25.14%) using AI with citrate-egg yolk extender. The overall mean improvement in CR in the two AI groups was higher ($P < 0.01$) than NM by 35.1%. CR in AI using Tris-citrate-glucose extender was higher than CR in AI using citrate-egg yolk extender by 9.9% (Table 1).

Effect of artificial insemination on progesterone (P4) levels

Levels of P4 hormone at 15 and 28 days from mating were 14.4 ± 0.60 and 6.1 ± 0.11 ng/mL in NM and increased ($P \leq 0.01$) to 21.4 ± 0.25 and 8.9 ± 0.12 ng/mL in AI using Tris-citrate-glucose extender and to 17.5 ± 0.23 and 6.8 ± 0.12 ng/mL in AI using citrate-egg yolk extender, respectively. The corresponding overall means in the P_4 level due to AI were 19.5 ± 1.97 and 7.85 ± 1.06 ng/mL. The percentage increases in P4 level due to AI were 35.4 and 29.5 at days 15 and 28 post-mating, respectively. P4 level was less than 1 ng/mL in the four experimental groups during the previous day of mating and no significant difference in P4 level between groups. Levels of P4 hormone at 15 and 28 days from mating were higher significantly in AI using Tris-citrate-glucose extender than those levels in AI using citrate-egg yolk extender (Table 2).

Effect of artificial insemination on litter number and litter weight at birth

Averages of litter number, bunny weight and litter weight at birth increased significantly by 19.4, 47.4 and 76.08%, respectively, due to AI with Tris-citrate-glucose extender in comparison with the NM group. The corresponding increasing percentages due to AI with citrate-egg yolk extender were 8.11, 26.17 and 36.43%, respectively, in comparison with the NM group. The overall mean averages

Table 1: Effect of artificial insemination on conception rate (CR)

Reproductive traits	Conception rate				
	Natural mating (NM)	Artificial insemination (AI)			†Change % due to AI & significant
		Tris-citrate-glucose extender	citrate-egg yolk extender	AI overall mean	
No of does at mating	15.0	15.0	15.0	15.0	----
Pregnant No.	8.0	11.0	10.0	10.5	+31.3**
CR (%)	53.3[c]	73.3[a]	66.7[b]	72.0	+35.1**
CR increase %		37.52	25.14	+31.7	

a, b…Means in the same row within each item having different superscripts differ significantly at $P < 0.05$
†Change = [(AI - NM)/ NM] X 100. **=significant at $P < 0.01$

Table 2: Progesterone (P_4) levels (ng/mL) in NZW does use natural mating or artificial insemination at different post-mating days

Post-mating days	Progesterone (P_4) levels (ng/mL)				
	Natural matting (NM)	Artificial insemination (AI)			†Change % due to AI & significant
		Tris-citrate-glucose extender	citrate-egg yolk extender	AI overall mean	
0	0.50 ± 0.17	0.51 ± 0.15	0.53± 0.13	0.52 ± 0.01	-4.0[NS]
15	14.4[c] ± 0.60	21.4[a] ± 0.25	17.5[b] ± 0.23	19.5 ± 1.97	+35.4**
28	6.10[c] ± 0.11	8.9[a] ± 0.12	6.8[b] ± 0.12	7.85 ± 1.06	+29.5**

a, b. Means in the same row within each item having different superscripts differ significantly at $P < 0.05$. †Change = [(AI-NM)/NM] X 100, NS = not significant, ** = significant at $P < 0.01$

Table 3: Effect of artificial insemination on litter size and litter weight at birth

Reproductive traits	Litter size and litter weight at birth				
	Natural mating (NM)	Artificial insemination (AI)			†Change % due to AI & significant
		Tris-citrate-glucose extender	citrate-egg yolk extender	AI overall mean	
Average litter size	4.07[c] ± 0.29	4.86[a] ± 0.39	4.40[b] ± 0.42	4.63	+13.8*
Bunny weight, g	48.9[c] ± 2.44	72.1[a] ± 6.3	61.7[b] ± 2.7	66.9	+36.8**
Litter weight, g	199.0[c] ± 10.0	350.4[a] ± 16.4	271.5[b] ± 12.2	311.0	+56.3**

a, b. Means in the same row within each item having different superscripts differ significantly at $P < 0.05$. †Change = [(AI-NM)/NM] X 100.* = significant at $P < 0.05$, ** = significant at $P < 0.01$

Table 4: Effect of artificial insemination on litter size and litter weight at weaning

Reproductive traits	Litter size and litter weight at weaning				
	Natural matting	Artificial insemination			†Change % due to AI & significant
		Tris-citrate-glucose extender	citrate-egg yolk extender	AI overall mean	
Average litter size	3.50[c] ± 0.17	4.74[a] ± 0.15	4.16[b] ± 0.13	4.45	+27.1**
Bunny weight (g)	493.4[b] ± 11.6	543.4[a] ± 24.4	547.2[a] ± 27.4	545.3	+10.5*
Litter weight (g)	1727[c] ± 77	2576[a] ± 179.5	2278[b] ± 135.22	2427	+40.5**
Increase in LW (g)		849.1	551.1	+700.1	
Increase in LW (%)		49.16	31.91	40.5	
*Mortality rate (%)	14.00[a]	2.47[c]	5.45[b]	3.96	-71.7**

a, b. Means in the same row within each item having different superscripts differ significantly at $P < 0.05$. *Statistical differences between Change = [(AI - NM)/ NM] X 100. * = significant at $P < 0.05$, ** = significant at $P < 0.01$

of litter size, bunny weight and litter weight in the two AI groups were higher significantly ($P < 0.05$; $P < 0.01$) than in the NM group by 13.8 and 36.8 and 56.3%, respectively. Using Tris-citrate-glucose extender in AI was better than using citrate-egg yolk extender in averages of litter size, bunny weight and litter weight by 10.5, 16.9 and 29.1%, respectively (Table 3).

Effect of artificial insemination on litter size and litter weight at weaning

Averages of litter size, bunny weight and litter weight at weaning improved significantly due to AI compared with the NM group. Litter size values at weaning increased significantly ($P \leq 0.01$) due to AI using Tris-citrate-glucose extender and citrate-egg yolk extender with an increasing percentage of 35.43 and 18.9 respectively. Litter size at weaning in AI using Tris-citrate-glucose extender was better than using citrate-egg yolk extender by 13.94%. Average bunny weight and litter weight at weaning improved significantly ($P < 0.05$; $P \leq 0.01$) due to AI using citrate-egg yolk extender by 10.2% and 49.17 and using Tris-5 citrate-glucose extender by 10.9% and 31.9%, respectively, compared with NM. The overall means of improvement in litter size, bunny weight and litter weights at weaning due to AI were 27.1, 10.5 and 40.5 respectively. The increases in LW values at weaning due to AI using Tris-citrate-glucose extender and citrate-egg yolk extender were 849.1 and 551.1 g with a percentage of 49.16 and 31.91 respectively, compared with NM. The overall mean increase in LW at weaning due to AI was 700.1 g with a percentage of 40.5 compared with NM. Using Tris-citrate-glucose extender in

AI was better in LW at weaning than using citrate-egg yolk extender by 298 g (54.1%) as presented in Table (4).

The mortality rate in bunnies during the suckling period decreased ($P \leq 0.01$) by 82.4, and 61.1% due to AI with Tris-citrate-glucose extender and citrate-egg yolk extender, respectively, with an overall mean of 71.7 compared with NM. The mortality rate in kits during the suckling period in AI using Tris-citrate-glucose extender was lower significantly than that of AI using citrate-egg yolk extender (Table 4).

Discussion

Under the hot summer season, the AI in female rabbits increased significantly the number of pregnant does by 37.5% using Tris-citrate-glucose and by 25.0% using citrate-egg yolk extenders compared with the NM. The decrease in the number of pregnant rabbits under heat stress during NM may be due to the reduction in sexual desire of male rabbits under heat-stress conditions.

AI using Tris-citrate-glucose extender enhanced ($P \leq 0.01$) CR from 53.3% in NM to 73.3% (+37.52%) and 66.7% (25.14%) using AI with citrate-egg yolk extender. Using the Tris-citrate-glucose extender was better by 13.13% than using the citrate-egg yolk extender. The improvement in CR in AI female rabbits may be due to the administration of GnRH which induction of ovulation. The priority of Tris-citrate-glucose than citrate-egg yolk extender may be due to the highest content of energy (glucose) available for sperms in the semen.

Tawfeek and El-Gaafary (1991) found a significant difference in conception rate between the AI as compared to NM. Injected NZW rabbits with 0.2 mL GnRH /doe at the time of insemination caused a significant increase in CR and gestation length (El-Ghaffar 1992; El-Gaafary and Marai 1994). The fertility rate for the does inseminate with semen diluted with Tris-extender was 71.5%, with an average of 8.1 higher than the does inseminate with semen diluted with the others extenders and it is a possibility that extender Tris-extender provides better energy and motility to rabbit sperms, thus developed fertility rates to rabbits does (Carluccio *et al.* 2004).

Heat stress has affects negatively most characteristics of individually male and female reproduction roles, such as the CR, pregnancy rate, estrous activity, embryonic mortality, sperm motility and sperms mortality (Hansen 2009). Heat stress impaired reproductive happenings by diminishing the appearance of estrous activities, changing ovarian follicular advance, cooperating oocyte capability and stopping embryonic progress (Mondal *et al.* 2017). Heat stress weakens several purposes related to beginning and maintaining pregnancy, containing changed follicular progress and domination patterns, corpus luteum deterioration, weakened ovarian function and oocyte superiority and capability, embryonic progress, enlarged embryonic mortality and early fetal loss, endometrial role, reduced uterine bloodstream and cheap expression of estrus activities like mounting (Schüller *et al.* 2017). The probability of successful AI might be shortened due to exposing the rabbits to heat stress because of altering the intrauterine environment and therefore CR extremely drops (Hansen *et al.* 2001). Heat load is also accompanied by lesser concepts of litter size, which may impact maternal recognition of pregnancy and keep of the corpus luteal role. Heat stress causes impaired the developing embryo and leads to lower CR and fertility (Gantner *et al.* 2011). The reduction in CR during the hot summer months can range between 20–30% as compared to the results obtained in the winter months (Raval and Dhami 2005).

The low CR during the hot summer season may be due to a complex set of actions that are communicated into the adverse effects on reproductively and also may be a cause of either fertilization failure or early embryonic death (Shehab-El-Deen *et al.* 2010). Many reasons for low CR during the hot summer season include reduced oocyte quality, failure of fertilization, reduced embryonic progress and diminished secretion of several hormones and reductions in embryonic weightiness (Sartori *et al.* 2002).

Levels of P_4 hormone at 15 and 28 days from mating were highly ($P \leq 0.01$) in AI technique using Tris-citrate-glucose extender or citrate-egg yolk extender than in NM technique. The percentage increase in P_4 level due to AI was 35.4 and 29.5 at days 15 and 28 post-mating, respectively. Administration of a GnRH analog in AI technique, as well as increasing the number of corpus luteums in pregnant rabbits, may be responsible for increasing the P_4 level. El-Ratel *et al.* (2017) reported that P_4 on 14-, 21- and 28-days post-mating was significantly higher in the group treated with GnRH during AI than in the control group.

Reproductive efficiency is influenced by the level of sexual hormones, mainly, testosterone in males and progesterone in females and may consequently be blocked when stressors interact with sexual hormones. There are many shreds of evidence that serious stressors impair animals reproductively during critical periods of the reproductive phase, especially, during early pregnancy and lactation (Habeeb 2020b, c). Heat stress also falls the secretion of estradiol and luteinizing hormones and a lower concentration of estradiol in the follicular fluid of dominant follicles must be a key to the decline in reproductive efficiency in animals (Wolfensen *et al.* 1997). The adverse effects of hot summer on semen characteristics could be explained by the deterioration of the germinal epithelium and to the partial deterioration of seminiferous tubules and defects of the spermatogenesis, particularly, in the last stage of differentiation of spermatids (Gantner *et al.* 2011). Rabbits also exposed to thermal conditions had lower libido (Theau-Clément *et al.* 2009).

At birth, the increasing percentages in litter number, bunny weight and litter weight were 19.4, 47.4 and 76.1% due to AI with Tris-citrate-glucose extender and were 8.11, 26.17 and 36.43%, due to AI with citrate-egg yolk extender,

respectively, in comparison with the NM group. At weaning, the increasing percentages in litter size, bunny weight and litter weight were 35.43, 10.2 and 49.17 due to AI with Tris-citrate-glucose extender and were 18.9, 10.9 and 31.9%, due to AI with citrate-egg yolk extender, respectively, in comparison with the NM group. The administration of GnRH in AI rabbits caused induction of ovulation and consequently improves the CR compared with NM rabbits. This improvement in CR may be associated with the increasing litter size and consequently increasing the litter weight of bunnies of female rabbits treated with AI in comparison with the NM rabbits. The difference between AI and NM in litter size is due to the hormonal treatment that precedes the AI technique which causes ovulation in female rabbits.

Litter sizes at birth increased by hormone stimulate injection which caused overstimulation of ovarian follicles owing to its long half-life (Sakr 2003). Litter size at birth in the NM technique was lower than that of AI for New Zealand white and Hyplus breed (Osman *et al.* 2012). At 21 days, Litter size in NM technique was 5.58 ± 0.21 lower than that of AI 7.03 ± 0.31 for New Zealand white and was 5.7 ± 0.29 in NM technique lower than that of AI 7.0 ± 0.33 for Hyplus breed. At weaning, litter size in NM technique (5.26 ± 0.19 and 5.45 ± 0.26) is smaller than that of AI (6.8 ± 0.35 and 6.8 ± 0.31) for New Zealand white and Hyplus breed, respectively (Osman *et al.* 2012). The doe of rabbits injected with GnRH equivalent 0.25 mL receptal at time of mating lead to significant progress in reproductive traits in expressions of greater kindling percentage, litter numbers and kids viability rate at birth and weaning (El-Ratel *et al.* 2017). Ovulation and conception rates in rabbits are the most vital traits which affect directly the sustainability of their offspring and litter number at birth (El-Ratel *et al.* 2017). However, Al and NM values were 8.3 and 8.9 in litter number and 64 and 63 g in bunny weight, respectively, without any significant differences between the two techniques (Alabiso *et al.* 1996).

The mortality rate increased in hot summer due to the high environmental temperature and straight effect of heat stress on the sensitive young rabbits and the decline of mothering milk yield as well as due to the broad-spectrum depression of metabolic activity in the animals (Habeeb 2019). The lower mortality rate in AI group than NM group may be due to higher P_4 level in AI group than in NM one.

Rabbits in tropical and subtropical countries are hurt from heat stress conditions during the long hot humid climate in the summer season from the beginning of May to the end of October months. Rabbits become uncomfortable suffering extremely in reproductive performances under this severe climatic stress. Rabbits also are extra sensitive to heat stress on the reason of their condensed fur and erratic efficient sweat glands that considerably delay heat loss from the skin surface (Habeeb 2020a). The use of AI for diminishing the harmful effects of heat stress conditions with injections of GnRH and $PGF_{2\alpha}$ to regulator the synchronization of ovulation time that is accurate sufficient to achieve acceptable pregnancy rates. Besides, AI allows well work organization as well as decreased manpower costs. Therefore, AI is considering one of the important methods for improving the reproductively of female rabbits during the hot summer season in Egypt, due to a reduction in the interval to the first service and increased pregnancy rates (Habeeb 2019).

Conclusion

It is concluded from this study that artificial insemination technique is better than NM for increasing the fertility rate of female rabbits as well as litter size, bunny weight as well as litter weight at birth and weaning, particularly, under heat stress conditions of the hot summer season in Egypt. In addition, using a Tris-citrate-glucose extender in AI during the hot summer season was more suitable than using a citrate-egg yolk extender.

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Conflict of Interest

No potential conflict of interest was reported by all the authors. All authors decided that no acknowledge any financial interest or benefit we have arising from the direct applications of our research.

Interest Statement

The direct benefits from the subject of this manuscript are that AI technique is better than NM in improving the fertility rate of female rabbits as well as litter size, bunny weight and litter weight at both birthing and weaning, especially, under heat stress conditions of the hot summer season in Egypt. In addition, using a Tris-citrate-glucose extender in AI during the hot summer season was more suitable than using a citrate-egg yolk extender.

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Disclosures and Declarations

Our study was approved by the appropriate ethics of the

Egyptian Atomic Energy Authority committee for research involving animals and a statement on the welfare of animals. Our work submitted for publication does not have any implication for public health or general welfare.

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