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Effects of collection frequency and age on semen characteristics of crossbred buck rabbit (New Zealand White × Local)

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ABSTRACT

The study involved 24 crossbred bucks (New Zealand White × Local) in a factorial design to assess the impact of semen collection frequency and age on semen quality. Two factors were considered: the age of the bucks (5-6 months for young and 9-10 months for mature) and the frequency of semen collection (daily (Fre-1), every three days (Fre-3), every five days (Fre-5), and weekly (Fre-7)). The study was conducted over 12 weeks. Results from the last 7 weeks indicated that weekly semen collection yielded the best outcomes, with a concentration of 346×10^6 sperm/mL, 66.7% sperm motility, 70.5% membrane integrity, and 66.8% live sperm rate. Furthermore, semen volume and the number of motile sperm increased as the collection interval increased, with mature bucks (9-10 months) showing better semen characteristics than younger bucks. Younger bucks had smaller testicular dimensions than mature bucks. In conclusion, the optimal semen collection frequency for New Zealand White × Local crossbred bucks was once a week, and the recommended age for semen collection was 9-10 months.

1. INTRODUCTION

Rabbits have great potential for growth in the Mekong Delta (Trung et al., 2022) because they reproduce efficiently and have short production cycles. They utilise natural feedstuffs and industrial by-products, resulting in economic advantages. Recently, studies have concentrated on improving an important aspect of rabbit farming, like nutrition and reproduction, to increase productivity (Abdel-Wareth et al., 2015; Al-Sagheer et al., 2017). Artificial insemination (AI) is a popular technique used worldwide (Ambriz et al., 2002), and it has attracted attention in Vietnam recently. The objective is to decrease the number of bucks needed for mating (Nizza et al., 2003), choose male rabbits with exceptional genetic characteristics and

excellent semen quality (Castellini & Dal Bosco, 1998), and enhance mating procedures.

The frequency of semen collection was indicated as an important factor that can impact the quantity, quality, and concentration of semen (Nizza et al., 2002). Insufficient semen doses might result from infrequent collection, which could be problematic for farm-scale operations. Rabbit semen volumes were usually small, ranging from 0.3 to 0.6 mL, with concentrations of $200-600 \times 10^6$ sperm/mL per ejaculation (Battaglini et al., 1993), leading to around 20-50 AI doses (Viudes-de-Castro et al., 1998). Some studies indicated that an increase in the frequency of semen collection could lead to a decrease in semen volume and concentration (Levin et al., 1986; Nizza et al., 2003), as well as on semen quality, such as live sperm rate and motility

(Bencheikh, 1995). On the other hand, some studies indicated that the frequency of semen collection might not affect semen quality (Carlsen et al., 2004). Nevertheless, higher collection frequencies were associated with higher pregnancy rates than lower frequencies (Conrad et al., 1981), and semen production increased with a collection frequency of 5 times per week compared to 3 times every 2 weeks (Kemp et al., 1991).

The findings emphasize the significance of determining the optimal frequency for collecting semen from bucks for AI. Previous studies have primarily focused on purebred rabbits such as New Zealand White or Californian and have utilized pellets as rabbit feed. However, this study utilized crossbred rabbits and local forage for feeding. Moreover, the ideal age for semen collection from New Zealand White (NZW) crossbred rabbits in the Mekong Delta has not been established. This study aims to determine the appropriate frequency and age for semen collection from NZW crossbred rabbits in the Mekong Delta and assess its impact on semen quality.

2. MATERIALS AND METHOD

2.1. Experimental animals and location

This study was involved 24 crossbred buck rabbits (New Zealand White x Local), with 12 rabbits aged 5-6 months weighing 2.35 ± 0.07 kg (young rabbits) and 12 rabbits aged 9-10 months weighing 2.72 ± 0.12 kg (mature rabbits). All rabbits were vaccinated against parasitic and respiratory diseases. The research was conducted at an experimental farm in Thoi Hoa ward, O Mon district, Can Tho City, Vietnam. Feed and semen quality were analyzed in the laboratories of the Faculty of Animal Sciences, College of Agriculture, Can Tho University.

2.2. Experimental design and data collection

The study was arranged in a factorial design with two factors and 3 replications. The first factor was the frequency of semen collection: once daily (Fre-

1), every 3 days (Fre-3), every 5 days (Fre-5), or once weekly (Fre-7). The second factor was the age of the bucks used for semen collection: 12 young (5-6 months old) and 12 mature (9-10 months old). (mature bucks).

The bucks were raised for 12 weeks, and two age groups of bucks were fed the same diet. The diet included soya waste (200 g), soybean extraction meal (27 g), vitamin E (6.4 mg), and *Pennisetum purpureum* (*ad libitum*). The chemical composition of the feed used in the experiment is shown in Table 1. The quality of the experimental rabbits' semen was assessed weekly by collecting and analysing it. Table 1 presents all the details of the experimental feed and chemical compositions.

All feed utilized in the study was fresh. Prior to commencing the experimental diet, the rabbits were given the diet *ad libitum* for one week to observe feed consumption and establish the necessary dry matter (DM) amount for the rabbits. The experimental diet was designed based on DM, with nutritional values fine-tuned to achieve a stable percentage of crude protein (CP) and metabolizable energy (ME). Samples of the feeds and refusals were collected for analysis of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Ether Extract (EE), and Total mineral (Ash) according to the methods of the Association of Official Analytical Chemists (2000). The content of Neutral Detergent Fiber (NDF) was determined using the methods described by Van Soest et al. (1991). The metabolizable energy (ME) values of feeds were calculated according to:

$$ME = DE \times \left(0.995 - 0.048 \frac{DCP}{DE} \right) \text{ (MJ/KgDM)}$$

(Maertens et al., 2002) in which:

$$DE = 14.9 - 0.22 \times ADF + 0.35 \times EE \text{ (MJ/KgDM) (De Blas et al., 1992)}$$

$$DCP = (-1.15) + 0.82 \times CP - 0.06 \times ADF \text{ (%/DM) (Fernández-Carmona et al., 2004)}$$

Where: DCP is digestible crude protein

Table 1. The chemical composition of feed used in the experiment (%DM)

Feed ingredients	DM	OM	CP	EE	NDF	Ash	ME, MJ/kgDM
Soya waste	16.9	95.5	17.6	5.23	31.9	4.50	10.5
Soybean extraction meal	89.8	89.7	43.1	2.50	27.8	10.3	11.5
<i>Pennisetum purpureum</i>	17.6	87.2	9.50	4.15	57.0	12.8	7.76

DM: dry matter, OM: organic matter, CP: crude protein, EE: Ether extract, NDF: Neutral detergent fiber, Ash: total mineral, ME: metabolizable energy.

Semen samples were obtained from individual bucks using a plastic artificial vagina described by Ewuola et al. (2014). The artificial vagina was warmed in water at a temperature between 50°C and 55°C to ensure a temperature of 40-42°C during collection. The inner sleeve was lubricated with vaseline, and a teaser doe was brought to the buck's pen during collection. As the buck mounted the teaser doe, the artificial vagina was introduced, and the ejaculate was collected. Fresh semen samples were diluted in a medium at a ratio of 1:10 and stored at a cold temperature of 12-17°C for analysis of sperm characteristics.

Semen evaluation was conducted according to the method outlined by Ax et al. (2000). The volume of ejaculate was measured directly from the calibrated collection tube, and the portion without gel was noted. The pH of the ejaculate was promptly assessed using pH paper (SpezialIndikatorpapier pH 5.5-9.0, Macherey-Nagel, Germany). All semen analyses were performed under a microscope at 400x magnification in accordance with the guidelines of the World Health Organization (WHO, 2021). Sperm concentration was determined by haemocytometric counts, and the sample was then diluted 1:4 with 5% NaHCO₃. The formula $C=N \times D \times 50,000$ was used to calculate the sperm concentration per milliliter ($\times 10^6/\text{mL}$), where C represents the concentration of sperm per ml, N is the number of sperm counted, and D is the dilution factor (20).

The percentage of live sperm was calculated by dividing the number of unstained sperm by the total sperm count from stained smears using eosin 1% and nigrosin 10%. Sperm motility (%) refers to the forward progressive movement of sperm out of the total counted in each sample, while other forms of motility, such as non-progressive and immotile sperm, were also assessed. Membrane integrity (%) was determined by counting the number of tail-bulging sperm out of the total observed, using the Hos test osmotic swelling method. The total number of motile and immotile sperm per ejaculation was calculated based on semen volume, sperm concentration, and motility rate. Testicular measurements were taken at the end of the experiment from each buck and averaged for the two testicles, measuring length and width using a calibrated measuring tape.

The data was initially processed in an Excel spreadsheet, then analyzed using the General Linear

Model in the Minitab 16 program (Minitab, 2016). Differences between the tests were assessed using the Tukey method in the same program. The average of the two experimental periods was compared using a T-test, with significance set at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. The effects of the collection frequency of semen and the age of bucks on feed intake, nutrient intake, growth performance, and testicular size in the experiment buck rabbits

The results of feed intake (Table 2) indicated a significant difference ($P < 0.05$) between the two age groups of experimental rabbits. The young bucks had a higher ($P < 0.05$) feed intake compared to the mature bucks, resulting in a higher nutrient intake as well. According to Vogel et al. (2017), the nutritional needs of animals were influenced by age. The young bucks, aged 5-6 months, had higher growth requirements, leading to increased feed intake. In contrast, the mature bucks, aged 9-10 months, were at sexual maturity and had a slower growth rate, resulting in lower feed intake, with nutrients primarily supporting maintenance and semen production. Similarly, our previous study found that dietary vitamin E supplementation increased feed intake in young bucks compared to mature bucks (Trung et al., 2024a). Salisu and Iyeghe-Erakpotobor (2014) also found that rabbits at 19 weeks of age had greater body weight but lower feed intake than those at 15 weeks of age.

There were no significant differences ($P > 0.05$) in feed intake among bucks with different semen collection frequencies (Table 2). However, nutrient intakes tended to increase ($P > 0.05$) with higher collection frequencies. Higher collection frequencies required greater energy and protein intake to support sperm production in bucks. The intake of crude protein (CP) and metabolizable energy (ME) was slightly higher for the daily collection frequency, while the other frequencies were similar. This could reflect that daily collection exceeds the physiological capacity of the rabbits and that increased feed intake compensates for the additional nutrient demands. Additionally, the results did not show any interaction between collection frequency and buck age on feed and nutrient intake ($P > 0.05$).

Table 2. The results of feed intake, nutrient intake, and metabolizable energy of the experiment bucks, g/head/day

Factor		Feed intake, gDM				Nutrient intake, g				
Age	Freq	SFW	SBE	PP	DM	OM	CP	EE	NDF	ME
Mature	-	30.2	21.2	17.4	69.5	63.6	16.1	2.86	25.9	0.70
Young	-	32.3	23.5	24.2	81.1	74.0	18.2	3.33	31.3	0.81
SEM		0.316	0.238	0.466	0.532	0.484	0.130	0.023	0.255	0.005
P (Age)		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
-	Freq-1	32.2	23.1	20.2	76.3	69.8	17.6	3.14	28.7	0.77
-	Freq-3	30.9	22.1	21.3	75.2	68.7	17.0	3.09	28.7	0.75
-	Freq-5	31.2	22.0	20.9	74.9	68.4	17.0	3.08	28.5	0.75
-	Freq-7	30.8	22.1	20.9	74.7	68.2	17.0	3.07	28.4	0.75
SEM		0.448	0.336	0.659	0.753	0.685	0.184	0.033	0.360	0.007
P (Freq)		0.151	0.127	0.719	0.432	0.390	0.109	0.501	0.889	0.236
SEM		0.633	0.475	0.932	1.064	0.968	0.260	0.046	0.509	0.010
P (Age×Freq)		0.619	0.262	0.071	0.104	0.119	0.241	0.147	0.059	0.167

SFW: Soya fermented waste, SBE: Soybean extraction, PP: Pennisetum purpureum, DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber, ME: metabolizable energy (MJ), Freq: Frequency.

Table 3. The growth performance, and testicular measurements results of bucks in the experiment

Factor		Live weight changing			Testicular size	
Age	Freq	ILW (g)	FLW (g)	WG (g)	TL (cm)	TW (cm)
Mature	-	2715	2751	36.1	6.17	1.20
Young	-	2353	2750	397	4.87	0.94
SEM		27.19	56.49	58.51	0.167	0.033
P (Age)		0.001	0.990	0.001	0.001	0.001
-	Freq-1	2585	2693	108	5.97 ^a	1.16 ^a
-	Freq-3	2519	2763	244	5.87 ^a	1.14 ^a
-	Freq-5	2579	2874	295	4.75 ^b	0.91 ^b
-	Freq-7	2453	2673	220	5.50 ^{ab}	1.07 ^{ab}
SEM		38.46	79.90	82.75	0.236	0.046
P (Freq)		0.090	0.314	0.460	0.009	0.007
SEM		54.39	113.0	117.0	0.334	0.065
P (Age x Freq)		0.940	0.556	0.521	0.344	0.397

ILW: The initial live weight, FLW: The final live weight, WG: Weight gain, TL: Testis length, TW: Testis width, Freq: Frequency.

At the start of the experiment, the body weight of mature bucks was 1.15 times greater (P<0.05) than that of young bucks. However, after 12 weeks of experimentation, there were no significant differences (P>0.05) in body weight between the two groups. The body weight of mature bucks remained relatively stable, whereas the young bucks exhibited an average weight gain 11 times greater than that of the mature bucks. This finding was consistent with the feed intake results (Table 2). Despite having similar final body weights, the testicular size of the young bucks remained smaller compared to the mature bucks (P<0.05). This suggests that early semen collection could impact testicular development. According to Chubb et al.

(1978), rabbit testes begin rapid growth from the fifth week of age and continue to develop until around 6 months of age, after which testicular development is minimal. The results for the testicular size of mature bucks in the experiment were consistent with our previous research, which involved the same subjects 9-10 month old crossbred rabbits (NZL×Local) showing testicular lengths ranging from 5.85 to 7.33 cm and widths from 1.13 to 1.42 cm (Trung et al., 2024b).

The experimental results did not show a significant (P>0.05) effect of semen collection frequency on the body weight of the experimental rabbits. The average weight gain with daily collection frequency

was less than half ($P>0.05$) compared to other frequencies. Testicular size was decreased ($P<0.05$) with less frequent collection. Testicular size was a key indicator of semen production capacity (Perry & Petterson, 2001; Ogbuewu et al., 2009). Similar results were observed in the study by Aguirre et al. (2007), where rams with higher frequency of semen collection had greater testicular length and circumference compared to those with lower frequency. Higher collection frequencies require the testes to produce more semen in the same period compared to other groups, potentially stimulating the development of accessory reproductive glands such as the seminal vesicles, ampullae, and prostate (Vásquez & del Sol, 2001). Additionally, increased testicular size was associated with greater diameter of the vas deferens, higher Sertoli cell counts, increased testosterone production, and increased semen production (Thompson & Berndtson, 1993; Bailey et al., 1996). The experiment did not record an interaction between collection frequency and age concerning the body weight and testicular size of the buck.

3.2. The effects of the collection frequency semen and age of bucks on sperm characteristics of experimental buck rabbits

The assessment of semen quality during the first 5 weeks of the experiment (Table 4) indicated that mature bucks had significantly higher ($P<0.05$) semen volume and concentration compared to young bucks. However, the live sperm rate and membrane integrity rates were indicated lower ($P<0.05$) in the mature group. According to Campos et al. (2014) found that semen volume was correlated with testicular size, which was associated with the testicular measurements of the bucks presented in Table 3. Additionally, our previous research showed similar results when vitamin E was supplemented in the diets of two age groups of bucks, with the mature group exhibiting higher semen volume and concentration (Trung et al., 2024a). This suggests that bucks aged 9-10 months have reached sexual maturity, resulting in higher semen volume and concentration, while the 5-6 month age range marks the late stage of sexual development, which may lead to lower semen quality.

Table 4. Spermatozoa characteristics of bucks during the first 5 weeks

Factor		Item						
Age	Freq	Vol (mL)	pH	Con ($\times 10^6$ /mL)	Moti (%)	Live (%)	SMI (%)	VMC (10^6)
Mature	-	0.68	7.17	337	59.6	64.4	63.9	140
Young	-	0.53	7.02	301	63.8	67.8	64.0	102
SEM		0.014	0.082	7.099	0.812	0.620	0.488	3.932
P (Age)		0.001	0.193	0.003	0.002	0.001	0.818	0.001
-	Freq-1	0.51 ^b	7.10	276 ^b	60.6 ^b	65.4 ^{ab}	64.4 ^{ab}	84.0 ^c
-	Freq-3	0.61 ^a	6.81	335 ^a	58.2 ^b	64.9 ^b	62.2 ^b	117 ^b
-	Freq-5	0.63 ^a	7.21	314 ^{ab}	62.9 ^{ab}	65.3 ^{ab}	63.9 ^{ab}	124 ^b
-	Freq-7	0.66 ^a	7.26	352 ^a	65.3 ^a	68.8 ^a	65.4 ^a	158 ^a
SEM		0.019	0.116	10.04	1.148	0.877	0.691	5.561
P (Freq)		0.001	0.061	0.001	0.003	0.022	0.028	0.001
SEM		0.027	0.165	14.20	1.624	1.241	0.977	7.865
P (Age x Freq)		0.041	0.609	0.007	0.003	0.004	0.001	0.033

Vol: Volume, Con: Concentration, Moti: Motility, Live: Live sperm, SMI: Sperm membrane integrity, VMC: The total motile sperm, Freq: Frequency.

The frequency of semen collection affected semen quality, with once every 7 days yielding better results ($P<0.05$) for semen volume, concentration, motility, live sperm rate, membrane integrity, and the number of motile sperm per ejaculate. Continuous daily collection resulted in the lowest volume and concentration, but there were no significant differences ($P>0.05$) in motility, live sperm rate, and membrane integrity compared to the

other two frequencies. Bahrawy et al. (2012) observed that higher collection frequencies reduced semen volume and concentration in dromedaries. Similarly, a study on this species reported decreased motility and live sperm rate when collected three times per week using an artificial vagina (Ferré et al., 2015). On the other hand, high-frequency collection raised concerns about decreased libido and sperm depletion to non-recoverable levels in

rabbits. However, the results presented are based on average values per ejaculate; thus, over the total experiment duration, daily collection still yielded good semen quality, with a total weekly volume of up to 3.5 mL and more than 900×10^6 sperm

produced. The average semen volume and concentration remained within the normal physiological range for rabbits. The results also indicated an interaction ($P < 0.05$) between collection frequency and rabbit age, affecting semen quality.

Table 5. Spermatozoa characteristics of bucks during the remaining 7 weeks

Factor		Item						
Age	Freq	Vol (mL)	pH	Con ($\times 10^6$ /mL)	Moti (%)	Live (%)	SMI (%)	VMC (10^6)
Mature	-	0.74	7.18	330	65.2	69.6	67.0	163
Young	-	0.64	7.16	306	63.9	69.2	63.7	126
SEM		0.013	0.033	3.175	0.381	0.392	0.483	2.515
P (Age)		0.001	0.643	0.001	0.022	0.501	0.001	0.001
-	Freq-1	0.53 ^c	7.09 ^b	286 ^c	64.0 ^b	67.8 ^b	62.9 ^b	97.0 ^c
-	Freq-3	0.67 ^b	7.14 ^{ab}	315 ^b	64.7 ^{ab}	70.0 ^{ab}	66.7 ^a	137 ^b
-	Freq-5	0.73 ^b	7.13 ^b	323 ^b	62.7 ^b	69.2 ^{ab}	64.9 ^{ab}	148 ^b
-	Freq-7	0.82 ^a	7.32 ^a	346 ^a	66.7 ^a	70.5 ^a	66.8 ^a	196 ^a
SEM		0.018	0.047	4.490	0.539	0.554	0.683	3.557
P (Freq)		0.001	0.016	0.001	0.001	0.018	0.003	0.001
SEM		0.026	0.066	6.349	0.763	0.784	0.966	5.031
P (Age x Freq)		0.001	0.822	0.002	0.001	0.001	0.029	0.001

Vol: Volume, Con: Concentration, Moti: Motility, Live: Live sperm, SMI: Sperm membrane integrity, VMC: The total motile sperm, Freq: Frequency.

During the subsequent 7-week period of the experiment, semen quality assessments in buck rabbits revealed minimal differences between the two stages. However, the mature bucks group demonstrated significant improvements ($P < 0.05$) in semen volume, motility, live sperm rate, and membrane integrity compared to the young bucks. Early sperm extraction at 5-6 months of age in buck rabbits could prevent the development and maturation of testicular structure, as the body prioritizes semen production over testicular growth. This is evidenced by the smaller testicular size observed in the young buck compared to the mature buck (Table 3). The experiment also revealed a significant interaction among sperm collection frequency, age, and sperm quality ($P < 0.05$).

Semen quality in the later stage of the experiment was highest ($P < 0.05$) at a collection frequency of once per week and lowest at once per day. The experimental trend remained stable throughout the 12-week evaluation. However, there was a notable improvement in semen volume, motility, live sperm rate, membrane integrity, and total sperm motility compared to the earlier stage. This indicated that semen collection at either a high frequency, such as once daily, or a lower frequency, such as once per week, does not lead to a deterioration in sperm quality in rabbits.

Interestingly, the experiment also found almost no difference in sperm quality between collection frequencies of once every 3 days and once every 5 days. The number of motile sperm between collection frequencies of once every 3 days, compared to once per day, and once per week, compared to once every 5 days, consistently exceeded 30 million sperm across both evaluation stages. Meanwhile, the difference in sperm count between collection frequencies of every 3 days and once every 5 days was minimal, only about 10 million sperm.

The relatively small increase in sperm density at a frequency of once every 5 days compared to once every 3 days may be due to the epididymis's capacity. At a certain limit, sperm are reabsorbed in the epididymis to make room for new sperm production. This hypothesis was supported by previous studies showing that the number of sperm retrieved at higher collection frequencies exceeds that at lower frequencies (Foote, 1969), and that epididymal sperm reabsorption has also been documented (Paufler & Foote, 1969). From an evolutionary perspective, the lower sperm quality observed in males with excessive collection frequencies could be seen as a competitive advantage for the species, allowing subordinate

males to contribute to the gene pool (Preston et al., 2001).

Overall, the results of sperm quality assessment indicate that in stage 2, there was an improvement ($P < 0.05$) in sperm quality parameters, including volume, motility, live sperm rate, and the number of sperm per ejaculate. However, sperm concentration remains almost unchanged across the two stages ($P > 0.05$). The significant increase in volume could be attributed to the stimulation of the development of secondary sexual organs, which have produced more seminal fluid. Similar findings were observed in the study by Paal et al. (2014), which investigated the impact of collection frequency on sperm motility.

According to the publication by Amann and Lambiase (1969) on the determination of daily sperm production using testicular homogenates, testicular spermatid reserves were concluded to represent 3.43 ± 0.03 days of sperm production. Sexually mature New Zealand White rabbits produce about 210×10^6 spermatozoa per day. This helps explain why there is no significant difference in sperm density between ejaculations every 3 days and every 5 days, but there is a significant difference compared to daily or weekly collection intervals.

Table 6. The comparison of semen characteristics between 2 periods (Mean±SD)

Items	First 5 weeks	Remaining 7 weeks	P
Vol (mL)	0.60±0.11	0.69±0.13	0.001
pH	7.09±0.32	7.17±0.13	0.287
Con ($\times 10^6$ /mL)	319±45.4	318±28.9	0.804
Moti (%)	61.7±4.95	64.5±2.52	0.008
Live (%)	66.1±3.58	69.4±2.11	0.001
SMI (%)	64.0±2.86	65.3±2.96	0.053
VMC ($\times 10^6$)	121±36.2	144±44.8	0.001

Vol: Volume, Con: Concentration, Moti: Motility, Live: Live sperm, SMI: Sperm membrane integrity, VMC: The total motile sperm.

Moreover, the live sperm rate, sperm motility, and sperm membrane integrity per ejaculation collected every 3 days versus 7 days showed almost no difference. These results are due to the close alignment between the collection frequency and the sperm storage cycle before renewal in rabbits. A collection frequency of once every 7 days theoretically skips one sperm renewal cycle, and the longer storage period contributes to a higher density and a higher percentage of mature spermatozoa.

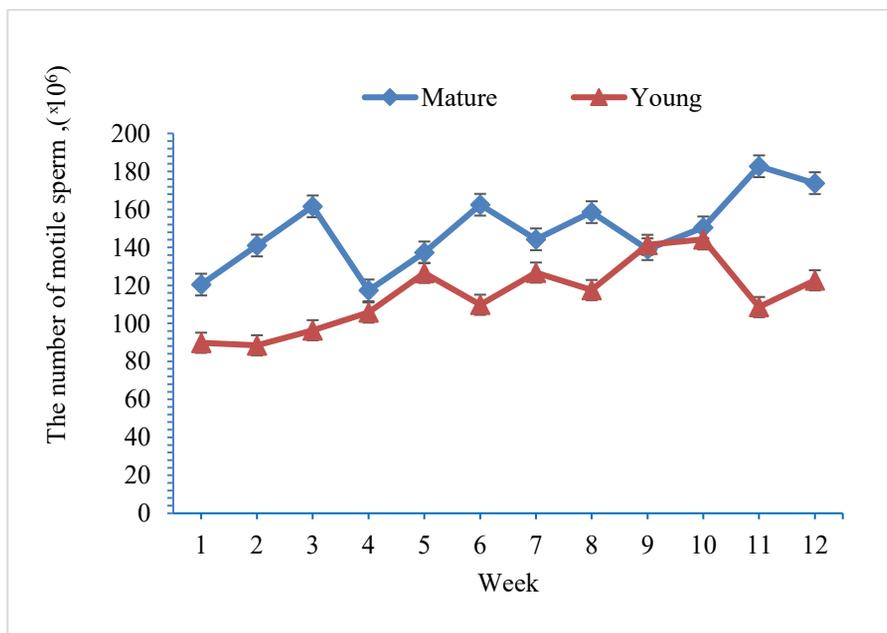


Figure 1. The number of sperm/ejaculation over the weeks of the experiment

The number of motile spermatozoa per ejaculate fluctuated over the 12-week follow-up period (Figure 1). Sperm count in mature bucks was consistently higher than in young bucks. This

fluctuation could be associated with the storage capacity of the epididymis. As shown in Table 5, the epididymal capacity was limited, leading to continuous sperm renewal and reabsorption, which

could account for differences in sperm viability during cyclic collection, as observed in the experiment. Additionally, sperm quality was influenced by environmental temperature (Corcuera et al., 2002), photoperiod factors (Theau-Clément et al., 1995), and the nutritional status of rabbits (Nizza et al., 2000). The age of the experimental rabbits could also contribute to this fluctuation, as the duration of the study increases and the rabbits age. Gogol et al. (2002) reported age-related differences in sperm quality.

4. CONCLUSION

The optimal age for collecting semen from crossbred buck rabbits (New Zealand White ×

Local) was 9-10 months. Sperm collected once a week showed the highest quality results and was appropriate for long-term collection.

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CONFLICT OF INTEREST

We certify that there is no conflict of interest.

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