Semen quality and physicochemical traits in rabbit breeds for artificial insemination in Vietnam

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Qualidade do sêmen e características físico-químicas em raças de coelhos para inseminação artificial no Vietnã

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Abstract

This study evaluated semen quality and physicochemical properties in five rabbit breeds raised under Vietnamese farming conditions to identify suitable candidates for artificial insemination and genetic improvement. A total of 220 ejaculates were collected from 25 healthy bucks aged 12 to 15 months. Data were analyzed using a repeated-measures mixed model. Significant breed effects were detected for most seminal traits. Flemish Giant bucks showed the most favorable profile, with the highest semen volume (1.04 mL), sperm concentration (3.02 × 10^9/mL), straight-line velocity, and beat cross frequency, together with superior osmotic pressure and buffering capacity. French Lop bucks had the lowest semen volume and semen resistance but exhibited the lowest proportion of abnormal spermatozoa. The Californian, Angora, and New Zealand White breeds showed intermediate and consistent characteristics. Overall, the Flemish Giant appears to be the most promising breed for artificial insemination and reproductive improvement under tropical Vietnamese conditions. These findings provide useful information for breeding selection and semen preservation strategies in rabbit production.

Keywords: Artificial insemination. Semen quality. Sperm motility. Flemish Giant. Rabbits.

Resumo

Este estudo avaliou a qualidade e as propriedades físico-químicas do sêmen de cinco raças de coelhos criadas em condições agrícolas vietnamitas, com o objetivo de identificar candidatos adequados para inseminação artificial e melhoramento genético. Um total de 220 ejaculados foram coletados de 25 machos saudáveis com idades entre 12 e 15 meses. Os dados foram analisados utilizando um modelo misto de medidas repetidas. Efeitos significativos da raça foram detectados para a maioria das características seminais. Os machos da raça Gigante de Flandres apresentaram o perfil mais favorável, com o maior volume seminal (1,04 ml), concentração espermática (3,02 × 10^9/ml), velocidade em linha reta e frequência de batimento cruzado, juntamente à pressão osmótica e capacidade tamponante superiores. Os machos da raça Lop Francês apresentaram o menor volume seminal e resistência ao sêmen, mas exibiram a menor proporção de espermatozoides anormais.

As raças Califórnia, Angorá e Nova Zelândia Branco apresentaram características intermediárias e consistentes. De modo geral, a raça Gigante de Flandres parece ser a mais promissora para inseminação artificial e melhoramento reprodutivo em condições tropicais vietnamitas. Esses resultados fornecem informações úteis para a seleção reprodutiva e estratégias de preservação de sêmen na produção de coelhos.

Palavras-chave: *Inseminação artificial. Qualidade do sêmen. Motilidade espermática. Gigante de Flandres. Coelhos.*

Introduction

Rabbits are widely distributed worldwide and have been domesticated for centuries, serving multiple purposes such as meat production, clothing, sport, scientific research, and companionship (Meredith, 2001; El-Deghadi et al., 2025). Originating from the European wild rabbit, domestic breeds are now widely used in both animal husbandry and laboratory research (Nath et al., 2016). In Vietnam, rabbit farming contributes approximately 3.24% to national agricultural output, placing the country among the leading producers in Southeast Asia (Lukefahr, 2007). This sector provides advantages for rural households because it requires low investment, makes use of locally available resources, and relies heavily on agricultural by-products as feed (Cheeke, 1986; El-Manylawi et al., 2005).

The sustainability of rabbit production is largely attributed to the species' capacity to consume high-fiber forages and adapt to diverse management systems. However, challenges such as maintaining breed diversity and improving reproductive performance remain significant. Conventional breeding methods are increasingly limited by inbreeding depression and high production costs (Ragab et al., 2015). To address these issues, artificial insemination (AI) was introduced into rabbit production in Europe during the 1980s (Theau-Clément, 2007). Since then, AI has become a valuable reproductive tool, enabling rapid genetic progress, limiting the spread of diseases, and allowing breeding across geographically distant herds (Leboeuf et al., 2008; Zhao et al., 2009; Costa et al., 2011). Advances in semen preservation and embryo technologies have further supported genetic improvement and the maintenance of diversity. Daniel and Renard (2010) emphasized the importance of using freshly collected semen for artificial insemination in rabbits, noting that the reduced fertility associated with frozen semen remains a major limitation for large-scale production.

One factor contributing to the inconsistent outcomes observed in rabbit sperm cryopreservation is the variability in sperm quality. Previous studies have shown that the ability of rabbit spermatozoa to withstand freezing differs markedly among individuals (Mocé et al., 2005) and between breeds (Kulíková et al., 2017). It is well established that semen output and sperm characteristics differ among rabbit breeds (Boulbina et al., 2025). Accurate assessment of sperm quality remains fundamental to reproductive biotechnology, as parameters such as concentration, motility, morphology, and viability are widely recognized as key indicators of male fertility and reproductive performance (Casares-Crespo et al., 2018). These traits are strongly influenced by genetic background, nutritional status, and environmental conditions, resulting in considerable variation among rabbit breeds. For example, Angora rabbits, although prized for their fiber, often exhibit reduced fertility (Onal et al., 2007), whereas New Zealand White rabbits are well known for their rapid growth and high reproductive efficiency (El-Azim and El-Kamash, 2011). French Lop rabbits, selected primarily for large body size, typically require greater reproductive management, while Californian rabbits are noted for their adaptability across production systems. Flemish Giants, despite their substantial body size, show relatively stable reproductive performance under diverse environmental conditions. As emphasized by Szendrő et al. (2012), artificial insemination in commercial rabbit farming relies heavily on high-performing breeds such as New Zealand White and Californian rabbits, which have been selectively improved for superior reproductive output and overall productivity.

Breed-specific evaluation of sperm biology not only guides reproductive strategies but also informs the development of semen preservation protocols. Yet, cryopreservation continues to pose challenges, as it may cause acrosomal damage and compromise post-thaw viability (Nazif et al., 2022). A better understanding of sperm characteristics across breeds is therefore critical for refining preservation methods and improving the outcomes of AI in both commercial production and genetic conservation.

The present study was undertaken to assess sperm quality and physicochemical properties among rabbit breeds raised in Vietnam, with the aim of providing information to enhance reproductive performance, optimize AI practices, and support sustainable breeding programs adapted to local farming conditions.

Material and methods

This study involved a total of 25 healthy adult male rabbits (bucks), aged 12–15 months, representing five breeds commonly raised in the Mekong Delta of Vietnam: Flemish Giant, Californian, Angora, New Zealand White, and French Lop. For each breed, five bucks were selected. All animals were maintained at a rabbit breeding farm located in Nhon Ai Commune, Can Tho City, Vietnam.

The bucks were individually housed in flat-floor cages under controlled conditions with a photoperiod of 16 hours light and 8 hours dark. Ambient temperature and relative humidity during the experiment averaged 28.5 ± 2.1°C and 78.9 ± 5.3%, respectively. Their diet consisted of one daily feeding of fresh forage, followed by supplementation with a commercial pelleted feed containing 15% crude protein, approximately 2700 kcal/kg, and balanced mineral levels (Ca: 0.8–1.5%; P: 0.5–0.8%). The fresh forage primarily included Napier grass (P*ennisetum purpureum*) or water spinach (*Ipomoea aquatica*), depending on availability at the farm. The pelleted feed was formulated with ingredients such as corn, mung bean hulls, soybean hulls, and dried soybeans, and provided approximately 13 to 15% crude fiber according to the manufacturer's specification. No additional hay or straw was provided. Clean drinking water was supplied *ad libitum* through a nipple drinker system to ensure continuous and hygienic access.

Semen samples were collected at the farm and immediately diluted using a Tris-citrate-glucose extender containing Tris buffer, citric acid, glucose, and antibiotics (penicillin and streptomycin). The extender was used solely to maintain sperm viability during handling and laboratory evaluation.

Formal ethical approval was not required for this study because no invasive procedures were conducted. All animal handling, care, and semen collection followed the Law on Animal Husbandry (No. 32/2018/QH14) issued by the National Assembly of the Socialist Republic of Vietnam. Animal welfare was closely monitored and maintained throughout the experimental period.

Semen collection and experimental design

Semen samples were collected using an artificial vagina pre-warmed to 42–45 °C, following Morrell (1995). Each buck was collected every three days throughout a 30-day sampling period, ensuring repeated measurements across different collection days. Only ejaculates showing ≥ 60% total motility were used for laboratory evaluation. All qualified samples were immediately diluted using a citrate-based extender containing trisodium citrate, glucose, fructose, streptomycin, penicillin, and distilled water (100 mL).

Semen volume

Volume (V, ml) was measured using a graduated pipette or collection funnel placed on a flat surface. The volume was read at the lower meniscus level.

Sperm motility

Progressive motility was assessed under 200× magnification at 2, 3, and 5 hours post-collection. The percentage of motile spermatozoa was recorded manually through visual observation of forward movement. Additionally, sperm motility characteristics were evaluated using a Computer-Assisted Sperm Analysis (CASA) system (Hamilton Thorne, USA). This automated system provided a comprehensive and objective analysis of sperm kinetics. The CASA system was calibrated daily using a stage micrometer (10 µm scale) to ensure measurement accuracy and reproducibility. The following CASA parameters were recorded:

Straight line velocity (VSL, µm/s): linear distance travelled per unit time.

Curvilinear velocity (VCL, µm/s): total velocity along the actual path of the sperm.

Straightness (STR, %): ratio of VSL to VAP, indicating directional accuracy.

Amplitude of lateral head displacement (ALH, µm): Lateral movement of the sperm head during progression.

Beat cross frequency (BCF, Hz): Frequency with which the sperm head crosses the average path.

Sperm concentration

Sperm concentration (C, $\times 10^9$/mL) was measured using an SDM1 sperm densimeter (Minitube, Germany). Before measurement, each ejaculate was diluted at a fixed 1:4 ratio using the citrate-based extender to ensure readings within the optimal measurement range of the device. Each diluted sample was measured three times, and the mean value was used for analysis.

Abnormal sperm morphology

Abnormal sperm morphology rate (K, %) was assessed using methylene blue staining. A semen drop was mixed with 0.85% NaCl, smeared on a glass slide, fixed by gentle heating, and stained. After rinsing, the slide was examined under 400× magnification using an Olympus microscope. A total of 300–500 sperm were counted, and abnormalities were recorded. The formula used was: K (%) = n/N x100, where n = number of abnormal sperm and N = total sperm observed.

Sperm resistance

Sperm resistance (R) was evaluated using Milovanov's (1962) serial dilution method with 1% NaCl solution. The dilution continued until no forward motility was observed. The formula used was: $R = r_0 + r \times n$, where r_0 = initial dilution, r = dilution factor per addition (r = 200), n = number of additional dilutions.

pH value

Semen pH was measured using a calibrated pH/Ion meter (Winlab, Japan). Triplicate measurements were taken, and the mean was reported.

Osmotic pressure

Osmotic pressure (mOsm/kg) was measured using an osmometer (Osmometer BKD-30SMC, Biobase).

Density

Density (d) was determined with a pycnometer using the formula: $d = M/(M_0)$, where M = mass of water and M_0 = mass of semen.

Viscosity

Viscosity (η) was measured at 20°C using a micropipette-based viscometry method, and the formula: $\eta = (d \times t)/(d_0 \times t_0)$, where d = semen density, t = flow time of semen, d_0, t_0 = density and flow time of distilled water.

Buffering capacity

Buffering capacity (β) was determined by titration using 0.1N HCl, following Salisbury et al. (1978). The amount of acid needed to lower the pH to 4.0 was recorded. The formula used was: $\beta = (a \times n \times 1000)/(dpH \times v) \times 100$, where a = acid volume added, n = acid normality, dpH = pH difference before and after addition, v = sample volume.

Statistical analysis

Semen data consisted of repeated measurements because three ejaculates were collected from each male during the experimental period. Therefore, a repeated-measures mixed model was applied to properly account for the non-independence of observations. In this model, breed was treated as a fixed effect, whereas individual male and collection day were included as random effects.

Model assumptions of normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene tests, respectively. When significant effects were detected, Tukey's post-hoc test was used for pairwise comparisons at a significance level of $p < 0.05$.

All statistical analyses were performed using SPSS software, version 26.0 (IBM Corp., Armonk, NY, USA). Results are presented as mean ± standard error (SE) to ensure appropriate interpretation of variance associated with the mixed model.

Results

Significant breed-related differences ($p < 0.05$) were observed in most semen quality and quantity traits (Table 1). Flemish Giant bucks recorded the highest semen volume, VSL, BCF, and sperm concentration, whereas French Lop bucks exhibited the lowest values for these parameters.

Angora and New Zealand White bucks showed intermediate performance, while Californian bucks demonstrated balanced values across most traits. Abnormal sperm morphology increased progressively from French Lop to Flemish Giant. Sperm resistance showed a narrow range among breeds.

Breed had a significant effect on osmotic pressure, buffering capacity, viscosity, and density ($p < 0.05$), while semen pH remained stable across all groups (Table 2). Flemish Giants had the highest osmotic pressure and buffering capacity, indicating stronger seminal plasma stability. French Lop bucks consistently presented the lowest values for most physicochemical traits. Angora, Californian, and New Zealand White bucks exhibited moderate and relatively uniform properties.

Table 1 - Selected semen quality and quantity parameters in male rabbits of five breeds (mean ± SE)

Evaluation criteria	Rabbit breeds				
	French Lop	Angora	Californian	New Zeland White	Flemish Giant
Semen volume (V, mL)	0.82 ± 0.04^{c}	0.97 ± 0.05^{ab}	0.95 ± 0.03^{b}	0.88 ± 0.05^{bc}	1.04 ± 0.05^{a}
VSL (μm/second)	48.12 ± 0.35^{bc}	49.55 ± 0.39^{b}	49.89 ± 1.41^{ab}	47.12 ± 1.45^{c}	50.06 ± 0.46^{a}
VCL (μm/second)	82.85 ± 0.99	83.06 ± 1.01	83.01 ± 1.00	82.94 ± 1.02	83.98 ± 1.08
Straightness (%)	74.46 ± 0.76	74.53 ± 0.76	74.54 ± 0.77	74.48 ± 0.80	74.57 ± 2.81
ALH (μm)	2.49 ± 0.02^{ab}	2.35 ± 0.02^{c}	2.38 ± 0.03^{bc}	2.43 ± 0.02^{b}	2.57 ± 0.03^{a}
BCF (Hz)	10.73 ± 0.34^{bc}	10.82 ± 0.32^{b}	10.88 ± 0.33^{ab}	10.66 ± 0.37^{c}	11.04 ± 0.38^{a}
Concent. (C, $\times 10^{9}$/mL)	2.32 ± 0.04^{c}	2.82 ± 0.05^{ab}	2.63 ± 0.04^{bc}	2.71 ± 0.06^{b}	3.02 ± 0.05^{a}
AM (K, %)	9.27 ± 0.29^{c}	10.41 ± 0.23^{bc}	11.29 ± 0.35^{ab}	10.98 ± 0.34^{b}	12.59 ± 0.44^{a}
Sperm resistance (R)	$2{,}648.09 \pm 13.42^{c}$	$2{,}711.14 \pm 13.05^{ab}$	$2{,}652.09 \pm 12.46^{b}$	$2{,}648.25 \pm 12.61^{bc}$	$2{,}718.64 \pm 13.59^{a}$

Note: Means with different superscripts in the same rows differ significantly ($p < 0.05$). SE = standard error; VSL = straight line velocity; VCL = curvilinear velocity; ALH = amplitude of lateral head displacement; BCF = beat cross frequency; Concent. = concentration; AM = abnormal morphology.

Table 2 - Physicochemical properties of rabbit semen across five breeds (mean±SE)

Evaluation criteria	Rabbit breeds				
	French Lop	Angora	Californian	New Zeland White	Flemish Giant
OP (mOsm/kg)	313.98 ± 1.24^{c}	385.05 ± 1.54^{ab}	320.49 ± 1.14^{bc}	368.11 ± 2.22^{b}	398.24 ± 2.34^{a}
Buffering capacity (β)	$1{,}138.02 \pm 18.39^{c}$	$1{,}198.83 \pm 8.39^{ab}$	$1{,}146.18 \pm 5.04^{bc}$	$1{,}176.85 \pm 4.19^{b}$	$1{,}264.17 \pm 7.89^{a}$
Density (d)	1.02 ± 0.01	1.03 ± 0.01	1.04 ± 0.01	1.03 ± 0.02	1.05 ± 0.01
Viscosity (η)	2.82 ± 0.01^{ab}	2.81 ± 0.01^{b}	2.95 ± 0.01^{a}	2.78 ± 0.0^{1c}	2.80 ± 0.01^{bc}
pH	6.97 ± 0.01	6.98 ± 0.02	6.97 ± 0.03	6.98 ± 0.01	6.98 ± 0.01

Note: Means with different superscripts in the same rows differ significantly ($p < 0.05$). OP = osmotic pressure

Discussion

Semen volume represents an important indicator of reproductive capacity in artificial insemination programs and is influenced by multiple factors including breed, age, nutrition, and testicular development (Salisbury et al., 1978). In the present study, Flemish Giant bucks yielded the greatest semen volume (1.04 mL), which is consistent with their larger body and gonadal size. In contrast, French Lop bucks produced the smallest ejaculates (0.82 mL), suggesting reduced spermatogenic output. These findings fall within the range reported by Paál et al. (2014), who observed an average volume of 0.68 mL, and align

with Macari and Machado (1978), who noted that semen volume increases with age and frequency of collection. However, the volumes recorded here remain lower than the 1.3 - 1.6 mL described by Vintoniv and Havrysh (2022), possibly due to breed-specific characteristics or environmental effects associated with tropical climates. Bencheikh (1995) found that collecting semen once per week, with a second ejaculate obtained 15 minutes later, improved several qualitative traits such as volume, pH, sperm concentration, motility, and the proportion of live sperm. Although the total weekly sperm output increased when semen was collected two or three times per week, the improvement was modest, reaching only about 28% of total sperm instead of the expected 300% increase. However, spermatozoa production is known to be stimulated by regular semen collection, while excessively frequent collection may reduce hormonal secretion and enhance the reabsorption of germ cells along the epididymis (Boussit, 1989).

These breed-related differences in sperm motility are believed to be linked to variations in pituitary gland activity (El-Deghadi et al., 2025). Flemish Giant bucks also showed superior sperm kinetics, with the highest straight-line velocity (VSL: 50.06 μm/s) and beat cross frequency (BCF: 11.04 Hz), together with the greatest sperm concentration (3.02 × 10^9/mL). High sperm velocity is a key indicator of fertilizing potential in AI and cryopreservation programs (Nagata et al., 2018), and the present findings are in line with Castellini et al. (2006), who emphasized the influence of collection rhythm on semen quality. Despite their advantages, Flemish Giants also exhibited the highest proportion (12.59%) of abnormal spermatozoa, indicating that high ejaculate output can sometimes be accompanied by structural defects in spermatogenesis (Shamsuddin et al., 1997). Conversely, French Lop bucks, although producing the lowest semen volume and motility values, showed the lowest percentage of abnormalities (9.27%), suggesting that reduced production does not necessarily compromise morphological integrity.

According to El-Azim and El-Kamash (2011), rabbit bucks of different breeds show clear differences in several reproductive traits, including reaction time, semen pH, ejaculate density and color, as well as mass motility and progressive motility. Californian, Angora, and New Zealand White bucks displayed intermediate performance. Californian bucks showed balanced semen characteristics, with moderate volume (0.95 mL), concentration (2.63 × 10^9/mL), and motility parameters. Angora bucks produced slightly larger ejaculates (0.97 mL) and higher sperm concentration (2.82 × 10^9/mL), but their abnormality rate remained relatively elevated (10.41%). Sperm cell concentration is considered a key parameter that indirectly reflects the overall quality of an ejaculate (Hagen et al., 2002). New Zealand White bucks demonstrated consistent semen quality with a concentration of 2.71 × 10^9/mL and stable velocity parameters, reflecting their widespread use in commercial rabbit breeding. The motility values observed for these breeds exceeded those reported by Nazif et al. (2022), who recorded a progressive motility rate of 45.81%, indicating that local management conditions may have supported improved seminal performance. Sperm resistance to dilution ranged from 2648.09 to 2718.64, with no significant differences among breeds, suggesting a generally uniform tolerance to osmotic stress and supporting the suitability of commonly used semen extenders across the evaluated genotypes (Ndors et al., 2015).

Breed-related differences were further reflected in the physicochemical properties of semen. Flemish Giant bucks exhibited the most robust seminal plasma profile, characterized by the highest osmotic pressure (398.24 mOsm/kg), buffering capacity (1264.17), and density (1.05). These traits indicate strong seminal stability and are compatible with the optimal osmotic range (333–336 mOsm/kg) recommended by Roca et al. (2000) for promoting sperm survival. Likewise, the high buffering capacity suggests enhanced pH maintenance during storage, in agreement with Seleem and Rowida (2005). In contrast, French Lop bucks had the lowest osmotic pressure (313.98 mOsm/kg) and buffering capacity (1138.02), which may contribute to reduced seminal resilience. Angora, Californian, and New Zealand White bucks showed intermediate yet satisfactory physicochemical values, with Californian bucks exhibiting the highest viscosity (2.95), a characteristic that may provide additional protection to spermatozoa but could impede motility if excessive. Nonetheless, viscosity values across all breeds (2.78–2.95) remained within acceptable physiological limits. Semen pH was highly consistent among breeds (6.97–6.98), slightly acidic but within the optimal range required to maintain sperm viability,

aligning with the findings of Alonge et al. (2019) and Alvarez and Storey (1992), who highlighted the role of membrane lipid composition in protecting sperm against oxidative stress during cryopreservation. Moreover, differences in accessory gland secretions between breeds may also contribute to the observed pH variation (Brun et al., 2002).

Overall, Flemish Giant bucks demonstrated the most advantageous semen characteristics, combining high ejaculate volume, strong sperm kinetics, high concentration, and superior physicochemical stability. New Zealand White and Californian bucks also exhibited reliable semen profiles suitable for artificial insemination programs. Although French Lop bucks expressed the least favorable quantitative traits, their lower abnormality rate underscores a potential advantage in morphological quality. These results provide a strong basis for developing breed-specific reproductive strategies and optimizing semen preservation protocols in rabbit production systems.

Conclusion

This study clearly demonstrated breed-related variability in semen quality and seminal plasma characteristics among rabbit breeds raised under Vietnamese farming conditions. Flemish Giant bucks consistently exhibited the most advantageous seminal profile, characterized by high ejaculate volume, superior sperm velocity parameters, elevated concentration, and strong physicochemical stability, reinforcing their suitability for artificial insemination and genetic improvement programs. New Zealand White and Californian bucks also showed reliable and well-balanced semen traits, supporting their continued use in reproductive management. By contrast, French Lop bucks displayed the lowest seminal output and stability, despite having a lower abnormality rate.

These findings provide practical implications for selecting sires with improved reproductive potential and offer a scientific foundation for breed-specific strategies in rabbit semen preservation and AI programs, particularly in tropical production systems. Future research integrating proteomic, metabolic, and oxidative-stress biomarkers is warranted to identify physiological mechanisms underlying semen resilience across breeds and to further refine selection criteria for reproductive efficiency.

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Authors´contributions

NTMP conceived, designed, and analyzed the data. PN wrote the draft, and performed the experiments.

Data availability statement

The research data are not publicly available.

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