



Annual dynamics of semen-dose production in boars for commercial artificial insemination*

Dinámica anual de la producción de dosis seminales en verracos para inseminación artificial comercial

Francisco Sevilla^{1,2}, Ignacio Araya-Zúñiga², José Rafael Alvarado-Herrera², Rafael Molina-Montero³,
Julio Rodríguez³, Felipe Vaquerano-Pineda⁴, Anthony Valverde²

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¹ Instituto Tecnológico de Costa Rica, Universidad Nacional, Universidad Estatal a Distancia, Doctorado en Ciencias Naturales para el Desarrollo (DOCINADE). Alajuela, Costa Rica. f.sevilla@itcr.ac.cr (<https://orcid.org/0000-0003-1480-4141>).

² Instituto Tecnológico de Costa Rica, Campus Tecnológico Local San Carlos, Escuela de Agronomía, Centro de Investigación y Desarrollo en Agricultura Sostenible para el Trópico Húmedo (CIDASTH), Laboratorio de Reproducción Animal. Alajuela, Costa Rica. igaraya@estudiantec.cr (<https://orcid.org/0000-0002-4292-2287>); joser0421@estudiantec.cr (<https://orcid.org/0009-0005-8171-6178>); anvalverde@itcr.ac.cr (corresponding author, <https://orcid.org/0000-0002-3191-6965>).

³ Instituto Tecnológico de Costa Rica, Campus Tecnológico Local San Carlos, Escuela de Agronomía, Programa de Producción Agropecuaria (PPA). Alajuela, Costa Rica. rafmolina@itcr.ac.cr (<https://orcid.org/0009-0009-3369-8058>); jurodriguez@itcr.ac.cr (<https://orcid.org/0000-0002-8426-8842>).

⁴ Instituto Tecnológico de Costa Rica, Campus Tecnológico Central Cartago, Escuela de Agronegocios, Centro de Investigación en Gestión Agroindustrial (CIGA). Cartago, Costa Rica. fvaquerano@itcr.ac.cr (<https://orcid.org/0009-0008-5299-1463>).

Abstract

Introduction. Semen-dose production in boar artificial insemination centers depends on the temporal stability of ejaculate quality, yet calendar-associated fluctuations remain insufficiently described under tropical commercial conditions. **Objective.** To characterize the monthly dynamics of semen-dose yield per ejaculate in boars managed under tropical commercial artificial insemination (AI) conditions and identify the annual periods associated with reduced ejaculate productivity. **Materials and methods.** The study was conducted on commercial pig farms in northwestern Costa Rica between 2020 and 2024. A total of 461 ejaculates from 119 sexually mature boars were evaluated. Each ejaculate was macroscopically assessed to determine and quantify the number of semen doses that could be prepared. A dose from each ejaculate was transported to the laboratory at 17 °C. Total and progressive motility were evaluated using ISAS® v1, and sperm morphology was assessed after sample fixation with the Trumorph® system. Only ejaculates containing ≥75 % morphologically normal spermatozoa were analyzed. Monthly patterns were analyzed using mixed-effects models that included month, farm, year, and age at collection as fixed effects and boar as a random effect. **Results.** Sperm concentration, total and progressive motility, and semen-dose yield varied markedly throughout the year. Concentration increased early in the year, peaked in March, declined toward June, and remained low until October before recovering. Total and progressive motility showed a distinct monthly pattern, with lower values during the first half of the year and improved performance thereafter. Semen-dose yield showed a prolonged low-output period from February through July, followed by recovery from August onward. **Conclusions.** Ejaculate productivity in tropical boars followed a marked annual pattern. Identifying the low-output period may allow AI centers to anticipate adjustments in collection schedules, boar allocation, and semen-dose availability.

Keywords: assisted reproduction, boar sperm, monthly dynamics, reproductive biotechnology, swine industry, thermal stress, tropical climate.



Resumen

Introducción. La producción de dosis de semen de verraco depende de la estabilidad de la calidad del eyaculado; sin embargo, sus fluctuaciones anuales están poco descritas en condiciones comerciales tropicales. **Objetivo.** Caracterizar la dinámica mensual del rendimiento de dosis de semen por eyaculado en verracos bajo condiciones comerciales de inseminación artificial (IA) e identificar los períodos de menor productividad. **Materiales y métodos.** El estudio se realizó en granjas porcinas comerciales del noroeste de Costa Rica entre 2020 y 2024. Se evaluaron 461 eyaculados de 119 verracos sexualmente maduros. Cada eyaculado se evaluó macroscópicamente para determinar el número de dosis que podían preparar. Una dosis se transportó al laboratorio a 17 °C. La motilidad total y progresiva se evaluó mediante ISAS® v1, y la morfología espermática, mediante Trumorph®. Solo se analizaron eyaculados con ≥ 75 % de espermatozoides morfológicamente normales. Los patrones mensuales se analizaron mediante modelos de efectos mixtos con el mes, la granja, el año y la edad al momento de la recolección como efectos fijos, y el verraco como efecto aleatorio. **Resultados.** La concentración espermática, la motilidad total y progresiva y el rendimiento de dosis variaron durante el año. La concentración alcanzó su máximo en marzo, disminuyó hacia junio, permaneció baja hasta octubre y luego se recuperó. Las motilidades total y progresiva presentaron una dinámica mensual diferenciada, con valores inferiores durante la primera mitad del año y una mejora posterior. El rendimiento de dosis mostró un periodo prolongado de menor producción entre febrero y julio, seguido de una recuperación a partir de agosto. **Conclusiones.** La productividad del eyaculado siguió un patrón anual marcado. La identificación del período de menor producción podría facilitar ajustes en los programas de recolección, la asignación de verracos y la disponibilidad de dosis en los centros de IA.

Palabras clave: reproducción asistida, espermatozoides de verraco, dinámica mensual, biotecnología reproductiva, industria porcina, estrés térmico, clima tropical.

Introduction

The biological value of an ejaculate in the swine industry translates into an operational output: the number of usable semen doses that can be produced from an ejaculate while maintaining acceptable quality standards (Rocha et al., 2024). For this reason, ejaculate productivity is a practical endpoint of major importance for artificial insemination (AI) centers (Walter et al., 2020). It directly influences dose availability, collection planning (Goldberg et al., 2013), boar utilization (Waberski et al., 2019), and the overall efficiency of semen production (Lopez Rodriguez et al., 2017).

Semen-dose output is the result of a biological process shaped by the combined effects of sperm concentration (Sevilla et al., 2023), motility (León et al., 2025), and ejaculate suitability (Dcunha et al., 2022). However, research on semen-dose production in the swine industry has focused on ensuring that farms have access to high-quality semen doses (Quirino et al., 2023). The quality of these doses is influenced by multiple factors, including breed-related characteristics (Žaja et al., 2016; Zak et al., 2017), sperm quality (Sevilla et al., 2025; Tsakmakidis et al., 2010), herd health status (Boeters et al., 2023; Kuster & Althouse, 2016), environmental conditions (Lopez-Rodriguez et al., 2017; Suriyasomboon et al., 2004), the type of extender used (Karageorgiou et al., 2016; Lucca et al., 2021; Schulze et al., 2019), and adherence to good laboratory practices in semen-dose production (Schulze et al., 2013, 2015).

Temporal variation in semen characteristics has been documented in boars (*Sus scrofa domesticus*, family Suidae) (Argenti et al., 2018; Kondracki & Górski, 2025). Seasonal changes in environmental conditions may influence sperm production (Kunavongkrit et al., 2005) and sperm function (Toledo-Guardiola et al., 2025),

ultimately affecting the number of semen doses that can be produced from each ejaculate (Flowers, 2022). Under tropical conditions, boars are exposed to recurrent heat- and humidity-related stressors that can alter reproductive performance (Jongbo et al., 2026). From an operational standpoint, even moderate declines in sperm concentration or motility may have operational consequences when they persist for several months (Sui et al., 2022), reducing the efficiency of dose production and increasing uncertainty in semen-dose supply (Kommisrud et al., 2002; Quirino et al., 2023).

Studies addressing temporal variation in boar semen have emphasized seasonal comparisons or biological descriptors of sperm quality, whereas less attention has been given to the monthly dynamics of ejaculate productivity under routine commercial conditions (Bronson, 2009; Fraser et al., 2016; Knecht et al., 2014; Sevilla et al., 2025; Zasiadczyk et al., 2015). However, for the management of AI centers, month-to-month variation may be more informative than broad seasonal averages (Mengistu et al., 2026). A seasonal mean can mask shorter biologically unfavorable periods, whereas a monthly approach can identify the specific periods during which ejaculate productivity declines, recovers, or stabilizes (Dehghanbanadaki et al., 2025). Such information is directly relevant for planning semen collections, allocating boars, anticipating dose shortages, and adjusting management strategies during low-output periods (Lopez-Rodriguez et al., 2017).

Dose production depends on whether ejaculates maintain adequate functional quality according to the acceptance criteria used by AI programs (Waberski et al., 2008). Thus, a calendar-associated decline in dose output may reflect a combined deterioration in quantitative and functional semen traits rather than changes in a single isolated parameter (Odinius et al., 2024). Identifying this pattern is especially useful in tropical systems, where the biologically limiting period may span multiple consecutive months and thereby affect both reproductive logistics and boar management decisions (Padilla et al., 2020; Pascal et al., 2024).

The aim of this study was to characterize the monthly dynamics of semen-dose yield per ejaculate in boars managed under tropical commercial artificial insemination conditions and identify the annual periods associated with reduced ejaculate productivity.

Materials and methods

Ethics statement

All procedures were conducted in accordance with the laws and regulations governing the use of live animals in research in Costa Rica. Animal handling throughout the study was performed with due regard for animal welfare and with the aim of minimizing unnecessary stress. The study complied with ethical principles for animal experimentation, including the Three Rs. It was approved by the Technical Committee of the Centro de Investigación y Desarrollo en Agricultura Sostenible para el Trópico Húmedo (CIDASTH; Research and Development Center for Sustainable Agriculture in the Humid Tropics) at the Costa Rica Institute of Technology (ITCR) in session 08/2020, Article I (DAGSC-100-2020 and VIE-368-2020), and in session 20/2023, Article 1.0 (DAGSC-188-2023 and CIE-206-2023). Reporting of the study also followed the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines.

Study period, sites and climatic context

This longitudinal observational study was conducted on commercial pig farms in northwestern Costa Rica between 2020 and 2024. The study involved 20 commercial pig farms and 119 boars included in the study were distributed among these farms, with an average of 5.95 boars per farm, although the number of animals varied

among establishments. A total of 461 ejaculates were obtained under routine commercial production conditions. During the rainy season, mean temperature, mean monthly rainfall, and relative humidity were 31.0 ± 0.7 °C, 128.4 ± 27.9 mm, and 91.8 ± 7.7 %, respectively, whereas the corresponding values during the dry season were 30.6 ± 0.7 °C, 38.7 ± 36.3 mm, and 41.8 ± 22.5 %, respectively. At the time of the study, all farms held a valid Certificado Veterinario de Operación (CVO, Veterinary Operation Certificate). Animal health was routinely monitored through vaccination and deworming programs, and comprehensive production records were maintained for all animals.

Animals and facilities

Boars were housed individually in well-ventilated pens, with ambient temperatures of 22.9 – 31.2 °C throughout the study period. Boars were fed 2.5 kg day⁻¹ of a commercial corn-soybean meal-based diet containing 3.02 Mcal kg⁻¹ of metabolizable energy and 12.0 % crude protein, supplemented with a mineral mix and salt to meet the nutritional requirements of swine (National Research Council, 2012). Water was available *ad libitum*.

A total of 119 sexually mature and clinically healthy boars, aged 8–35 months (18.1 ± 9.9 months, mean $\pm$ standard deviation), were included in the study. All boars were trained for semen collection. Boars were assigned to breed groups according to their breed composition, established from pedigree records in herds with controlled mating or based on their origin when the boars were acquired directly from a selection nucleus. On this basis, seven breed groups were defined: Duroc ($n = 21$ boars; 91 ejaculates), Yorkshire ($n = 6$; 21 ejaculates), Landrace ($n = 11$; 32 ejaculates), Large White ($n = 2$; 13 ejaculates), Pietrain ($n = 13$; 26 ejaculates), F₁ Pietrain $\times$ Duroc ($n = 64$; 272 ejaculates), and F₁ Yorkshire $\times$ Landrace ($n = 2$; 6 ejaculates). At least two ejaculates were collected from each boar, yielding a total of 461 ejaculates.

Semen collection and processing

Ejaculates were collected once weekly in the morning using the double-glove technique (Hancock & Hovell, 1959) and were immediately diluted 1:1 (v/v) with a commercial extender. Before collection, boars were moved to a dedicated pen equipped with a dummy, and semen was obtained by manual manipulation of the penis after mounting. The last three fractions of each ejaculate were collected in graduated containers and filtered through three layers of sterile gauze to remove bulbourethral secretions from the remaining seminal components. After each collection, a routine macroscopic evaluation of volume, color, and consistency was performed. Sperm concentration ($\times 10^6$ spermatozoa) was measured spectrophotometrically following established procedures (Sevilla et al., 2023). An aliquot (<1 mL) of the semen diluted 1:1 (v/v) was transferred to a microcuvette using a Pasteur pipette.

During packaging and sperm concentration assessment, the filtered semen fraction was maintained in a water bath at 37 °C. After sperm concentration determination, the number of semen doses produced from each ejaculate was recorded. One dose per ejaculate was transported to the laboratory at 17 °C in a light-protected cooler. On arrival, samples were stored horizontally at 17 °C for 24 h, equilibrated at room temperature (24 °C) for 30 min, and then divided into 1.5-mL aliquots in microtubes. The aliquots were incubated at 37 °C for 30 min on a heating block before sperm analysis.

Semen evaluation

Each ejaculate was assessed for total motility, progressive motility, and sperm morphology, and only those containing at least 75 % morphologically normal spermatozoa were retained for analysis. Because sperm morphology was used as an *a priori* inclusion criterion, the range of morphological variation in the analytical dataset was necessarily restricted. Therefore, morphology-related findings should be interpreted as applying only

to ejaculates meeting the predefined threshold of $\geq 75\%$ morphologically normal spermatozoa. Morphology was evaluated by a single technician using the Trumorph® system, following criteria previously described for boar semen studies (Calderón-Calderón et al., 2022). A total of 200 spermatozoa per slide were examined, and the mean value was accepted when the difference between fields was $< 5\%$. For this analysis, a $10\ \mu\text{L}$ aliquot of semen was placed on a glass slide and covered with a coverslip before evaluation with the Trumorph® system.

Sperm motility was assessed using the ISAS® v1 system (Integrated Semen Analysis System, Proiser R+D, Paterna, Spain). An ISAS® D4C20 disposable counting chamber ($20\ \mu\text{m}$ deep, four fields; Proiser R+D, Paterna, Spain) was prewarmed to and maintained at $37\ ^\circ\text{C}$. After thorough homogenization, $2.7\ \mu\text{L}$ of diluted semen was loaded into the counting chamber by capillary action following the recommended loading procedure. Sperm tracking was analyzed using a computer-assisted semen analysis system coupled to a Proiser 782M video camera operating at $50\ \text{Hz}$ and acquiring 25 frames per field at a final resolution of 768×576 pixels. The imaging system was mounted on a microscope equipped with a $1\times$ eyepiece, a $10\times$ negative phase-contrast objective (NA 0.25), and an integrated heated stage set at $37.0 \pm 0.5\ ^\circ\text{C}$.

Statistical analysis

Assumptions of normality were evaluated by inspection of normal probability (Q–Q) plots and by the Shapiro–Wilk test, whereas homogeneity of variances was assessed using Levene’s test. Continuous outcomes were analyzed with linear mixed-effects models. Boar was included as a random intercept to account for the non-independence of repeated ejaculates from the same individual.

The primary focus was the monthly pattern of semen-dose yield per ejaculate, whereas sperm concentration, total motility (TM), and progressive motility (PM) were analyzed as supporting indicators of ejaculate suitability. Because season was derived from calendar month, only month-based models were fitted. Fixed effects comprised month, commercial breeding-purpose category, farm, and year, with age at collection included as a covariate. The two-way interactions commercial breeding-purpose category $\times$ farm, commercial breeding-purpose category $\times$ year, and farm $\times$ year were also included in the model. Boar was included as a random intercept to account for repeated ejaculates from the same individual. For the monthly analysis, the following model was fitted (Equation 1):

$$Y_{ijklmn} = \mu + M_i + L_j + F_k + Y_l + \beta \text{Age}_{ijklmn} + (L \times F)_{jk} + (L \times Y)_{jl} + (F \times Y)_{kl} + B_m + \varepsilon_{ijklmn} \quad (1)$$

Where Y_{ijklmn} is the observed value of the response variable, μ is the overall mean, M_i is the fixed effect of month (January–December), L_j is the fixed effect of commercial breeding-purpose category (maternal, paternal, or terminal), F_k is the fixed effect of farm, Y_l is the fixed effect of year, $\beta \text{Age}_{ijklmn}$ is the covariate for age at collection, B_m is the random effect of boar, and ε_{ijklmn} is the residual error. The two-way interactions evaluated were commercial breeding-purpose category $\times$ farm, commercial breeding-purpose category $\times$ year, and farm $\times$ year. Nonsignificant interactions were not interpreted further, and the presentation of results focused on effects relevant to the primary objective of the study.

Repeated measurements within each boar were modeled using an appropriate covariance structure selected according to model-fit criteria. When an overall effect was significant, pairwise comparisons of estimated marginal means were performed using the Tukey–Kramer adjustment. Statistical significance was set at $p < 0.05$. Data are reported as mean $\pm$ standard error of the mean (SEM). All statistical analyses were performed using IBM SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, NY, USA).

Results

Monthly sperm concentration varied markedly throughout the year. Values rose from January, peaked in March, and then declined steeply through June, when they reached the annual minimum (approximately 235×10^6 spermatozoa mL^{-1}), 45–50 % lower than the March peak. Concentrations remained depressed from July to October and then recovered during November–December (Figure 1).

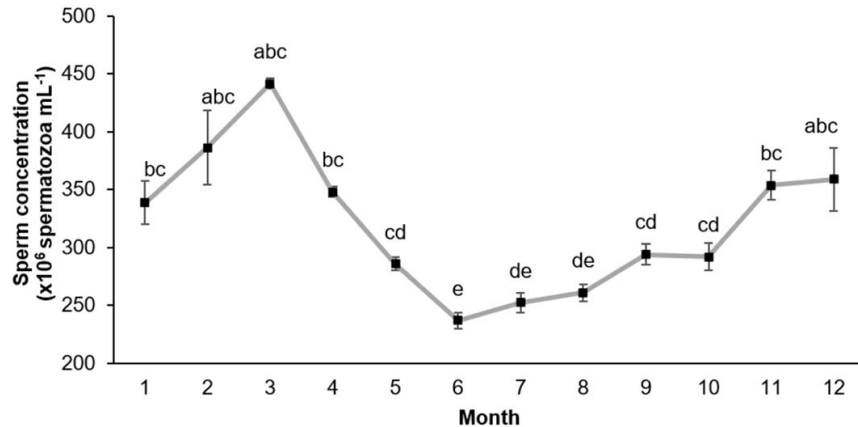


Figure 1. Monthly variation in sperm concentration in boars (mean $\pm$ standard error of the mean). Northwestern Costa Rica, 2020–2024. a-e Different letters indicate differences between months for sperm concentration ($p < 0.05$).

Figura 1. Variación mensual de la concentración espermática en verracos (media $\pm$ error estándar de la media). Noroeste de Costa Rica, 2020-2024.

a-e Letras diferentes indican diferencias entre meses para la concentración espermática ($p < 0,05$).

Total motility varied markedly among months. Values declined from January to March, partially recovered by May, declined again in June, and then rose sharply in July, reaching an annual peak in August. Values from September through December did not differ significantly from the August value, whereas those from February through July were significantly lower. The annual range was approximately 33 percentage points between March and August (Figure 2).

Progressive motility (PM) varied markedly among months. It declined from January to an annual minimum in March. Thereafter, PM rose sharply through July and reached an annual peak in August. Values in September and October did not differ significantly from the August value. PM then decreased modestly in November and stabilized in December. The annual range between the March minimum and the August maximum was approximately 54 percentage points. Values from February through June were significantly lower than those recorded from August through October (Figure 3).

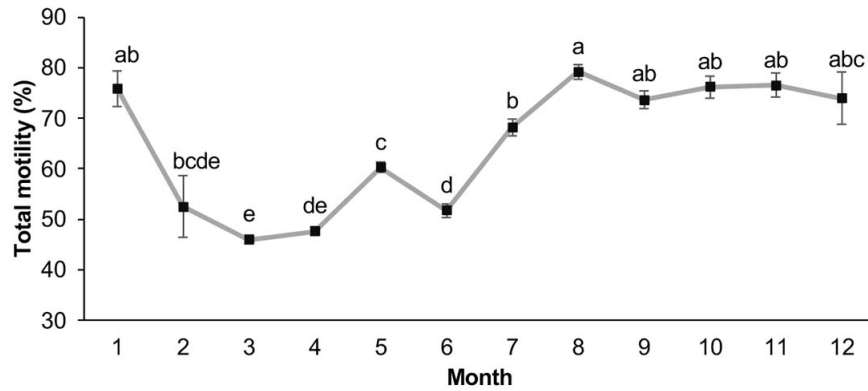


Figure 2. Monthly variation in total sperm motility in boars (mean $\pm$ standard error of the mean). Northwestern Costa Rica, 2020–2024.

^{a-c} Different letters indicate differences between months for Total Motility ($p < 0.05$).

Figura 2. Variación mensual de la motilidad espermática total en verracos (media $\pm$ error estándar de la media). Noroeste de Costa Rica, 2020-2024.

^{a-c} Letras diferentes indican diferencias entre meses para la motilidad total ($p < 0,05$).

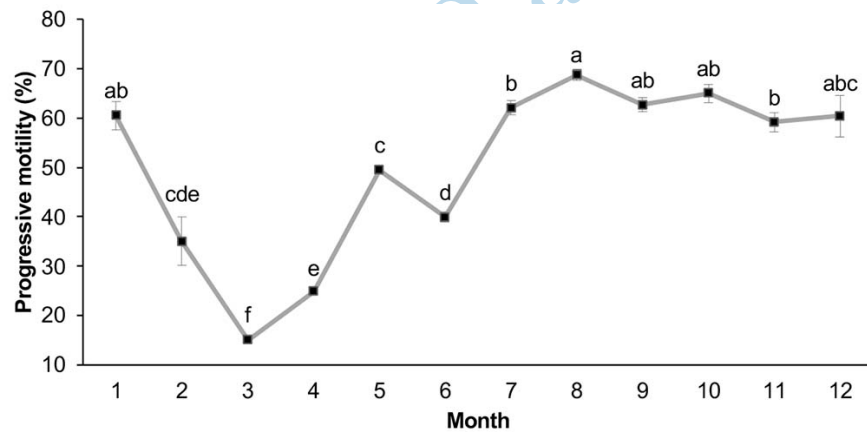


Figure 3. Monthly variation in progressive sperm motility in boars (mean $\pm$ standard error of the mean). Northwestern Costa Rica, 2020–2024.

^{a-f} Different letters indicate differences between months for Progressive Motility ($p < 0.05$).

Figura 3. Variación mensual de la motilidad espermática progresiva en verracos (media $\pm$ error estándar de la media). Noroeste de Costa Rica, 2020-2024.

^{a-f} Letras diferentes indican diferencias entre meses para la motilidad progresiva ($p < 0,05$).

Monthly semen-dose yield was analyzed as the number of doses produced per ejaculate under the AI-center criteria used in this study. The numerically lowest mean was observed in February, while several months from February through July showed comparatively low values. According to the Tukey–Kramer groupings, February did not differ significantly from several other months within this low-output interval. Semen-dose yield generally increased from August onward; however, pairwise differences between late-year and mid-year months were not uniform. October showed an intermediate response and shared statistical groupings with both later months and several months within the February–July period. January, September, November, and December were among the months with the highest semen-dose yields (Figure 4).

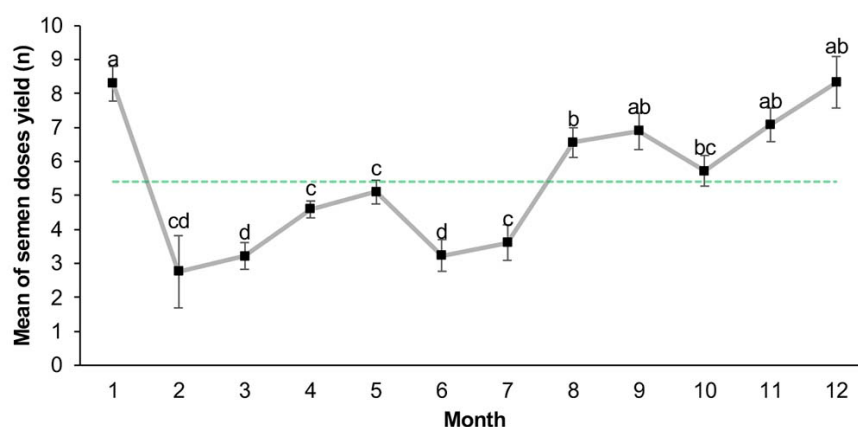


Figure 4. Monthly variation (mean $\pm$ SEM) in the number of boar semen doses produced per ejaculate according to the sperm-quality criteria used in this study. Northwestern Costa Rica, 2020–2024.

^{a-d} Different letters indicate differences between months for production of boar semen doses ($p < 0.05$). The dashed line represents the overall mean semen-dose yield calculated across all ejaculates included in the study.

Figura 4. Variación mensual (media $\pm$ error estándar) en el número de dosis de semen de verraco producidas por eyaculado según los criterios de calidad espermática utilizados en este estudio. Noroeste de Costa Rica, 2020-2024.

^{a-d} Letras diferentes indican diferencias entre meses en la producción de dosis de semen de verraco ($p < 0.05$). La línea discontinua representa la dosis media total de semen calculada a partir de todos los eyaculados incluidos en el estudio.

The monthly variation in semen-dose yield was pronounced. However, mean semen-dose yield per ejaculate did not differ significantly among seasons ($p > 0.05$; Figure 5A). Furthermore, mean semen-dose yield per ejaculate did not differ significantly among commercial breeding-purpose categories ($p > 0.05$; Figure 5B).

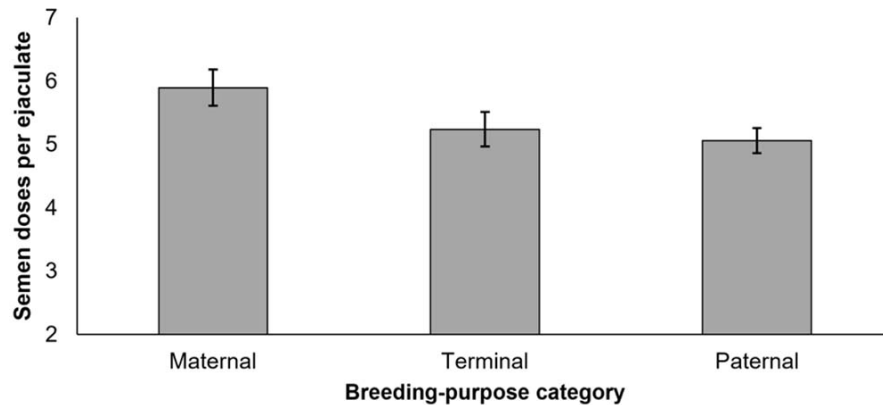


Figure 5. Number of semen doses produced per ejaculate (mean $\pm$ standard error of the mean [SEM]) (A) according to commercial breeding-purpose category). Northwestern Costa Rica 2020–2024.

No significant differences were detected ($p > 0.05$).

Figura 5. Número de dosis seminales producidas por eyaculado (media $\pm$ error estándar de la media) según la categoría de propósito reproductivo comercial. Noroeste de Costa Rica 2020-2024.

No se detectaron diferencias significativas ($p > 0,05$).

Discussion

Month-to-month differences were observed in sperm concentration, total motility, and progressive motility, suggesting that spermatogenesis and post-testicular processes influencing sperm movement may be sensitive to seasonal stress (Petrocelli et al., 2015). Together, these temporal patterns suggest that semen-dose yield varies substantially throughout the year under routine commercial conditions in the tropics (Suliman et al., 2020). This variation is relevant to AI centers because a semen sample can meet a numerical concentration threshold and still exhibit lower motility during certain months (Henneberg et al., 2023; Valverde et al., 2021).

The biologically limiting window extends beyond a short heat peak and encompasses an extended multi-month trough in ejaculate quality (concentration and motility) and semen-dose yield. This is particularly relevant in commercial systems because the production of acceptable semen doses depends on maintaining both a sufficient sperm count and adequate sperm motility according to the criteria used by the AI center (Harstine et al., 2018). Collectively, these results suggest related but distinct temporal patterns. The trough coincided with periods during which both concentration and motility decreased, potentially reducing ejaculate productivity during dose preparation. Overall, these results indicate that boar semen quality exhibits biological variation across the year despite processing under routine AI-center conditions (Kondracki & Górski, 2025; Krupa et al., 2023; Sá et al., 2025).

Temporal differences in the semen-quality parameters of the analyzed doses may be associated with changes in environmental conditions throughout the year. Under tropical conditions, boars are particularly susceptible to heat stress during periods of elevated ambient temperature (Kunavongkrit et al., 2005). Moreover, the effects of high temperature may be exacerbated when accompanied by high relative humidity, as commonly occurs under humid

tropical conditions (Sevilla et al., 2026). This combination of relatively high temperature and relative humidity is particularly evident from May to October, corresponding to the rainy season. Previous studies have reported that heat stress can significantly reduce sperm concentration in boar ejaculates (Peña et al., 2019). In contrast, relatively high sperm concentrations were observed during some of the warmer months in the present study. A decrease in sperm motility, particularly total and progressive motility, was also observed during periods of greater thermal stress, consistent with previous evidence showing that heat stress negatively affects boar sperm motility and kinematic characteristics (Sui et al., 2022).

Broad seasonal classifications provide useful environmental context but may mask shorter periods of biological vulnerability (Murphy et al., 2018). In contrast, monthly analysis provides greater temporal resolution, allowing the onset, duration, and recovery of reductions in sperm concentration, motility, and semen-dose yield to be identified more precisely (Mellado et al., 2014). From an AI-center management perspective, this temporal resolution may be more informative for collection planning and dose forecasting than broad seasonal averages alone.

Future research should incorporate reproductive performance data and heritability estimates for motility and kinematic parameters derived from computer-assisted semen analysis (CASA). It should also include direct environmental and physiological measurements, such as microclimate, testicular temperature, and oxidative stress (Li et al., 2023; Singh et al., 2025; Sui et al., 2022). The integration of these datasets would facilitate the genetic selection of males based not only on carcass or maternal traits but also on reproductive resilience to seasonal stress under tropical conditions (Hensel et al., 2024).

Conclusions

Semen-dose yield in boars managed under tropical commercial conditions exhibited a marked monthly pattern throughout the year, with a prolonged low-output period from February through July that partially coincided with periods of lower sperm concentration and motility, followed by recovery during the later months. The monthly analysis allowed identification of the onset, duration, and recovery of the prolonged low-output period in greater temporal detail. Recognition of this low-output window may help commercial AI centers plan adjustments to collection schedules, boar allocation, and semen-dose inventories. Further studies incorporating individual environmental and physiological measurements are required to determine the mechanisms underlying these temporal changes.

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Data availability statement

The raw data supporting the conclusions of this article are available in the TECdatos Repository at <https://doi.org/10.18845/RDA/YOYEDC>.

Conflict of interest

The authors declare that there are no conflicts of interest related to this study.

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