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Crossbred boars achieve greater semen-collection training success than purebreds, independent of environmental enrichment

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Efficient training of boars for semen collection is crucial for artificial insemination (AI) programs, yet the relative contributions of genetics, rearing enrichment, and endocrine indicators remain uncertain. We compared sexual performance of purebred and crossbred males reared with environmental enrichment (EE) or in barren housing (BE) during growth and tested whether serum testosterone and salivary cortisol were associated with training success. Initially, 128 males were randomly allocated to EE (three alternating objects) or BE; after growth, 32 genetically approved boars were randomly selected and evaluated in a 2x2 factorial (line: pure vs. cross; rearing: EE vs. BE; n=8 pigs) across three sessions on alternate days (6 days; 96 sessions). "Trained" was defined as mounting the dummy and allowing collection. In total, 19/32 (59.37%) were trained; 41.66% of sessions featured unassisted mounts during acclimation, only 7.29% succeeded after handler stimulation/assistance, and 48.95% failed to mount despite assistance. Crossbreds showed higher libido scores and training rates than purebreds ($P<0.001$). Mean libido scores did not differ between EE and BE ($P = 0.276$), but EE groups yielded fewer trained animals, suggesting reduced mounting dexterity after enriched rearing. Ejaculate volume did not correlate with ejaculation time ($P>0.05$). Testosterone was higher in fasted than post-feeding samples ($P<0.001$) and higher in EE than BE ($P<0.05$), yet training success did not explain its variation ($P = 0.50$). Cortisol showed a genetic-line effect (crossbreds>purebreds; $P = 0.034$), no enrichment effect, and no association with trained/not-trained outcome ($P>0.05$); pre- vs. post-session means did not differ ($P = 0.784$). Our results suggest that crossbred boars improve semen-collection training performance, the EE regime tested provided no benefit and was associated with lower operational success, and single-time endocrine measures have low predictive value; prioritizing handler practices, acclimation routines, and standardized, welfare-focused protocols is recommended.

KEYWORDS
libido score, pig, salivary cortisol, semen-collection training, sexual behavior

Introduction

Advances in pig production are closely tied to reproductive performance and to the efficiency of gene dissemination for herd genetic improvement. Currently, more than 95% of females in major swine-producing countries are bred via artificial insemination (Schulze et al., 2014). To meet this demand, boars are raised and selected to produce high-quality semen at scale. In pursuit of higher productivity, natural mating has largely been replaced by manual or automated semen collection using dummy mounts, eliminating the need for a female (Knox, 2016). As a result, a boar that previously mated with about 20 females per year can, through artificial insemination, sire offspring with approximately 200 females (Fávero and Figueiredo, 2009). Operationally, it is desirable that boars ejaculate larger volumes in shorter periods, thereby reducing handling time.

Despite the economic relevance, relatively little is known about boar sexual behavior during dummy collection (Levis and Reicks, 2005). Libido is generally not included among selection criteria; however, its absence carries substantial economic costs, often leading to culling regardless of genetic merits (Hemsworth and Tilbrook, 2007). It is estimated that 1% to 20% of breeding boars are culled due to lack of libido (Robinson and Buhr, 2005). Behavioral assessment typically considers the latency from entry into the collection pen to mounting, ejaculation duration, and number of mounts (Hemsworth et al., 1978; Levis and Reicks, 2005; Frydrychová et al., 2011; Oberlender et al., 2010; Wysokińska and Kondracki, 2014). Responses to the dummy are nonetheless highly individual, indicating the need to understand why some animals mount quickly while others are slower or fail to mount, as well as which factors modulate learning (Levis and Reicks, 2005).

Boar sexual behavior emerges from interactions between physiological and environmental factors (Hemsworth and Tilbrook, 2007; Wysokińska and Kondracki, 2014). Prior work has documented effects of social isolation (Hemsworth et al., 1977a, Hemsworth et al., 1977b, Hemsworth et al., 1983), human–animal interactions (Hemsworth et al., 2011; Dysart, 2015), pharmacological interventions with prostaglandins (PGF2α/lutalyse) (Kozink et al., 2002; Estienne et al., 2007; Ryu et al., 2007; Zamora et al., 2010), and climatic variation (Suriyasomboon et al., 2005; Freneau et al., 2012; Savić and Petrović, 2015) on libido. Yet, there remains a gap regarding environmental manipulations before puberty and their downstream effects on adult reproductive performance (Flowers, 2015). In this context, investigating boar behavior and deploying environmental enrichment are promising strategies. Although cognitive and emotional processes in livestock were historically underexplored, applied research has grown (Puppe et al., 2007). Several studies have demonstrated that environmental enrichment promotes improvements in behavior and productive performance, while also contributing to enhanced bone strength and biomechanics, as well as neural development and learning capacity in pigs (Sneddon et al., 2000; Puppe et al., 2007; Jansen et al., 2009; Day et al., 2008; Zwicker et al., 2013; Tavares et al., 2023a; Tavares et al., 2023b). However, its specific impact on breeding males and their mounting proficiency remains poorly characterized (Van de Weerd and Day, 2009a), including which object features might influence libido.

Accordingly, we hypothesize that environmental enrichment during boar growth can modify sexual behavior, shorten reaction time, and thereby facilitate semen-collection training. Our objective was to compare the sexual performance of purebred and crossbred boars during collection training as a function of having been raised, during the growth phase, in enriched versus barren environments.

Materials and methods

Animals, housing and management

The study was conducted at a commercial farm. Purebred and crossbred boars were reared during growth in enriched or barren environments, composing a 2 × 2 factorial design. The purebred group consisted of Landrace (Line 1010) and Large White (Line 1020) genetic lines. The crossbred group consisted of Line 65, a synthetic line derived from Pietrain, Duroc, Landrace, and Large White breeds, and Line 415, a commercial cross obtained from Line 65 females mated with Pietrain Line 62 boars. The growth phase lasted approximately 90 days and included 128 boars (mean age: 70 days; age range: 65–75 days) housed in eight pens (16 animals per pen).

In enriched environments (EE), three objects were presented sequentially for 30 days each to sustain exploratory interest: suspended chains (month 1), a suspended 5-L jerrycan (month 2), and a 50-L jerrycan loose on the floor (month 3). Once a week, the floor jerrycan was impregnated with sow urine to provide sexual stimuli and could be mounted/manipulated (Silambarasan et al., 2019). Chains and the suspended jerrycan targeted oronasal exploration (chewing), whereas the floor jerrycan promoted motor and cognitive engagement (Van de Weerd and Day, 2009b).

At the end of growing period, company staff conducted individual genetic selection according to internal quality standards. After selection, approved boars waited five days before transfer to the breeding unit; rejected boars were culled. A random sample of 32 boars (8 per treatment) was then assigned to semen-collection training, moved to the breeding unit, and housed individually three days prior for acclimation. At the first training session, mean age was 174 days (range: 168–180 days).

Training spanned six consecutive days: each boar trained three times on alternate days (total: 96 sessions). Animals were split into two groups and trained between 08:00 and 15:00, with daily randomized order. A single trained handler conducted all sessions without auxiliary animals or materials.

Animals were kept in two adjacent buildings with individual crates, a training pen with a dummy mount, and a laboratory for semen analysis. Buildings (~80 × 12 m; 3m eave height; ceramic roof tiles; Figures 1a, b) featured automated roof-mounted forced ventilation, triggered when temperature exceeded 25.5 °C. During the experimental period, mean indoor temperature and relative humidity were 20.77 °C ± 1.2 °C and 60.03% ± 6.1%. Crates measured 0.55 × 2.0 m and had partially slatted floors, with the slatted area corresponding to approximately 35% of the total floor area. Feed allowance was 4 kg/animal/day (2 kg at 08:00; 2 kg at

FIGURE 1
Facilities used for housing and semen-collection training of young boars. **(A)** Internal view of the housing facility showing individual crates and the automated ventilation system; **(B)** front view of the individual crates equipped with automatic feeders; **(C)** training pen used for adaptation and semen-collection training with a dummy; and **(D)** boar mounted on the dummy during semen collection.

15:00) delivered automatically; water was provided *ad libitum* via nipple drinkers. The training pen measured 3.45 × 2.36 m and included a handler escape area. The dummy measured 1.20 × 0.30 m with adjustable height.

Response variables

After acclimation, the following outcomes were recorded: (a) boar behavior; (b) human–animal interactions; (c) ejaculate volume; (d) serum testosterone; (e) salivary cortisol.

a. Boar behavior

Behavior was recorded by direct observation using focal sampling and continuous recording, adapted from Hemsworth et al., (1977a; Hemsworth et al., 1978; Hemsworth et al., 1981; Hemsworth et al., 1983; Table 1). All observations were conducted by a single observer throughout the study. Prior to data collection, preliminary observation sessions were performed to define and standardize the behavioral categories and recording procedures. We quantified number of mounts, latency to mount/ejaculate, ejaculation duration, and handling proficiency, using a digital stopwatch. Protocol: 5 min alone in the pen for familiarization, followed by up to 15 min of handler stimulation, comprising verbal cues (auditory stimulation) and physical interactions to encourage approach and mounting of the dummy, including showing, guiding, or positioning the boar onto it (tactile stimulation); the boar left after semen collection or at 15 min. A libido score (1–6; Table 2) was assigned per session, adapting Kozink et al. (2002); Okere et al. (2005); Estienne et al. (2007), and Frydrychová et al. (2011).

b. Human–animal interactions

In all sessions, handler behavior was evaluated by direct continuous observation (15 min), adapting Breuer et al. (2000); Lensink et al. (2000), and Hemsworth et al. (2011). Categories were "physical" (with contact) and "non-physical" (no contact) interactions (Seabrook and Bartle, 1992; Table 3), with frequency recorded. To preserve spontaneity, the handler knew observation would occur but not its specific nature (Lensink et al., 2000).

c. Semen collection

Following mounting, the prepuce was cleaned (emptying preputial diverticula and wiping). Semen was collected by the gloved-hand technique using a thermal cup with a disposable liner and filter to remove the gel fraction. Ejaculate volume was determined by weighing after gel removal on an electronic balance (Maes et al., 2011).

d. Salivary cortisol

Saliva was sampled on days 1 and 6, before and after each session, using 25-cm sticks with cotton tips. Chewed cotton was compressed; fluid was transferred to tubes, centrifuged (10 min; 1500 rpm), and stored at −20 °C then −80 °C until analysis. Cortisol

TABLE 1 Ethogram used to record animal behavior during training.

Category	Register	Component	Description
Courtship behavior	Y/N	Singing	Short series of jaw grunts, with excessive courtship behavior.
	Y/N	Jaw banging	Repetitive chattering of the jaws.
	F	Smelling the mannequin	Naso–nasal contact with the mannequin.
	Y/N	Excessive salivation	Foaming at the mouth.
Performance during mounting	F	Total number of jump attempts	Number of times the animal tries to jump on the dummy, but positions itself incorrectly.
	F	Total number of correct jumps	Number of times the animal mounts the dummy, with both front legs positioned on the front or back of the dummy.
	Y/N	Penis exposure	The exteriorization of the penis completely from within the foreskin.
	Y/N	Ejaculation (interrupted)	Moment when the male ejects semen for a period less than 1.5 minutes.
	Y/N	Ejaculation (complete)	Moment when the male ejects semen for a period greater than 1.5 minutes.
	T	Latency at first mount	Time elapsed between the animal entering the training stall and the first mount.
Skill during mounting	T	Mounting attempt	Time elapsed from entry into the training stall until the first mounting attempt.
	T	Latency to ejaculation	Time elapsed between the animal's entry into the training pen and ejaculation.
	T	Ejaculation	Ejaculation time.
	F	Escape attempt	Moved away or distanced himself from the dummy and the trainer in order to leave the stall.
Others	Y/N	Vocalizing	Making a loud, shrill sound, excluding courtship grunts.
	F	Urinating	Act of urinating.
	F	Defecating	Act of defecating.
	Y/N	Interacting positively with the trainer	Follow and allow to be touched by the trainer.
	Y/N	Interacting negatively with the trainer	Attacking, threatening or avoiding the trainer.

Y/N, Yes or No; F, Frequency; T, Time. Based on the methodologies described by: Hemsworth et al. (1977a, Hemsworth et al., 1978, Hemsworth et al., 1981, Hemsworth et al., 1983).

was quantified with a commercial ELISA (DiaMetra, Italy; Ref. DK0020; limit of detection 0.12 ng/mL); inter- and intra-assay CVs were 8.3% and 10% (Thomsson et al., 2014) (Escribano et al., 2012).

e. Serum testosterone

Blood samples were collected by jugular vein puncture using a vacuum collection system, in glass tubes without anticoagulant, as described by Moreno et al. (1997). Prior to venipuncture, adult boars were restrained in a standing position using a 6-mm rope placed behind the canine teeth and around the snout, held by an assistant, with the head slightly elevated and the body aligned. Puncture was performed on the right side of the cervical region, at the jugular fossa, laterally to the manubrium cartilage and cranially to the scapular joint contour, with the needle inserted 3–6 cm in a dorso-caudal and slightly medial direction. Two blood draws per animal (jugular venipuncture; vacuum system; plain tubes): morning fasting and 1 h post-feeding (fixed order). Serum was obtained by centrifugation (10 min; 1500 rpm) and stored at −20 °C and subsequently transferred to −80 °C until hormonal analysis (Naskar et al., 2016). Total testosterone was measured by solid-phase RIA (Immunotech, France; Ref. IM1087; sensitivity 0.03 mg/mL); intra and inter-assay CVs were 8.6% and 11.9%.

Two blood draws per animal were collected by jugular venipuncture using a vacuum collection system and plain tubes. During sampling, animals were manually restrained in their individual crates to allow safe blood collection. Samples were obtained in the morning under fasting conditions and again 1 h after feeding (fixed order). Serum was obtained by centrifugation (10 min; 1500 rpm) and stored at −20 °C and subsequently transferred to −80 °C until hormonal analysis. Total testosterone was measured by solid-phase RIA (Immunotech, France; Ref. IM1087; sensitivity 0.03 mg/mL); intra- and inter-assay CVs were 8.6% and 11.9%.

TABLE 2 Description of libido scores.

Libido score	Action
1	Lack of interest in the dummy.
2	Animal showed interest in the dummy, but did not attempt to mount it.
3	Animal tried to mount the dummy, but was unsuccessful.
4	Animal mounted, but did not allow semen collection.
5	Animal mounted and semen was collected (Note.: time between entry into the stall and the onset of ejaculation was greater the 5 minutes.
6	Animal mounted the dummy during the adaptation period without trainer assistance, and semen was successfully collected (Note: time between entering the stall and the start of ejaculation less than 5 minutes).

Based on the methodologies described by: Kozink et al. (2002); Okere et al. (2005); Estienne et al. (2007); Frydrychová et al. (2011).

Statistical analysis

A completely randomized 2×2 factorial design (lineage: pure vs. crossbred; environment: enriched vs. barren) with eight replicates per treatment (experimental unit = animal) was adopted. Behavioral data were summarized descriptively and by Pearson correlations among quantitative variables. Libido scores were compared across treatments by Kruskal–Wallis (α=0.05) and, when applicable, by Mann–Whitney tests (α=0.05) for pairwise contrasts (lineage and enrichment). Quantitative outcomes were analyzed by ANOVA with *post-hoc* multiple comparisons; assumptions were checked by Shapiro–Wilk and Bartlett tests (α=0.05). Cortisol was log-transformed to stabilize variances. Analyses were conducted in R (R Development Core Team, 2005).

TABLE 3 Work ethogram used to record the trainer's behavior during animal training.

Category	Behavior	Description
Physical interactions	Touching	Carefully touch any part of the animal.
	Pushing	Vigorously push the animal.
	Beating	Using force when leaning on the animal.
	Massaging the foreskin	Lightly press the animal's prepuce in order to stimulate it.
	Surrounding the animal	Preventing the animal from moving away.
Non-physical interactions	Talking	Pronounce words in a soft voice.
	Whistling	Produce a high–pitched sound with your mouth.
	Screaming	Pronounce words out loud.
	Walking	On the move.
	Clapping	Strike the palms of each hand against each other to produce a characteristic noise.
	Hitting something	Hitting part of the stall or the mannequin with your hands or feet to produce noise.

Based on the methodologies described by: Breuer et al. (2000); Lensink et al. (2000); Hemsworth et al. (2011).

Results and discussion

Assessment of animal behavior

During training, a male was classified as "trained" when it successfully mounted the dummy and allowed semen collection. Once collected for the first time, these males showed no difficulty in subsequent sessions. Of the 32 animals evaluated, 19 (59.37%) were successfully trained, meaning 40.63% were not collected after three attempts. At the first exposure to the dummy, 12 males (37.50%) mounted and were collected; three more succeeded at the second session, and four at the third. Figure 2 displays, by treatment, the percentage of animals that succeeded across the three semen-collection trainings.

Based on Figure 2, environmental enrichment during the growth phase did not improve training outcomes; thus, the observed response contradicts the initial hypothesis. Conversely, males reared in barren environments exhibited a higher proportion of trained animals across sessions.

In this study, animals previously kept in enriched group pens were transferred to individual cages. This environmental change may have induced stress. Douglas et al. (2012) reported that animals are sensitive to declines in environmental quality; swine moved from enriched to barren housing can show changes in emotional state and behavior. Broom (1991) similarly noted that the absence of environmental stimuli may render some animals extremely inactive and unresponsive (apathetic behavior).

Douglas et al. (2012) further examined whether a cognitive paradigm could assess the emotional effects of enrichment. Pigs housed in enriched and barren environments were trained to discriminate auditory cues indicating reward (positive) or punishment (negative). Enriched-housed animals responded faster to the positive cue; when relocated to barren housing, they became more sensitive to reduced environmental quality and slower, indicating a negative emotional state.

It is also possible that the enrichment objects used here offered limited cognitive function for breeding males. Tönepöhl et al. (2012) made a similar assumption when no enrichment effects on cognitive function were detected in pigs.

Figure 2 also shows that crossbred lines outperformed pure lines in all semen-collection trainings. Differences in sexual activity between breeds have been reported by Neely and Robison (1983); Sonderman and Luebbe (2008); Wysokińska and Kondracki (2014), and Savić and Petrović (2015). Consequently, male sexual activity varies among breeds or genetic lines (Flowers, 2008).

Our findings align with Wysokińska and Kondracki (2014), who found differences in sexual activity among Duroc and Pietrain boars, both purebred and crossbred. Crossbreds exhibited higher libido and shorter latencies from pen entry to mounting than

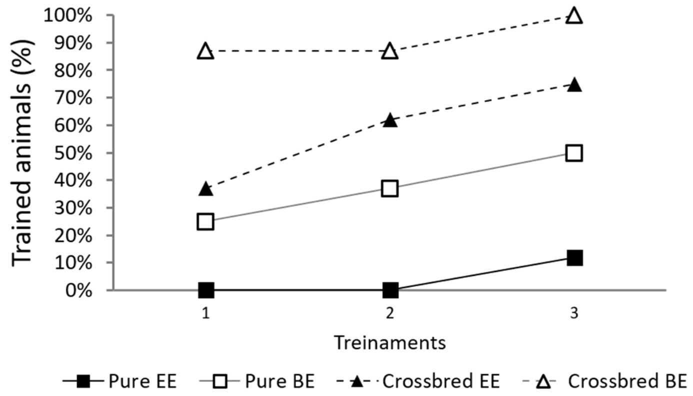

FIGURE 2
Percentage of trained boars across training sessions.

purebreds. Sonderman and Luebbe (2008) likewise reported a higher proportion of Landrace and Yorkshire purebred boars failing training due to low libido, whereas Duroc × Hampshire crossbreds trained more readily, reaching a trained rate of 99.4%. These observations suggest that the superior performance of crossbreds relative to purebreds may reflect heterosis (Wysokińska and Kondracki, 2014).

Levis and Reicks (2005) emphasized that the sexual behavior of AI boars has received limited scientific attention; thus, no standardized procedure exists to assess libido and sexual performance. The binary outcome "trained/not trained" is insufficient on its own; regardless of the final outcome, one must assess the male's reaction to the dummy and account for individual variability.

Progression of sexual activity across trainings

Behavior was analyzed following Kozink et al. (2002); Okere et al. (2005); Estienne et al. (2007), and Frydrychová et al., (2011, Frydrychova et al., 2014), which grade libido from low to high using scores (0–6) that consider interest in the dummy, mounting ability, and collection ease. Recorded variables were used to assign libido scores: 0 (no sexual interest) to 6 (marked sexual interest with rapid erection and ejaculation). Figure 3 depicts libido-score progression across the three sessions.

In session one, scores 1, 2, 3 (18 animals) and 6 (11 animals) predominated. Hence, most animals either showed no interest and failed to mount (scores 1–3) or mounted during the acclimation period prior to handler entry and were collected (score 6). Only one animal mounted during the first training with handler assistance (score 5), and two mounted correctly but did not allow collection (score 4).

In session two, score-1 frequency declined and score-2 increased, indicating greater interest among those previously unsuccessful. Score-5 counts also rose (four animals), whereas scores 6 and 4 remained constant.

In the final session, score-6 frequency increased (18 animals) and scores 2, 3, 4, and 5 decreased. The rise in score 6 indicates that males mounting correctly in the previous session (scores 4–5) mounted without assistance and were collected. After three training attempts, three animals showed no interest, and seven—despite interest—did not attempt to mount.

At the individual level, animals scoring 6 maintained that performance subsequently, signifying that once mounting and collection occurred, further assistance was unnecessary. Males scoring 5 later reached 6. Among eight animals scoring 1 initially,

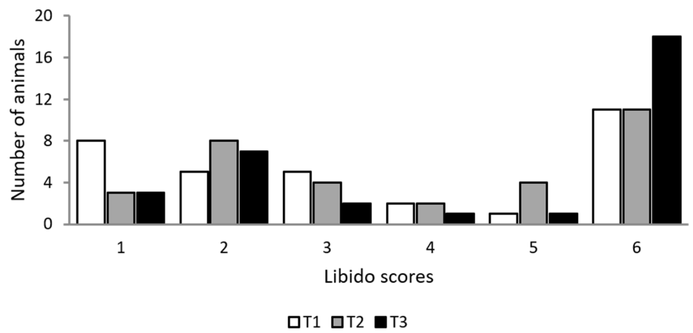

FIGURE 3
Number of boars assigned to each libido score during the first (T1), second (T2), and third (T3) semen-collection training sessions. Libido scores were defined as follows: 1 = no interest in the dummy; 2 = interest in the dummy without mounting attempt; 3 = mounting attempt without success; 4 = mounted the dummy but semen collection was not achieved; 5 = mounted the dummy after 5 min and semen was collected; 6 = mounted the dummy within 5 min and semen was collected.

only two mounted and were collected (scores 5 and 6 in session three). In total, across 96 trainings, 40 mounts occurred during acclimation without assistance; in the remaining 56, animals were stimulated, but only seven succeeded.

Training failure may stem from low sexual motivation or limited mounting skill/dexterity (Hemsworth and Tilbrook, 2007). Ease and speed of mounting and collection are crucial for future use in studs or farms, as they determine handling time.

Several authors have measured reaction times to the dummy—latency to first mount attempt, correct mount, onset and total ejaculation time (Savić and Petrović, 2015; Wysokińska and Kondracki, 2014; Dysart, 2015)—as indicators of dexterity. Given their importance, Table 4 presents mean latencies for first mount, mount attempt, and ejaculation among animals scoring 3, 4, 5, and 6. Score-6 males had the shortest reaction times, confirming quicker erection, mounting impetus, and ejaculation onset, and they ejaculated longer overall—i.e., they mounted rapidly and remained in ejaculation longer. Animals scoring 3 and 4 were slower and less adept. Overall, ejaculation time ranged from 111 to 501 s (mean 280.28 s). Ejaculate volume ranged from 321 to 528 mL. Considering session three only (most collections), time–volume correlation was not significant at the 5% level.

Libido scores by treatment

We next identified which treatments yielded greater dexterity and the corresponding scores. Figure 4 displays the total frequency (24 observations/treatment) of each libido score by treatment. Descriptively, pure lines with and without enrichment appeared less active (scores 1–2). The Pure EE treatment uniquely exhibited correct mounts without allowing collection (score 4), as animals dismounted when the handler approached. This treatment had the lowest frequency of trained animals, with only one collected in the third session (score 6). In Pure BE, two distinct animals mounted with assistance in two assessments (score 5), and seven observations involved mounts during acclimation (score 6). Four animals in Pure BE were trained.

Among crossbreds, Crossbred BE performed best. As shown in Figure 4, there were no scores 1–2, indicating interest in all observations. In 21 of 24 observations, animals mounted without assistance (score 6); in sum, all animals in Crossbred BE were trained. Conversely, Crossbred EE showed higher frequencies of scores 1–3; there were 11 observations of unassisted mounts (score 6) and three with stimulation (score 5). In total, six animals were trained.

TABLE 4 Average values in seconds realted to dexterity during training according to the animals' libido score.

Behaviours (average latency in seconds)	Scores 3	4	5	6
First attempt to mount (s)	518.45	494.00	327.00	48.16
First correct mount (s)	–	709.75	672.50	78.57
Beginning of ejaculation (s)	–	–	706.83	140.52
Total ejaculation time (s)	–	–	277.00	283.57

Because treatment effects on libido scores tend to magnify over time, we analyzed only the final session. The Kruskal–Wallis test indicated that at least one pair of treatments differed in the distribution of libido scores ($P = 0.0037$). Mann–Whitney tests showed that pure (mean 3.187) and crossbred lines (5.562) differed, confirming higher scores in crossbreds ($P < 0.001$). No significant difference emerged between EE (3.937) and BE (4.812) ($P = 0.276$). Pairwise comparisons revealed only one significant contrast: Pure EE (2.75) versus Crossbred BE (6.00) ($P < 0.05$). In summary, crossbreds achieved higher mean libido scores (better sexual performance), and growth-phase enrichment (EE vs. BE) did not affect libido scores during training.

Other behaviors observed during training

The following behaviors were commonly observed during dummy exposure. Their interplay likely underlies the libido score, yet the scoring method does not detail them; thus, we describe them here. The number of mount attempts was defined as the number of times an animal tried to jump but positioned incorrectly; correct mounts were defined as the number of times the male mounted with both forelegs on the dummy's rear or front. Over the 20-minute training period, males—either following stimulation or spontaneously—attempted or correctly mounted several times (Figure 5).

Pure EE animals showed the highest frequencies of attempts and correct mounts. Despite poorer sexual performance, these males displayed libido, attempting to jump about ten times and correctly mounting about nine times on average in the first session. Contrary to expectations, the number of attempts decreased while correct mounts remained constant throughout sessions, suggesting reduced activity and interest. Training duration varies with libido, breed, and handler experience. According to Knox et al. (2008), 52% of producers reported a one-week training period, 41% two to three weeks, and 7% up to one month. If a male shows no libido despite repeated exposures, culling may follow. Robinson and Buhr (2005) reported culling rates of 1–20% for lack of libido across seven AI units.

Crossbred BE and EE males, which exhibited greater dexterity, attempted and correctly mounted less often because they usually succeeded on the first jump (Figure 5b). In Crossbred BE—the best-performing group—means were 1.89 attempts and 1.28 correct mounts, indicating superior skill. Attempts and correct mounts may indirectly reflect reaction/interest toward the dummy and mounting ability. As shown in Figure 5, although they did not complete training, Pure EE males had the highest frequencies of attempts and correct mounts, suggesting they could jump and position correctly but did not allow collection.

During the study, some males attempted to escape the training pen for two apparent reasons: to avoid the pen/handler (fear) or to seek other males (strong sexual attraction). Escape-attempt frequencies across sessions are shown in Figure 6. Variation across treatments and sessions was large. Only in Crossbred BE did no animal attempt escape during the third training, when all were collected and the mean time from pen entry to ejaculation onset was 104.50 s. Pure EE (6.6) and Crossbred BE (6.5) showed the highest escape-attempt frequencies in sessions 1 and 2, respectively. As

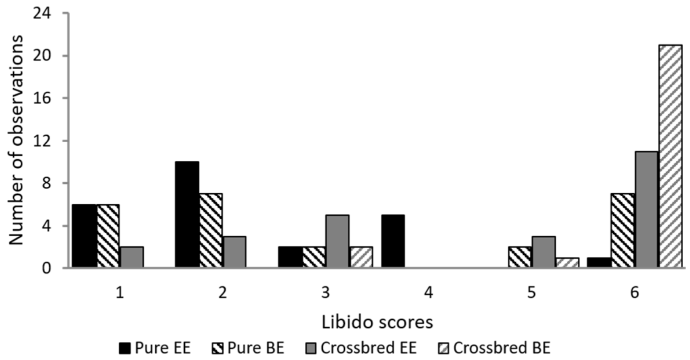

FIGURE 4
Number of observations assigned to each libido score according to treatment group. Animals were classified as pure or crossbred and reared in enriched (EE) or barren (BE) environments. Libido score definitions are described in Figure 3.

Knox et al. (2008) noted, some boars require multiple exposures to grasp the dummy's purpose.

From crate to pen, libido in some males increased after visual/physical contact with other males, which benefited training as they entered the pen more sexually motivated and mounted immediately. However, when the dummy did not elicit interest, attention shifted to males housed opposite the pen, hindering training. Male-to-male sexual interest was also reported by Levis and Reicks (2005), who found that pre-training visual contact increased libido; Wysokińska and Kondracki (2014) considered it sexually stimulating to allow males to observe another male mounting the dummy beforehand.

Courtship behaviors typical of natural mating also occur during dummy collection (Hemsworth and Tilbrook, 2007). Thus, libido can be evaluated by singing/grunting, jaw-champing, and excessive salivation, whereas lack of libido may be inferred from stress vocalizations (Figure 7). Here, "vocalization" was defined as high-pitched stress calls. As shown in Figure 7, treatments with enrichment—both pure and crossbred—exhibited more vocalizations (58.33% and 50%, respectively), possibly indicating greater stress. Singing and jaw-champing (chewing-like movements) occurred in roughly 40–50% of observations. Jaw-champing and salivation are related, as chewing stimulates saliva production (Signoret, 1971). Crossbred BE animals showed less salivation, consistent with faster performance and less time in courtship.

According to Hughes et al. (1985), behaviors such as singing, jaw-champing, and nose-to-nose contact release auditory and olfactory stimuli that attract females. Excess salivation and urination can also stimulate sexual interest via pheromones, volatile compounds produced by both sexes; in males, they are produced in the preputial pouch and submaxillary salivary glands (Signoret, 1976; Swenson and Reece, 1996; Perry et al., 1980). In this study, urination frequency averaged 1–1.5 times per session. Differences between purebreds and crossbreds are further explained by Sonderman and Luebbe (2008), who highlighted variation in susceptibility to seasonal infertility, age at puberty, libido, and conditioning period. Purebred males may reach puberty later (8–9 months) than crossbreds (6–7 months) and exhibit lower libido (Sonderman and Luebbe, 2008).

In summary, sexual behavior differs between purebred and crossbred males during semen-collection training. Crossbreds display greater mounting dexterity, whereas purebreds appear to require more training. Moreover, environmental enrichment did not yield positive effects; animals reared in enriched environments showed poorer dexterity and required more time, stimulation, and effort. These animals may be more sensitive to declines in environmental quality and, therefore, less responsive. There is a clear need to identify and test alternative enrichment materials for breeding males.

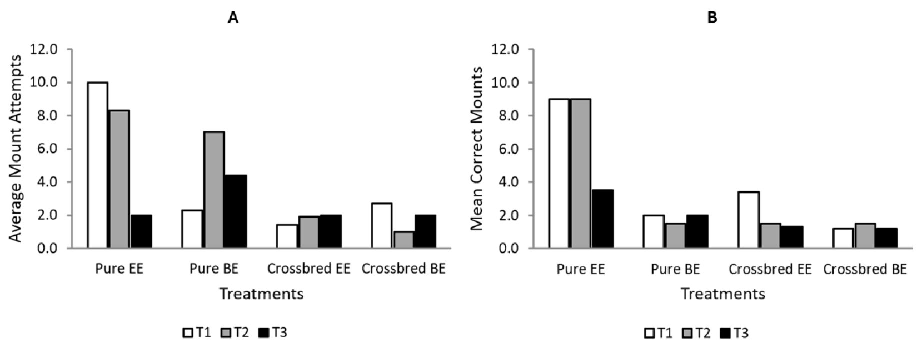

FIGURE 5
Average frequency of **(a)** mounting attempts and **(b)** correct mountings for each treatment in all training sessions.

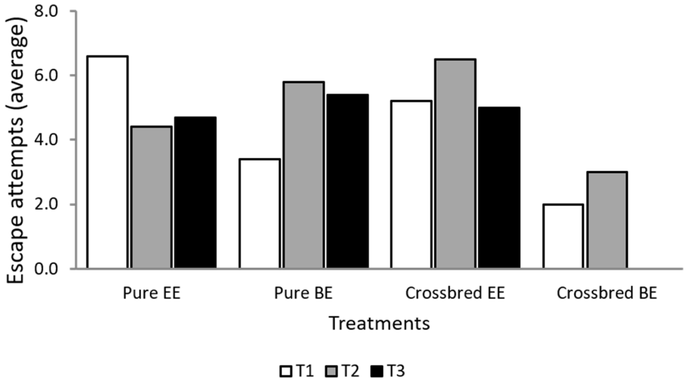

FIGURE 6
Average frequency of escape attempt behavior for each treatment during all training sessions.

Human–animal interactions

Semen-collection training involves critical human–animal interactions. Handlers engage multiple senses to facilitate mounting; training success thus depends partly on these interactions. Figure 8 shows mean frequencies of negative interactions, preputial massage, and assistance during mounting by treatment.

Negative interactions refer to hitting or using force when touching the animal. They were more frequent in Crossbred EE (2.09). No negative interactions were recorded in Crossbred BE, likely because those animals performed better and, consequently, required less handler intervention. Fear and reactivity are greater in animals subjected to aversive handling (Hemsworth et al., 1999). If an animal fears or avoids the handler, collection becomes unfeasible, and the male may become agitated and attempt to escape. Negative interactions should be avoided not only during training but throughout life.

Hemsworth et al. (1986) investigated pleasant, unpleasant, and minimal-contact handling regimes and found that pleasantly handled males entered a designated area faster (0.4 min), stayed closer to the researcher (0.5 m), and interacted more. Unpleasantly handled males achieved first mating later than pleasantly handled ones (192 vs. 161 days). The authors concluded that unpleasant handling induced chronic stress and impaired reproduction.

Assistance during mounting includes showing, stimulating, or positioning the animal on the dummy. Preputial massage and

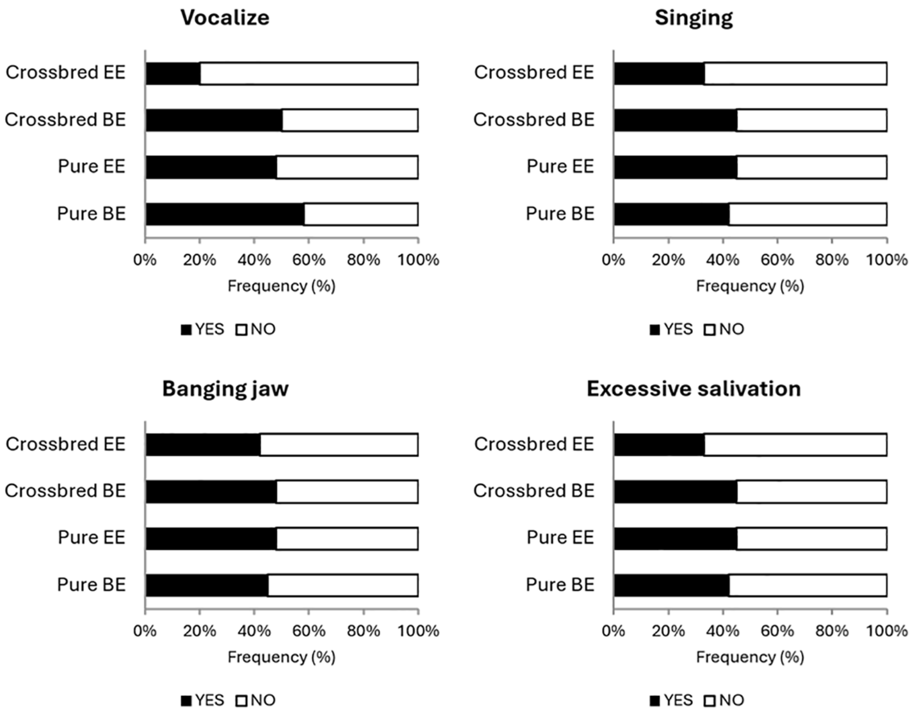

FIGURE 7
Percentage of animals reared in enriched (EE) or barren (BE) environments that displayed mating-related behaviors and stress-related vocalizations during semen-collection training. Data represent observations recorded across all training sessions.

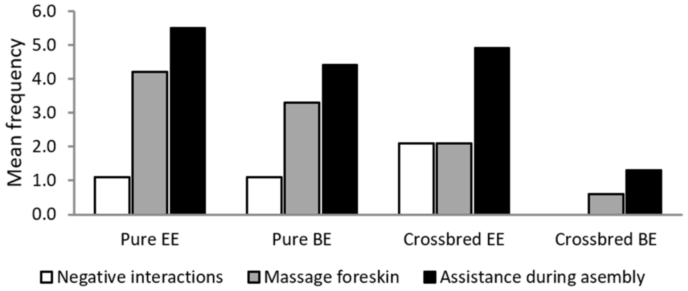

FIGURE 8
Average frequency of behaviors: negative interactions; massaging the foreskin and assistance during mounting.

mounting assistance were more frequent in Purebred EE, consistent with the poorer sexual performance observed, which required greater stimulation. Despite the costs involved, continuous staff training is essential to enhance handlers' knowledge of animal behavior. Protocols dedicated to semen-collection training may accelerate learning and improve the efficiency of semen providers (Kozink et al., 2002).

Testosterone levels

Figure 9 depicts mean testosterone levels at the first collection (fasted; testosterone 1) and the second (post-feeding; testosterone 2), as well as the overall mean. Fasted levels were higher, ranging from 5.63 to 39.41 ng/mL (mean 17.72 ng/mL). Post-feeding levels ranged from 3.90 to 18.11 ng/mL (mean 10.40 ng/mL). Differences between fasted and post-feeding means were significant ($P < 0.001$), with a positive within-animal correlation ($r = 0.563$; $P < 0.01$).

ANOVA indicated no significant difference in testosterone between pure and crossbred lines ($\alpha = 0.05$), but significant differences between EE and BE ($P < 0.05$). Figure 10 suggests higher mean testosterone in enriched than barren environments; nevertheless, EE animals performed worse during training. To assess testosterone effects on libido, mean values ranged from 6.00 to 25.52 ng/mL (overall mean 14.06 ng/mL). Figure 10 shows mean testosterone by animal and the trained/not-trained outcome. Variation existed across animals and treatments: some with low testosterone succeeded, whereas others with high levels did not. Accordingly, training success did not explain testosterone variation (ANOVA, $P = 0.50$).

Testosterone underpins spermatogenesis and male sexual traits, directly influencing libido (Hafez and Hafez, 1995; Correa et al., 2001; Flowers, 2008; Estienne et al., 2007). However, libido is difficult to quantify and is shaped by genetic, seasonal, social, sexual, and psychological factors (Hemsworth and Tilbrook, 2007). Reported testosterone levels vary widely due to genetics, age, sampling schedule, and assay methods (Ren et al., 2009). For instance, Ren et al. (2009) found mean levels of 27.9 ng/mL in 10-month-old boars; Park and Yi (2002) reported 0.73–3.06 ng/mL (Duroc) and 2.48–5.11 ng/mL (Yorkshire) at 15–22 months, varying seasonally; Li et al. (2015) observed 2.62–4.62 ng/mL in 27 month old Large White boars under heat exposure. Flowers (2008) noted that boars with low interest in the dummy (low libido) tend to have low testosterone. Ren et al. (2009) reported a significant correlation between libido score and serum testosterone ($r = 0.354$; $p < 0.01$). Bilskis et al. (2012) showed that immunocastration reduced testosterone (0.25–5.05 ng/mL) and sexual behavior. Conversely, when sexual behavior during growth, collection, or after hormonal treatment does not align with testosterone levels (Louis et al., 1994; Zamaratskaia et al., 2004; Zamora et al., 2010), results reflect the complexity of male sexual behavior (Ren et al., 2009). Moreover, sexual behavior is highly individual, and two males rarely respond identically during training (Dysart, 2015).

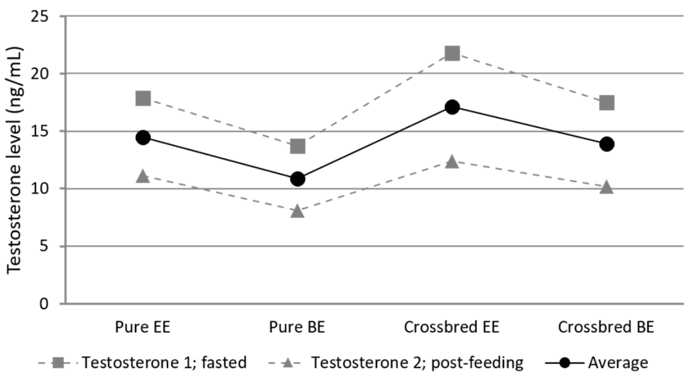

FIGURE 9
Average testosterone values for each treatment.

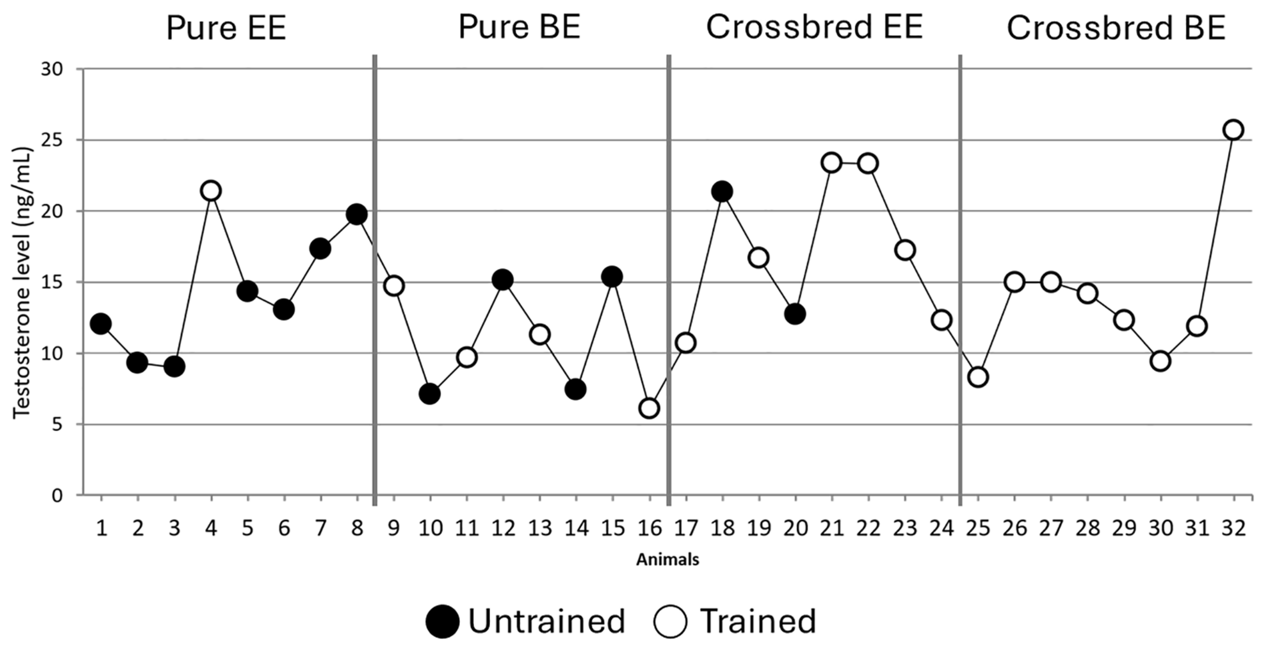

FIGURE 10
Variation in testosterone levels per animal and its relationship with responses: trained and untrained.

Although EE and BE differed significantly in testosterone, this variation likely did not suffice to alter libido, which also depends on other physiological and behavioral factors.

Salivary cortisol levels

Salivary cortisol was sampled immediately before and after each training, on the first and last day. A paired t-test showed no difference between the two periods ($P = 0.784$). We then tested for differences among treatments, between pre- and post-training levels, and for associations with training success. Figure 11 shows mean concentrations by treatment and the pre/post means for sessions 1 and 3.

Descriptively, pre-training cortisol means were lower for all treatments except Crossbred EE, which showed higher pre- (2.36 ng/mL) than post-training (2.20 ng/mL) concentrations and the highest overall means. The correlation between pre- and post-levels did not differ from zero to 5% ($r = 0.24$; $P = 0.073$). ANOVA indicated a significant effect of genetic line on log-cortisol ($P = 0.034$), with no significant effects of environmental enrichment or interactions. Thus, pure lines had lower mean cortisol than crossbreds, a difference confirmed by Tukey's test ($P < 0.05$). Crossbreds appeared more agitated during training; their higher cortisol may be related to this behavior.

Elevated cortisol is associated with stress and directly affects reproduction via hormonal changes (McDonald, 1989; De Jong, 2000). Cortisol rises within minutes of an acute stressor and returns to baseline in about an hour (Wendelaar Bonga, 1997). Figure 12 shows mean cortisol by animal and trained/not-trained outcome. Cortisol varied among animals, and the outcome did not appear

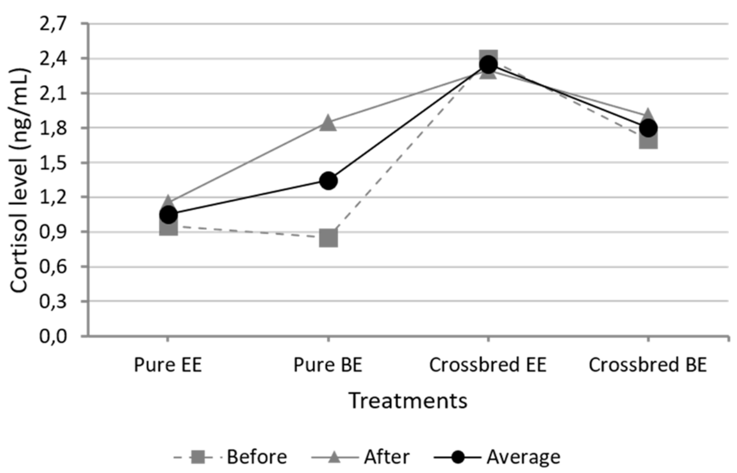

FIGURE 11
Salivary cortisol concentration (ng/mL): before and after semen collection training and overall average for each treatment.

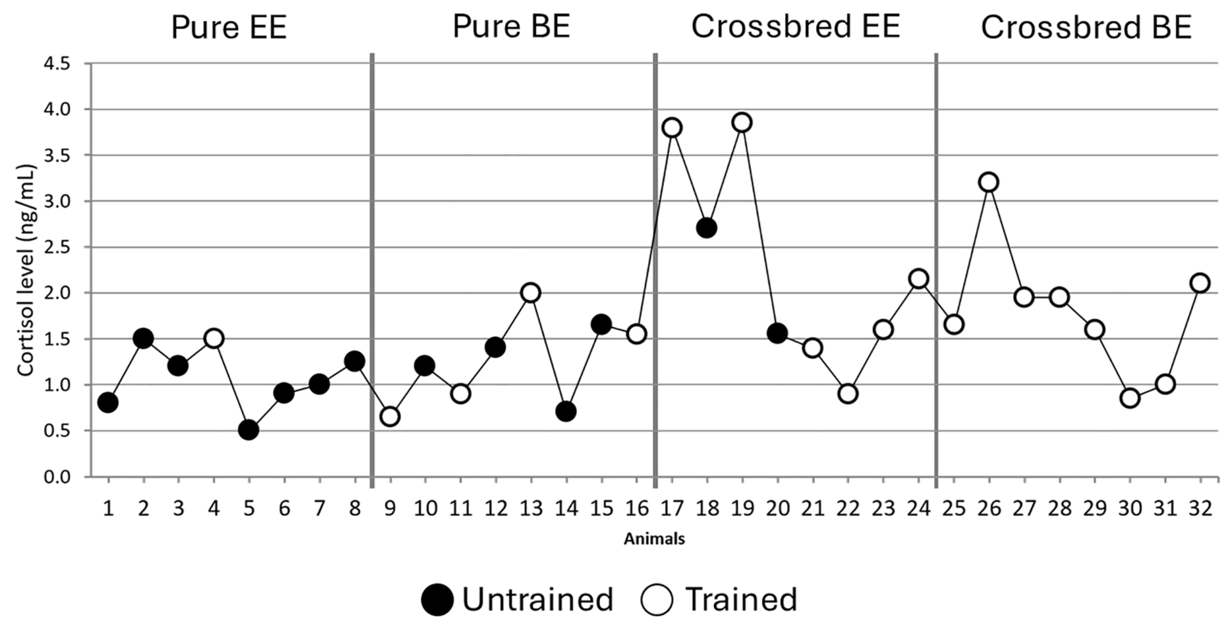

FIGURE 12
Variation in salivary cortisol levels per animal and its relationship with responses: trained and untrained.

associated with cortisol, as both high and low levels were observed among trained animals. ANOVA confirmed that training success did not explain cortisol variation ($P > 0.05$).

LH stimulates Leydig cells to produce testosterone; under stress, increased cortisol inhibits LH release, lowering testosterone production and negatively affecting spermatogenesis and sexual behavior (Barth and Bowman, 1994). However, this alteration is likely confined to acute stress.

In summary, the training setting and concurrent factors (human–animal interaction and unfamiliar environment) were insufficient to alter cortisol levels. The only variable affecting cortisol was genetic line: crossbred males exhibited higher mean cortisol and also performed better during training.

Conclusion

Crossbred boars consistently improved boar performance in semen-collection training, yielding higher libido scores and greater training success than purebreds, whereas the environmental enrichment regime used during growth conferred no benefit and aligned with lower operational success. Despite higher serum testosterone in fasted samples and in EE relative to BE, and a genetic-line effect on salivary cortisol (crossbreds > purebreds), single-time endocrine measures did not predict trained/not-trained outcomes. Together with the low proportion of sessions that succeeded only after stimulation, these findings indicate that intrinsic mounting dexterity and management factors—particularly handler practices and acclimation routines—are the principal levers for efficiency. We recommend prioritizing crossbred males for AI studs, standardizing welfare-focused training protocols that minimize aversive interactions and leverage controlled social/visual cues, and re-evaluating enrichment materials to ensure functional, context-relevant stimulation. Future work should test enrichment typologies with cognitive value, incorporate repeated endocrine sampling and fine-grained behavioral latencies, and apply multivariate modeling to improve prediction of training readiness and semen-collection success.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was waived because, at the time of data collection, institutional animal ethics committee approval was not mandatory at the host institution. All procedures were conducted in accordance with the national regulations and industry guidelines in force at the time and followed internationally recognized principles of animal welfare. The animals were managed under routine commercial conditions, and no harmful procedures beyond standard management and sampling practices were performed. Written informed consent was obtained from the animal owners.

Author contributions

AC: Data curation, Investigation, Writing – original draft. RS: Conceptualization, Validation, Visualization, Writing – review &

editing. IL: Formal Analysis, Writing – review & editing. DL: Visualization, Writing – review & editing. IS: Conceptualization, Supervision, Project administration, Validation, Writing – review & editing.

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To Agroceres Pig

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. The author(s) declared that generative AI was used in the creation of this manuscript. During the preparation of this work the author(s) used ChatGPT (GPT-5, OpenAI) in order to improve the readability of the text and enhance the language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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