EFFECT OF SPERM CONCENTRATION AND SITE OF INSEMINATION ON CONCEPTION RATE OF RABBITS

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Abstract

A total number of 16 mature bucks and 190 monoparous does' of New Zealand White rabbits were used in this work. The aim of this study was to evaluate some reproductive traits and semen quality. The traits measured were semen-ejaculate; sperm motility; sperm-cell concentration; the percentages of live spermatozoa; total-sperm abnormalities and acrosomal damages by conventional technique. Pooled semen sample was divided into two homogeneous parts then diluted with glucose yolk citrate diluents. The dilution rates were 1: 5 and 1: 10. The kindling rate and litter size at birth were recorded. Results of first experiment showed that the ejaculates with high percentage of motile spermatozoa was 74.42 ± 3.34; low percentage of dead spermatozoa was17.02 ± 2.71; sperm abnormalities (14.39 ± 1.03); acrosomal damages prior to their inclusion in the final pool (12.26 ± 0.96), and sperm cell concentration (450.41±43.94). Results of second experiment indicated that the average length of the vaginal tract was 14.5 ± 19.5 cm. Semen dilution rate at 1: 5 resulted high significant (P<0.05) conception and kindling rates compared to semen dilution rate of 1:10. Similarly, the conception rate was increased significantly (P<0.05), where the insemination catheter deeply deposits diluted semen into vaginal tract to depth 12 cm, giving better conception and kindling rates than in the case of 4 or 8 cm.

Key words: rabbit; semen; dilution rate; AI; straw length

INTRODUCTION

Recently, AI technique is favorable and most suitable for small and large commercial Rabbitries [11, 34]. AI can be a useful technique which shows a better performance rather than natural mating [47]. Success in this method of reproduction depends on many factors [5], of which the semen quality, insemination dose, time interval between semen collection and artificial insemination and depth of semen deposition in the female reproductive tract. Fertility of rabbit bucks is of great importance because the sperm is responsible for fertilizing the ova (Hafez, 1970). The most important parameters pertaining to fertility are the number of spermatozoa inseminated and their motility [6].

Success of AI depends on the extensive use of genetically superior males to impregnate a large group of females with relatively low doses of sperm per insemination. This requires high quality semen which can be assessed in various ways [6, 15]. The number of possible inseminations per ejaculate of semen depends on the number of viable sperm in the ejaculate and the number required inseminating each female. The required number per insemination depends on semen handling, and on the genetic strain and physiologic state of the female [6]. Meanwhile, semen samples were diluted ejaculate to (80–100) × 106 spermatozoa/ml (1 semen: 3 extender) [25], (dilution rate 1:5) consisting of 6 million sperms [33] and Semen samples was diluted at a ratio of 1:7-10, each dose contained at least 15 × 106 of spermatozoa [56]. Insemination with very small volumes may result in less effective mechanical drainage, while highly concentrate semen may be more irritating because of more contacts between spermatozoa and endometrium, resulting in intense inflammatory response [3].

One of the most important factor which was associated with obtaining high fertility with low sperm numbers was the site of

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semen deposition, as is known from many large studies with cattle and sheep [1, 31, 52]. There's no doubt about the benefits of semen deposition in the uterine body as compared to deposition in the cervix [36]. Louis (2009) found that the pipette should be inserted 10-15 cm into vagina to ensure appropriate delivery of sperm to female rabbit. Larsson and Larsson (2010) and Meirelles et al., (2012) recorded that the deposition of semen near to the uterus-tubal junction could reduce the spermatic loss, either by the retrograde flow of uterine mucus, or by the phagocytosis during uterine migration. Only one study has evaluated the impact of deep vaginal-cervical insemination on doe rabbit's fertility [27].

The present study was designed to evaluate the impact of different depth of vaginal-cervical insemination and sperm concentrations on some reproductive performance of NZW doe rabbits. Finally, to determine the best and beneficial sperm concentration and site of insemination that would improve doe rabbit's fertility.

MATERIAL AND METHOD

The present study was conducted in a private Rabbitry, located in El-Agezia Village, near Sakarah City, Giza Province, Egypt. A total number of 206 New-Zealand White (NZW) rabbits were used in this study. 190 monoparous does at 21 weeks of age, and 16 sexual mature bucks at 24 weeks of age were used.

The pelleted diets covered the nutritional requirements of the mature phase of rabbits according to NRC (1977) recommendations.

Each week, two ejaculates per 6 male were collected artificially using an artificial vagina as described by Boiti et al. (2005), with a minimum of 30 min between ejaculate collections. The ejaculated semen of each buck was evaluated microscopically. At the same time of insemination each female was intramuscularly injected with 20 µg Gn-RH to induce ovulation immediately before insemination as described by Lopez and Alvarino (2000). 180 NZW rabbit receptive females were divided into 2 groups (n=90). Group 1 and 2 will be inseminated with the first and second portions of diluted semen respectively. Also, the two groups will be subdivided (n=30) according to different length of insemination pipette to three depth of insemination inside genital tract (4, 8 and 12 cm depth). Insemination technique was applied according to Eschborn (1985) using especial devises disposable a plastic curved pipette (Imporvet, S.A., Barcelona, Spain). One pipette for each doe connected to 2 ml syringe through suitable rubber tube.

RESULTS AND DISCUSSIONS

Raw semen quality: Data presented in Table 1 showed the semen quality of NZW rabbit bucks. The quality is represented by percentages of each of advanced sperm motility, dead and abnormal spermatozoa, and acrosomal defects. The selected ejaculates contain high percentage of total motile cells (74.42±3.34), low percentage of dead (17.02±2.71) and abnormal spermatozoa (14.39±1.03). The acrosomal damages, prior to their inclusion in the final pool, was normal (12.26±0.96). The results also showed normal concentration of sperm cells (450.41±43.94 per ml.).

Semen quality after dilution: The effect of semen dilution rate on physical semen characteristics of buck rabbits is shown in Table 2. The 1:5 dilution rates showed the highest significant ($P \leq 0.01$) physical semen characteristics compared to 1: 10 dilution rate. The present results are in line with [13]. On the other hand, these results are different than that observed by Morton et al. (2009) who showed that sperm motility was higher after dilution 1:4 than 1:1 or 1:2 ($P \leq 0.05$). The present results also showed that the average numbers of the inseminated doe rabbit with semen obtained from 1:5 and 1:10 dilution were 33.520 and 16.760 million motile sperm, respectively.

These results demonstrate that there is a considerable reduction in rabbits' sperm motility and membrane functionality at high sperm dilution rates or with reduced numbers of spermatozoa per dose. This condition probably results from the lower concentration of seminal plasma at higher dilutions. At the highest dilution these beneficial elements could be diluted, reducing spermatozoa

protection. Similar results have been reported by [30, 2, 45].

So that, the selection of the ejaculates with high percentage of total motile cells (more than70%), low percentage of dead, and abnormal spermatozoa and acrosomal damages prior to their inclusion in the final pool. Heterospermic pools were used to perform the inseminations. Brun et al. (2002) results stress on the importance of selecting the ejaculates according to mass motility prior to insemination.

Fertility traits of NZW doe rabbits:

Good semen quality with normal sperm concentration and high libido are the strength of rabbit production.

Effect of dilution rate: The effect of dilution rate on some fertility traits of NZW doe rabbits was so clear, where diluted semen at rate of (1:5) caused significant ($P \leq 0.05$) increase in all fertility traits for doe NZW rabbits (Table 2). These results are in agreement with those of Castellini (2008) and Mircu et al. (2008) who observed that increasing the number of sperm inseminated generally has a positive effect on the number of animal born alive and litter size. On the other hand, the improvement of fertilizing ability in 1:5 dilution can be related to increase of sperm motility in thee same rate of dilution. However, Evans and Maxwell (1987) and Lavara et al. (2005) reported that a characteristic feature and prerequisite for the fertilizing capacity of spermatozoa is their motility. In addition, significantly ($P<0.01$) higher fertility levels in 1:5 dilution than 1:10 dilution may be related higher numbers of spermatozoa with not acrosomal damages in 1:5dilution than 1:10 dilution. However, Lavara et al. (2005) showed that only sperms that maintain an intact acrosome can take part in fertilizing an oocyte, thus, the percentage of sperm with damaged acrosome should be low in order to maintain high fertility levels. Accordingly, the percentage of sperm with damaged acrosome should be low in order to maintain high fertility levels. They added that number of sperm per insemination dose should be increased, in which an increase in the percentage of abnormal sperm is observed in the ejaculates to avoid fertility problems. Also, Brun (2002) results for KR seemed to depend on sperm quality, such as sperm motility, plus the cumulative effects of some other traits (concentration and volume), while litter size seemed to be more dependent on quantitative features such as the number of spermatozoa in the dose, via concentration.

Effect of insemination site: The present results also showed that CR for doe NZW rabbits was increased significantly, where the insemination catheter deeply deposits diluted semen into vagina to depth 12 cm, giving better CR than in the case of 4 or 8 cm. While the KR percentage was increased when the insemination catheter deeply deposits diluted semen into vagina to depth 12 cm and 8cm than 4 cm, as shown in Table 3. These results are in agreement with those of Hagen et al. (2003) who showed that sperm numbers required for optimal fertility can be reduced if care is taken to use deep vaginal-cervical insemination, an important factor in commercial AI of rabbits. It appears that effects on establishment of pregnancy are compensable with increased sperm numbers or deposition deeper into the reproductive tract (Tantasuparuk et al., 2011). Also, Anderson et al. (2004) and Kurykin et al. (2006) found that a low number of spermatozoa significantly increased the pregnancy rate, when the semen in the uterus body was deposited. On the other hand, Chelmońska et al. (2007) showed that deep penetration of the posterior region of the uterus creates unfavorable conditions for spermatozoa (reducing their transport along the oviduct) and, consequently, fertility efficacy. Similarly, Surai and Wishart (1996) reported that rooster semen deposited at a depth of 5-6 cm (not 2-3 cm, as is the usual practice) decreases fertility results.

Research carried out on AI depth in Turkey hens showed the best fertility could be obtained by inseminating deep (5-8 cm) in the oviduct (Ogasawara et al., 1968). On the contrary, Wentworth et al., (1975) reported that fertility in shallow insemination (2 cm) was superior to deep insemination (7 cm) whereas a non-significant effect was observed in fertility using either deep or shallow insemination by Rooney et al. (1966).

Table 1 Libido and physical raw semen characteristics of NZW rabbit bucks (Means±SE)

libido and physical semen characteristics	Measurements
Lipido (Sec.)	24.67 ± 3.64
Semen ejaculate volume (ml)	0.53 ± 0.03
Semen mass motility (Score)	3.84 ± 0.39
Advanced sperm motility (%)	74.42 ± 3.34
Dead spermatozoa (%)	17.02 ± 2.71
Sperm abnormalities (%)	14.39 ± 1.03
Acrosomal damages (%)	12.26 ± 0.96
Sperm cell concentration (N×106/ml)	450.41 ± 43.94
Total sperm output (N×106/ejaculate)	238.72 ± 27.43

Table 2 Effect of diluted semen on sperm quality

Sperm count in each insemination dose (0.5 ml)	Semen dilution rate	
	1: 5	1: 10
Total spermatozoa (N × 106/ 0.5 ml)	45.04±000	22.52±
Motile spermatozoa (N × 106/ 0.5 ml)	33.52±000	16.76±
Alive spermatozoa (N × 106/ 0.5 ml)	37.38±000	18.69±
Normal spermatozoa (N × 106/ 0.5 ml)	38.56±	19.28±
Spermatozoa with not acrosomal damages (N X 106/ 0.5 ml)	39.52±	19.76±

Table 3 Effect of insemination straw length and semen dilution rate on fertility traits of does AI (Means ± SE)

Items	Dilution rate	Straw length entered in genitalia tract (cm)			Mean ± SE
		4	8	12	
Ovulation rate (%)	1: 5	88.3 ±4.6	91.0 ±5.0	87.6 ±4.3	89.0±3.6
	1: 10	86.7 ±5.9	90.5 ±4.8	85.2 ±4.9	87.5±4.1
Mean ± SE		87.5±3.9	90.8±4.4	86.4±3.7	88.2±3.3
Conception rate (%)	1: 5	87.5	90.0	95.0	90.83 a
	1: 10	80.0	85.0	87.5	84.16 b
Mean ± SE		83.75 B	87.5 B	91.25 A	87.5
Kindling rate (%)	1: 5	87.5	90.0	90.0	89.17 a
	1: 10	80.0	85.0	85.0	83.33 b
Mean ± SE		83.75 B	87.5 A	87.5 A	86.25
Litter size at birth	1: 5	9.2 ±1.08	9.7 ± 1.15	9.6 ±1.24	9.5±0.92
	1: 10	8.4 ±0.98	9.2 ±0.92	8.8 ±1.07	8.8±0.63
Mean ± SE		8.8±0.7	9.5±0.8	9.2±0.6	9.17±0.6
Litter weight at birth (g)	1: 5	352.8 ±18.7	371.6 ±23.7	359.4 ±16.9	361.3±15.8 a
	1: 10	327.4 ±15.3	341.9 ±15.7	336.2 ±15.9	335.2±14.7 b
Mean ± SE		340.1±13.5 B	356.8±17.6 A	347.8±13.9 AB	348.3±12.4
Pre-weaning mortality	1 : 5	18.5±3.2	19.9±2.8	18.4±2.5	18.9±1.8
	1 : 10	17.7±2.7	18.4±2.4	18.9±2.8	18.3±1.6
Mean ± SE		18.1±1.9	19.2±1.7	18.7±1.3	18.7±1.2

Meanshave different letter superscripts, within the same row; or the same column are significantly (P ≤ 0.5)

CONCLUSIONS

It can be concluded that, AI depth at 8 cm with high diluents (1-10) in doe rabbits showed the best fertility rather than high sperm concentration in shallow insemination (4 cm). The importance of selecting the ejaculates according to mass motility prior to insemination, with regard to fertility.

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