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Research article

Quality control of semen processing in boar studs: A Brazilian scenario

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ABSTRACT: Artificial insemination success in swine is mainly associated with semen dose quality. Thus, this study compared quality control parameters in 11 Brazilian boar studs after applying audit services for 24 months (1,650 boars). An extensive checklist was applied in each audit, registering 'compliance' or 'noncompliance' for 75 items. Semen doses produced were analyzed as regards volume and sperm concentration, and microbiological analyses were conducted for semen and water samples collected at distinct production stages. On average, boar studs produced 112.9 semen doses per boar per month, and the odds of raw semen contamination increased when boars were inadequately housed and doses were collected under increased temperatures, with no anti-slip rubber mat or after a poor prepuce cleaning ($p < 0.05$). Collection from boars with locomotor problems and no regular change of reverse osmosis filters increased the contamination odds in semen doses produced and stored at the stud ($p < 0.05$). As regards the water submitted to the osmosis reverse process, contamination odds increased as a result of deficient cleaning and disinfection of the purification equipment ($p < 0.05$). Risk factors for reduced sperm motility (< 70 %) were: no anti-slip rubber mat for semen collection, no cleaning program for automatic feeding system (drops) and bins, and inadequate intervals between semen collections (≤ 2 days or > 7 days; $p < 0.05$). Two boar studs had the best results for compliance with the checklist items. Constant monitoring, appropriate hygiene of facilities and equipment, and periodical staff training are highlighted as non-negotiable points for boar semen dose quality.

Keywords: bacterial contamination, benchmarking, boar semen, semen doses, sperm quality

Introduction

Artificial insemination (AI) in swine offers several advantages, such as genetic gains, sanitary security, reduced breeding costs, better use of facilities, etc. (Bortolozzo et al., 2005). Nowadays more than 90 % of females worldwide are mated through AI, presenting satisfactory reproductive performance (Waberski et al., 2019). Most AI procedures in swine are performed with cooled semen stored at 15-18 °C for 3-7 days (Waberski et al., 2019; Mellagi et al., 2023), and the AI success has been associated mainly with boar semen dose quality (Bortolozzo et al., 2015; Knox, 2016).

Boar semen processing spans several steps; therefore, the quality produced can be associated with many factors (Rodriguez et al., 2017), such as bacterial contamination (Wolff et al., 1993; Prieto-Martínez et al., 2014; Nitsche-Melkus et al., 2020) and the temperature control over the semen processing, transport and storage of semen doses (Schulze et al., 2013). Because of these variables, boar studs require systematic quality control of semen processing, identifying and controlling the risk factors for decreased semen quality, which can be obtained through the implementation of Hazard Analysis and Critical Control Points (HACCP) systems, as prescribed for bull studs in Brazil (Goularte et al., 2015). It is noteworthy that quality control programs also promote the proper characterization of studs (Knox et al., 2008; Bennemann et al., 2020), as well as the setting of benchmarks, which are critical points for further improving the quality of boar semen doses.

The implementation of an HACCP system for boar studs has already been reported (Riesenbeck et al., 2015; Schulze et al., 2015), and to date, 40 European boar studs have been submitted to a solid science-based quality control program (Schulze et al., 2022). In Brazil, 42 boar studs are currently subject to the guidelines required by official agencies (MAPA, 2020), producing more than 9.5 million semen doses per year that results in an annual production of 4.9 million ton of pork (Bennemann et al., 2018; ABPA, 2023). Nevertheless, standardized quality control programs still have to be applied to Brazilian boar studs. Additionally, the little available information regarding Brazilian boar studs focuses mainly on the facilities and workflow process (Bennemann et al., 2020).

The data above reinforce that there is still room for characterizing Brazilian boar studs and identifying, monitoring, and controlling the critical issues of quality control programs. Therefore, this study aimed to implement a quality control system to evaluate routine practices in Brazilian boar studs, assessing critical points for semen dose quality and comparing the studs according to their compliance level.

Materials and Methods

Audits in boar studs

This study included data from 11 boar studs from five Brazilian states located in the mid-western and southern regions (Mato Grosso, Mato Grosso do Sul, Paraná, Santa Catarina, and Rio Grande do Sul), comprising records from 1,650 boars. The studs were periodically visited through the same systems of veterinary services for 24 months, totalizing 96 audits (53 audits in the autumn and winter, and 43 audits in the spring and summer). During each audit, a checklist containing 75 items was applied. The items were grouped into eight categories: boar housing; boar health; semen collection; laboratory structure; semen processing; semen quality; water quality; and cleaning and disinfection (Table 1), and were classified as compliant or noncompliant. The studs' general characteristics are summarized in Table 2.

Semen collection and processing

In all studs, boar semen was collected via a semi-automatic collection system (Magapor). After collection, ejaculate was processed and semen doses were produced with a long-term extender (Vitasem®, Magapor). They were stored in the boar stud and then transported to the destination farm at 16-18 °C. For each ejaculate collected, boar identification, date of collection, raw semen volume, number of semen doses produced, and type of semen dose (either for cervical AI, CAI; or post-cervical AI, PCAI) were recorded. The target volume for CAI and PCAI

Table 1 – Checklist (75 items) applied in the audits of 11 Brazilian boar studs over 24 months and the frequency of noncompliance by category considering all the audits (n = 96).

Category	Noncompliance
Boar housing and semen collection area	21 %
Pen structure: area of 3 × 2 m; non-retention of humidity; feeders and drinkers; adjustments regarding the height of the semen collection dummy; anti-slip rubber mat in the semen collection area; cleaning and disinfection: daily removal of feces; high-pressure washing 1-2 times per month; nebulization 1-3 times per week; temperature within 20-22 °C; environmental conditions: temperature; humidity; welfare.	
Boar health	10 %
Boar hygiene: preputial hairs, feces in the ventral region; annual replacement rate of 50 % (1,000 d-old maximum, average of 20 months in the boar stud or control of Estimated Breeding Values); maximum of 5 % incidence for locomotor problems: prevention and treatment; specific diet for boars; visual score of body condition within 3.0-3.5 (scale 0-5.0); vaccination, antibiotic treatment, and parasite control for boars; rodent control; cleaning of feeding system (drops) and bins.	
Semen collection	11 %
Daily cleaning and disinfection of semen collection areas; pre-cleaning area and use of an automatic system for semen collection; average of 1-2 semen collections/boar/week; length of preputial hairs: 0.5-1.0 cm; dry preputial cleaning with paper towels and gloves; no contact of the semen with the technician's glove during semen collection; duration of semen collection; discard of the pre-sperm fraction of ejaculate (first three jets); discard of the gel portion from the ejaculate; semen collection performed by an experienced technician; use of disposable material for semen collection.	
Laboratory structure	8 %
Adequate layout for semen processing; temperature control (24-25 °C; air conditioning); no humidity in walls and surfaces; no odor or aerial contaminants (dust, smoke, vapors); daily and weekly cleaning schedule with sodium hypochlorite, neutral detergent, and 70 % alcohol; cooling room (18 °C); proper laboratory clothing; use of 70 % alcohol before entering the laboratory.	
Water quality	11 %
Conductivity of the water entering the laboratory; water purification equipment; conductivity of the water leaving the laboratory; routine water quality analyses: conductivity and bacteriological; periodical replacement of deionizer and reverse osmosis filters; cleaning and disinfection of water purification equipment.	
Semen processing	7 %
Measurement of raw semen's temperature in laboratory; observation of raw semen's aspect; determination of viable sperm cells per semen dose; extender preparation: temperature control; volume; contamination; homogenization; pre-dilution for great volumes; water temperature for extender preparation: 35-37 °C; use of stabilized extender (prepared at least 30 min before using); semen weighing to determine the volume; evaluation of sperm motility before dilution; slow dilution of raw semen through the lateral wall of the recipient (for precise temperature control); maximum difference of 2 °C between raw semen and extender (extender temperature should be inferior); evaluation of total sperm motility before storage; adequate storage avoiding contamination; time of sample collection at stud to monitor the shelf life of the dose; real volume of the semen dose after packing matches the target volume; cooling curve: 45 min at room temperature for 1 h; protection of the semen doses from light exposure during the cooling curve; proper arrangement of semen doses during the cooling curve (no superposition); storage in a refrigerated cabinet with temperature control (15-18 °C; thermometer for max and min; daily record); homogenization of semen doses (twice a day); organization of semen doses according to the boar; evaluation of total sperm motility in semen doses before releasing their expedition; expedition of semen doses within a recipient protected from light exposure; transport of semen doses within a container/vehicle with temperature control.	
Semen dose quality	14 %
Longevity of semen doses (for all collections); monthly determination of sperm morphology; monthly bacteriological analyses of raw semen, extended semen, and stored semen doses; monthly determination of semen doses' sperm concentration; monthly determination of semen doses' osmolarity.	
Cleaning and disinfection	18 %
Use of disposable material for semen processing; proper storage of material used for semen processing (e.g., extender, blisters, bottles); cleaning and sanitation of equipment (using water, soap, and 70 % alcohol); calibration and maintenance of laboratory equipment; hose cleaning; washing/replacement of internal cleaning material (e.g., cloths, sponges); washing/replacement of external cleaning material (e.g., cloths, sponges); washing/rinse of slides with purified water, 70 % alcohol, and kiln drying.	

Table 2 – General characteristics of Brazilian boar studs (*n* = 11) audited by veterinary services over 24 months.

Boar stud	Audits	Number of boars	Mean total sperm number per dose* (billion)			Frequency of semen dose type (%)		Number of semen doses produced	
			Raw semen	Semen dose					
				PCAI	CAI	PCAI	CAI	Per month	Per boar per month
A	7	200	78.3	1.9	3.4	86.4	13.6	23,260	116.3
B	11	195	70.7	1.9	3.2	69.8	30.2	22,065	113.1
C	6	171	103.9	1.8	3.2	95.4	4.6	27,628	161.6
D	9	175	120.7	1.5	2.8	69.3	30.7	14,855	84.9
E	6	190	82.0	2.0	3.5	74.5	25.4	10,793	56.8
F	7	160	83.8	1.9	3.5	84.1	15.9	18,643	116.5
G	10	92	77.2	1.9	3.1	54.1	45.9	12,769	138.8
H	12	134	94.1	1.9	3.2	68.2	31.8	11,751	87.7
I	13	125	69.0	2.0	3.3	74.3	25.7	13,951	111.6
J	8	90	75.2	1.9	3.7	51.8	48.2	7,853	87.2
K	7	118	78.7	2.0	3.3	90.6	9.4	19,270	163.3
Total	96	1,650	.	.	.	.	.	182,848	1,237.8
Mean	9	150	84.9	1.9	3.3	74.4	25.6	16,622	112.9

*Semen doses were produced based on the total number of sperm cells with no morphological abnormalities. PCAI = post-cervical artificial insemination; CAI = cervical artificial insemination.

semen doses was 80 mL and 45 mL, respectively. Sperm motility, concentration, and morphological abnormalities in raw semen were evaluated through a computer-assisted semen analysis system (Magavision®, Magapor).

Semen doses analysis

Ten CAI semen doses and 16 PCAI semen doses were collected monthly from each boar stud and sent to the laboratory for sperm concentration and volume determination (totalizing 3,833 semen doses). The volume was verified by weighing the semen dose content, and sperm concentration was determined using a hemocytometer chamber (Neubauer Improved, Optik Labor) after diluting 100 µL of semen dose in formalin solution (900 µL).

Microbiological analyses

Sample collection

For each ejaculate collected in each boar stud, five samples of raw semen, extended semen, and semen doses stored in the boar stud were collected monthly for microbiological analyses. Moreover, five samples of boar semen doses were collected at the destination farm. Water samples from boar studs were also collected monthly: one sample of unpurified water from the stud entrance, another sample of water submitted to the reverse osmosis process, and a further sample of water submitted to the reverse osmosis process and stored for 24 h at room temperature (~ 24 °C; stored water). Additionally, one sample of the extender freshly prepared was obtained. All samples were collected using sterile containers and stored at 2-8 °C until analysis.

Counting and identification of aerobic mesophiles

At the laboratory, samples were submitted to serial dilution. Microbiological analysis was carried out using the spread plate technique, in plate count agar (PCA) which was incubated for 48 h at 37 °C. The number of colonies forming units per mL (CFU mL^{-1}) was determined after 48 h incubation at 37 °C. Raw semen samples presenting > 2,000 CFU mL^{-1} were considered contaminated, as well as the extended semen, boar semen doses (stored in the boar stud or the destination farm), and extender when counting was ≥ 1 CFU mL^{-1}. For water submitted to the reverse osmosis process (stored or not), samples with ≥ 1 CFU mL^{-1} were classified as contaminated, while for unpurified water samples, contamination was considered when counting > 1,000 CFU mL^{-1}.

Isolated colonies were evaluated by biochemical testing. Gram-positive and Gram-negative bacteria were identified using 3 % potassium hydroxide (KOH) and cultured in Rugai-modified medium with lysine. Gram-positive bacteria were submitted to the catalase, coagulase, 6.5 % sodium chloride (NaCl) and bile-esculin tests.

Statistical analyses

Statistical analyses were conducted using SAS® software (SAS Institute Inc. Release 9.4). Noncompliance items were tested as potential risk factors for 13 response variables of interest. Eleven of these response variables were dichotomous and, therefore, evaluated through logistic regression models: 1) contamination in raw semen; 2) in extended semen; 3) in semen stored in the stud; 4) in semen stored at the farm of origination; 5) in unpurified water; 6) in water submitted to the reverse osmosis process; 7) in stored water; 8) in the extender; 9) occurrence of semen doses with more than 3.4×10^9 sperm cells in 80 mL; 10) occurrence of ejaculates with more than 30 % sperm cells with morphological abnormalities; and 11) occurrence of ejaculates with sperm motility inferior to 70 %.

After checking for normality using the Shapiro-Wilk test, multiple linear regression was applied to evaluate the remaining two response variables (continuous variables): ejaculate volume (12) and the total number of sperm cells in ejaculates (13). For all responses (dichotomous or continuous), the period of analyses considered an interval of 60-days (30 days before and 30 days after applying each checklist). Differences were considered when $p \leq 0.05$.

Thereafter, the boar studs were ranked according to scores corresponding to the frequency of identified compliance items related to semen quality and semen or water contamination: score one represented the maximum frequency while score 11 represented the minimum frequency. Subsequently, a median score was determined to rank each stud according to its frequency of compliance items.

Results

On average, the audited boar studs presented an inventory of 150 boars and a monthly production of 16,622 semen doses during the period evaluated (112.9 semen doses per boar per month), most of them being produced for PCAI (74 %). The total number of sperm cells in CAI semen doses was 3.2 ± 0.2 billion; however, 35 % of these semen doses had more than 3.5 billion sperm cells. For PCAI semen doses, the average total number of sperm cells was 1.9 ± 0.1 billion, with 62.1 % of them presenting more than 1.8 billion sperm cells. The coefficient of variation for total sperm number was similar for CAI and PCAI semen doses (Table 2; Figure 1).

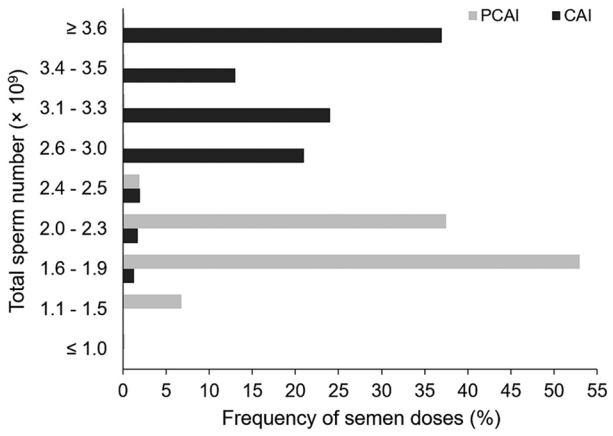

Figure 1 – Frequency distribution of PCAI and CAI semen doses (n = 1,420 and 2,143, respectively) produced by 11 Brazilian boar studs audited by veterinary services over 24 months, according to their total number of sperm cells. PCAI = post-cervical artificial insemination (semen doses with 1.6 ± 0.3 billion cells; coefficient of variation = 14.9 %). CAI = cervical artificial insemination (semen doses with 3.3 ± 0.5 billion cells; coefficient of variation = 14.1 %).

A decrease in the volume of raw semen was associated with the seasonal increase in temperature; however, the parameter increased when the interval between semen collections was inadequate (≤ 2 days or > 7 days; Table 3; $p < 0.01$). Seasonal increase in temperatures were also related to a decrease in sperm concentration of raw semen ($p = 0.03$), which was also associated with the lack of height adjustment of the semen collection dummy ($p < 0.01$) and the lack of anti-slip rubber mat in the semen collection area ($p = 0.03$).

No risk factor was associated with a total sperm number > 1.8 billion in PCAI semen doses or ≥ 3.4 billion in CAI semen doses ($p > 0.05$). The odds of observing > 30 % of sperm morphological abnormalities in raw semen were 1.4 times greater when the laboratory equipment was poorly calibrated or monitored ($p = 0.03$), 1.5 times greater when boar housing was inadequate, and 1.7 times greater when semen was collected by a non-experienced technician or when the interval between semen collections was inadequate ($p < 0.02$). The odds of raw semen presenting sperm motility < 70 % was 1.7 greater when no anti-slip rubber mat in the semen collection area was used, no cleaning program for the automatic feeding system and bins was followed, or the interval between semen collections was inadequate ($p < 0.01$; Table 4).

The risk factors associated with semen contamination are shown in Table 5. The odds of contamination in raw semen were more than two times greater when there was no anti-slip rubber mat in the semen collection area or when boar prepuce was poorly cleaned. When boars were not adequately housed or when the seasonal temperature increased, the odds of raw semen contamination were at least 1.7 times greater ($p < 0.03$). For contamination of extended semen, the odds were at least eight times greater when non-disposable material was used for semen processing, when laboratory equipment was not adequately cleaned, and when the extender was contaminated ($p < 0.01$). Contamination odds in semen doses stored in the boar stud were at least four times greater ($p < 0.01$) when

Table 3 – Linear regression coefficients for risk factors associated with raw semen volume and total sperm number in 11 Brazilian boar studs audited by veterinary services over 24 months.

Risk factor	Estimate	*p*-value
Ejaculate volume in raw semen (R^2 = 0.19)		
Intercept	271.24	-
High seasonal temperatures	–19.55	< 0.01
Inadequate interval between semen collections*	28.17	< 0.01
Total sperm number in raw semen (R^2 = 0.23)		
Intercept	90.60	-
High seasonal temperatures	–8.72	0.03
No height adjustment of the semen collection dummy	–21.10	< 0.01
Absence of an anti-slip rubber mat in the semen collection area	–12.59	0.03

* ≤ 2 days or > 7 days. R^2 = coefficient determination.

Table 4 – Risk factors for the raw semen presenting > 30 % of sperm morphological abnormalities or total sperm motility < 70 % in 11 Brazilian boar studs audited by veterinary services over 24 months.

Risk factor	OR	95 % CI	*p*-value
Raw semen with > 30 % of sperm morphological abnormalities			
Inadequate boar housing	1.51	(1.07-2.12)	0.02
Inadequate interval between semen collections*	1.69	(1.12-2.57)	0.01
Semen collected by a non-experienced technician	1.70	(1.10-2.62)	0.02
Poor calibration and maintenance of laboratory equipment	1.39	(1.02-1.88)	0.03
Raw semen with total sperm motility < 70 %			
Absence of an anti-slip rubber mat in the semen collection area	1.70	(1.17-2.48)	< 0.01
Absence of a cleaning program for the automatic feeding system (drops) and bins	2.12	(1.38-3.29)	< 0.01
Inadequate interval between semen collections*	2.93	(1.91-4.51)	< 0.01

*≤ 2 days or > 7 days. OR = odds ratio; CI = confidence interval.

Table 5 – Significative risk factors for the occurrence of contamination* in boar semen, water and extender from 11 Brazilian boar studs audited by veterinary services over 24 months.

Risk factor	OR	95 % CI	*p*-value
Contamination of raw semen (*n* = 94 audits)			
High seasonal temperatures	1.78	(1.10-2.89)	0.02
Inadequate boar housing	1.83	(1.07-3.15)	0.03
No anti-slip rubber mat in the semen collection area	2.17	(1.21-3.86)	0.01
Preputial cleaning	2.64	(1.61-4.32)	< 0.01
Contamination of extended semen (*n* = 94 audits)			
Use of non-disposable material for semen processing	9.40	(4.02-21.96)	< 0.01
Inadequate cleaning of laboratory equipment	8.81	(2.94-26.03)	< 0.01
Extender contamination	17.14	(7.80-37.72)	< 0.01
Contamination of semen extender (*n* = 92 audits)			
Inadequate cleaning of laboratory equipment	4.97	(1.22-20.21)	0.02
Contamination of semen doses stored in the boar stud (*n* = 94 audits)			
Boar with locomotor problems	4.23	(1.72-10.38)	< 0.01
No regular change of reverse osmosis filters	4.57	(1.90-11.01)	< 0.01
Contamination in semen doses stored in the destination farm (*n* = 75 audits)			
Deficient evaluation of semen motility	2.71	(1.53-4.83)	< 0.01
Semen collected by a non-experienced technician	5.85	(2.23-15.33)	< 0.01
Use of non-disposable material for semen processing	1.96	(1.18-3.25)	0.01
Inadequate cleaning of laboratory equipment	2.21	(1.09-4.50)	0.03
Extender contamination	3.47	(2.01-5.98)	< 0.01
Contamination in unpurified water (*n* = 75 audits)			
High seasonal temperatures	2.00	(1.11-3.60)	0.021
Contamination in water submitted to the reverse osmosis process (*n* = 93 audits)			
Equipment of purification not cleaned and disinfected	4.87	(2.17-10.92)	< 0.01
Contamination in stored water** (*n* = 88 audits)			
Inadequate cleaning of laboratory equipment	6.93	(1.25-38.30)	0.03
Water contamination after the reverse osmosis process	4.33	(2.07-9.08)	< 0.01

*Contamination: raw semen (> 2,000 CFU mL^{-1}); extended semen (≥ 1 CFU mL^{-1}); semen stored in the stud or the destination farm (≥ 1 CFU mL^{-1}); unpurified water (> 1,000 CFU mL^{-1}); water submitted to reverse osmosis process then stored or not for 24 h at room temperature (≥ 1 CFU mL^{-1}); extender (≥ 1 CFU mL^{-1}). OR = odds ratio; CI = confidence interval; CFU = colonies forming units. **Water submitted to reverse osmosis process then stored for 24 h at room temperature.

there was no regular change of reverse osmosis filters or when the herd presented an incidence of locomotor problems > 5 %.

For semen doses stored at the destination farm, the odds of contamination were 1.96 times greater when non-disposable materials were used for semen processing ($p = 0.01$), and more than two times greater when the laboratory equipment was not adequately cleaned ($p = 0.03$) or when the evaluation of semen motility was deficient ($p = 0.01$). In addition, the contamination odds of semen doses stored at the destination farm increased by 3.5 and 5.8 times when the extender was contaminated and the semen was collected by a non-experienced technician, respectively ($p < 0.01$). Seasonal temperature increases doubled the odds of contamination in the unpurified water ($p = 0.02$; Table 5).

The odds of contamination in water submitted to the reverse osmosis process were more than 4.8 times greater when the purification equipment was not cleaned and disinfected ($p = 0.01$). For water submitted to the reverse osmosis process and stored at room temperature for 24 h, the odds of contamination were 4.3 times greater when water submitted to the reverse osmosis process (but not stored) was contaminated and 6.9 times greater when the laboratory equipment was not adequately cleaned ($p < 0.01$). Inadequate cleaning of laboratory equipment was also associated with extender contamination, increasing the odds of extender contamination by almost five times ($p = 0.02$; Table 5). In the raw semen and water samples, 25 distinct microorganisms were isolated, with a higher frequency of Gram-negative bacteria (Table 6).

Comparing the sperm quality items among the boar studs (Table 7), more than 90 % compliance was observed for items related to reduced contamination in semen doses. Compliance higher than 90 % was also observed for items associated with sperm quality, such as frequency of raw semen with total sperm motility > 70 % or morphological abnormalities < 30 %, and frequency of semen doses (CAI or PCAI) containing the target total sperm number. On the other hand, lower compliance (≤ 65.5 %) was observed for items such as contamination of water submitted to the reverse osmosis process and contamination of raw semen. Overall, median scores indicating a higher frequency of compliance were observed in three boar studs (identified in Table 7 as boar studs 'J', 'C', and 'G'; median scores: 3, 4, and 4; respectively). Additionally, three boar studs presented median scores indicating a lower frequency of compliance (boar studs identified in Table 7 as 'E', 'D' and 'I', with median scores 8, 9, and 10, respectively).

Discussion

Our results showed that indicators of semen quality (e.g., sperm motility, morphological abnormalities, and concentration in raw semen) were negatively influenced by several risk factors: poor calibration and maintenance of laboratory equipment; inadequate cleaning of the automatic feeding system and bins; semen collection by a non-experienced technician; no adjustments on dummy according to the boar's height; and an inadequate interval between collections (≤ 2 days or > 7 days). Considering a two-day interval as a minimum period between consecutive semen collections, it should not be excessively shortened in periods of increased semen demand to prevent the

Table 6 – Frequency of bacterial agents isolated in raw semen and water samples* from 11 Brazilian boar studs audited by veterinary services over 24 months.

Agent	Frequency per boar stud, %										
	A	B	C	D	E	F	G	H	I	J	K
Acinetobacter sp.	-	-	-	22.1	-	-	0.9	-	-	-	-
Citrobacter sp.	-	0.8	12.5	-	-	2.3	5.2	-	10.9	-	-
Edwardsiella sp.	-	0.8	12.5	-	-	6.8	0.9	-	-	-	-
Enterobacter sp.	20.0	16.2	4.2	24.3	-	15.0	11.3	-	10.9	-	-
Enterococcus sp.	20.0	1.7	-	1.0	-	-	0.9	-	-	-	-
Escherichia coli	6.7	6.0	2.1	2.1	-	4.5	9.6	-	9.4	-	-
Klebsiella pneumoniae	-	6.8	8.3	1.0	-	-	0.9	-	4.7	-	-
Klebsiella sp.	20.0	10.3	-	3.2	-	4.5	2.6	-	4.7	-	-
Micrococcus sp.	-	2.6	2.1	-	-	4.5	-	-	-	-	-
Morganella morganii	13.3	11.1	18.8	15.8	15.4	21.6	8.7	-	20.3	100	-
Proteus mirabilis	-	7.7	12.5	9.5	23.1	4.5	15.7	-	20.3	-	-
Proteus sp.	-	-	-	2.1	-	-	0.6	33.3	-	-	100
Proteus vulgaris	-	1.7	4.2	-	-	-	3.5	-	1.6	-	-
Providencia sp.	-	0.8	-	2.1	-	4.55	3.5	-	3.1	-	-
Pseudomonas aeruginosa	20.0	19.1	2.0	11.6	23.1	6.8	16.7	-	7.8	-	-
Salmonella sp.	-	0.8	12.5	4.2	7.7	2.3	11.3	-	-	-	-
Salmonella thyphi	-	-	-	-	-	-	1.7	-	-	-	-
Serratia marcescens	-	1.7	-	-	7.7	2.3	2.6	-	-	-	-
Staphylococcus aureus	-	1.7	-	-	-	9.1	1.7	-	1.6	-	-
Staphylococcus epidermidis	-	-	-	-	-	4.5	-	33.3	-	-	-
Staphylococcus haemolyticus	-	0.8	-	-	-	-	-	33.4	-	-	-
Staphylococcus saprophyticus	-	6.8	8.3	-	15.4	4.5	0.9	-	3.1	-	-
Streptococcus agalactiae	-	1.7	-	-	7.6	-	-	-	1.6	-	-
Streptococcus pyogenes	-	0.9	-	-	-	2.2	0.8	-	-	-	-
Vibrio parahaemolyticus	-	-	-	1.0	-	-	-	-	-	-	-

*Unpurified water, water submitted to reverse osmosis process, and water submitted to reverse osmosis process and stored for 24 h at room temperature.

Table 7 – Frequency of compliance items according to boar semen and semen doses quality in 11 Brazilian boar studs audited by veterinary services over 24 months.

Response	Boar stud											Mean (%)
	A	B	C	D	E	F	G	H	I	J	K	
I	98.3 (1)	96.0 (2)	93.9 (5)	93.9 (6)	95.3 (3)	92.9 (7)	84.7 (11)	92.6 (8)	85.8 (10)	95.2 (4)	91.6 (9)	92.9
II	96.0 (5)	96.0 (4)	96.7 (3)	97.9 (2)	90.9 (9)	93.1 (8)	86.9 (11)	94.5 (6)	88.1 (10)	94.1 (7)	98.5 (1)	94.1
III	100.0 (1)	99.5 (8)	100 (1)	90.1 (11)	99.7 (7)	99.8 (6)	100 (1)	100 (1)	99.2 (10)	99.4 (9)	100 (1)	98.6
IV	99.1 (2)	95.8 (5)	95.8 (4)	87.0 (11)	93.3 (9)	94.9 (7)	94.0 (8)	95.4 (6)	91.5 (10)	98.6 (3)	100 (1)	94.3
V	4.3 (11)	66.7 (5)	75.9 (4)	37.5 (10)	93.8 (1)	65.6 (6)	76.7 (3)	37.9 (9)	87.5 (2)	44.8 (8)	46.2 (7)	60.2
VI	84.0 (10)	86.7 (9)	96.6 (6)	65.6 (11)	100 (1)	93.8 (7)	100 (1)	100 (1)	100 (1)	100 (1)	92.9 (8)	92.6
VII	67.0 (7)	70.3 (6)	74.9 (4)	43.2 (9)	60.4 (8)	38.4 (10)	81.9 (2)	70.7 (5)	35.5 (11)	98.3 (1)	76.1 (3)	65.5
VIII	85.6 (8)	91.5 (7)	79.4 (9)	96.9 (3)	78.7 (10)	92.3 (6)	95.1 (4)	93.2 (5)	98.2 (1)	97.1 (2)	56.1 (11)	87.2
IX	85.8 (8)	93.6 (4)	87.0 (7)	97.4 (3)	76.9 (11)	84.1 (9)	92.3 (5)	91.0 (6)	98.0 (2)	98.4 (1)	77.5 (10)	89.5
Median score	6	5	4	9	8	7	4	6	10	3	7	-

I = Frequency of sperm morphological abnormalities < 30 %; II = Total sperm motility > 70 %; III = Total sperm number > 1.8 × 10^9 sperm cells in semen doses for post-cervical artificial insemination; IV = Total sperm number > 3.4 × 10^9 sperm cells in semen doses for cervical artificial insemination; V = Contamination in water submitted to the reverse osmosis process; VI = Contamination in extender; VII = Contamination in raw semen; VIII = Contamination in extended semen; IX = Contamination in stored semen doses. Values are presented as the frequency of compliance items and the scores for each response within parenthesis.

reduction in the ejaculate's volume and impairment in semen quality, since a short transit of the semen through the epididymis would jeopardize sperm maturation (Pruneda et al., 2005).

In the present study, approximately 74 % of the semen doses produced in studs evaluated were for post-cervical AI (45 mL), containing an average of 1.9 billion sperm cells. The PCAI shows a wide-spread commercial use for sows, mainly in South America (García-Vázquez et al., 2019), and is currently being performed with semen doses containing 1.0-2.0 billion sperm cells in 40-50 mL (Bortolozzo et al., 2015; Waberski et al., 2019). The use of semen doses with 2.5-4 billion sperm cells in 70-100 mL have been mostly used for CAI (Soriano-Úbeda et al., 2013; Bortolozzo et al., 2015; Knox, 2016; Roca et al., 2016), and the average value observed in this investigation for CAI semen doses (80 mL) was 3.3 billion sperm cells. Overall, it is essential to highlight that both PCAI and CAI semen doses presented a sperm concentration (~ 40 million mL^{-1}) under the limit currently recommended (~ 60 million mL^{-1}) to avoid low sperm motility over the storage period (≤ 70 %; Quirino et al., 2023). Our data also showed some dispersion beyond the average values of total sperm number, which was observed for all studs evaluated, regardless of the type of semen dose, as indicated by coefficients of variation of 14-15 %. This information must be highlighted since this parameter is associated with semen dose quality and with the optimization of ejaculate use.

It has already been observed that high levels of bacterial contamination in boar semen are associated with sperm agglutination, damaged acrosomes, poor sperm motility, reduced shelf life of the extended semen product (Wolff et al., 1993; Auroux et al., 1991; Úbeda et al., 2013; Prieto-Martínez et al., 2014), and detrimental effects on reproductive performance (Maroto-Martín et al., 2010; Úbeda et al., 2013; Sepulveda et al., 2014). This scenario may be aggravated by the resistance of microorganisms isolated in semen samples to commonly used antimicrobials, as reported for boar (Schulze et al., 2015; Costinar et al., 2022) and bull semen (Goularte et al., 2020). Our data showed that a wide variety of microorganisms were isolated from raw semen samples, as has already been reported in other studies (Althouse et al., 2008; Maroto-Martín et al., 2010; Kuster and Althouse, 2016), which confirms the relevant prevalence of Gram-negative bacteria in raw semen (Úbeda et al., 2013; Costinar et al., 2022). As a collection of fully sterile ejaculates is nearly unfeasible (Schulze et al., 2015), some level of semen contamination is expected before processing. For this reason, in the present study, raw semen was considered contaminated when containing more than 2,000 CFU mL^{-1}. However, a previous study reported that boar sperm quality would be impaired in ejaculates containing more than 1,000 CFU mL^{-1} (Goldberg et al., 2013), which emphasizes that determining the tolerable contamination levels in raw boar semen is a complex task.

It may be expected that the risk factors for contamination in raw semen would be related to the collection process. The prepuce is a relevant source of contamination of ejaculates if basic hygiene procedures (e.g., dry cleaning of the prepuce before semen collection) are neglected (Goldberg et al., 2013), which was confirmed in the present study by contamination odds that were at least 2.6 times greater when hygiene procedures were not properly followed. Additionally, the odds of contamination in raw semen increased when there was inadequate boar housing and no anti-slip rubber mat in the semen collection area. Housing factors such as barn dimensions, drainage of manure, and humidity levels may influence the accumulation of dirt in the ventral abdomen, thereby affecting the chances of semen contamination during its collection (Althouse et al., 2000). Moreover, inappropriate housing can increase the occurrence of locomotor problems, which increases the odds of contamination in the semen

doses stored at the stud. Locomotor problems affect the capacity of boars to sustain themselves adequately during semen collection, consequently increasing the chances of semen contamination. Thus, boar housing conditions must be constantly monitored to prevent inadequate welfare conditions for boars. The absence of a non-slip rubber mat may result in a slippery floor in the semen collection area, making it difficult for the boars to sustain an adequate position during semen collection. Indeed, this risk factor was not only associated with higher contamination odds in raw semen but was also related to increased odds of occurrence of ejaculates with less than 70 % sperm motility.

The odds of contamination in raw semen also increased with seasonal increases in temperature, which were also associated with adverse effects on the volume and sperm concentration of raw semen, as well as water contamination. Although heat-stressed boars may have impaired semen quality and decreased fertility (Peña Jr. et al., 2019), only marginal effects of seasonal temperature increase on boar sperm quality in subtropical areas such as have been reported (Argenti et al., 2018). However, seasonal fluctuations in semen contamination impairing semen quality were reported, with multifactorial interactions with the extender quality, the prevalence of contaminant microorganisms, and the efficiency of antimicrobials (Althouse et al., 2008). All boar studs evaluated in this study presented temperature control through air conditioners; however, it did not prevent the potential effects of seasonal temperature increasing on the quality of the ejaculates. It suggests that adjustments in temperature control systems inside the studs throughout the year may be required to compensate for seasonal fluctuations in environmental temperatures.

As semen contamination after collection and processing is supposed to be minimal in commercial studs with strict biosecurity, extended semen was considered contaminated when containing ≥ 1 CFU mL^{-1}. The risk factors for contamination in extended semen were related to neglected laboratory procedures, such as the use of non-disposable material and the poor cleaning of equipment, as has been reported in other studies (Schulze, et al., 2015; Nitsche-Melkus et al., 2020). These risk factors were associated with semen dose contamination at the destination farm. In addition, the inadequate cleaning of laboratory equipment was identified as a risk factor for extender contamination, which was also a risk for contamination of extended semen and semen doses stored at the destination farm. These findings emphasize that procedures related to the semen processing routine at the boar studs influence semen quality at subsequent stages. As most microorganisms isolated in the present study can be considered opportunistic, their presence in the semen and in the extender may result from an inadequate cleaning process of laboratory material, leading to the formation of biofilm. (Waberski, 2019; Costinar et al., 2022).

As has been previously reported, failures in the water purification process and manipulation during the semen processing result in water contamination (Úbeda et al., 2013). Although the odds of contamination in unpurified water increased with high seasonal temperatures, purification processes could mitigate such contamination. Nonetheless, the odds of contamination in stored water were increased when the process of cleaning and disinfecting the purification system or cleaning the laboratory equipment were inefficient, leading to higher odds of water contamination even after the reverse osmosis process. Our results identified associations between water contamination and semen contamination, as the lack of a scheduled change of reverse osmosis filters was a risk factor for contamination in the semen stored at the stud. Those findings may reflect that the water supplied to the evaluated boar studs had not been treated with chloride since it was mostly from natural and artesian wells. Therefore, constant maintenance and cleaning of the equipment used for water purification is mandatory. It is also important to mention that, in this investigation, the composition of some materials, such as semen packages, was not assessed. Nevertheless, this approach can be considered in quality control programs since the contact of sperm cells with toxic compounds can result in additional detrimental effects on subsequent fertility after AI, even when adverse effects on semen quality are not evident (Nerin et al., 2014; Schulze et al., 2020).

The studs evaluated in this study represent nearly 20 % of Brazil's boar inventory and 15 % of the semen doses currently produced in the Brazilian swine industry. The quality control comparison across studs indicated that studs E, D, and I presented greater noncompliance frequencies, especially for items related to semen quality. However, Stud E ranked particularly worse in items related to semen contamination. Furthermore, studs D and E produced the lowest number of semen doses per boar per month. On the other hand, studs J, C, and G presented greater compliance frequencies. Although studs J and G had the lowest boar inventories (< 100 boars) and were among the studs with lower monthly production of semen doses, the semen produced in such studs was likely high-quality. Thus, as all studs evaluated were periodically audited by the same veterinary service, stud J, with a median score equal to three, would be a candidate as a reference to benchmark the remaining studs. However, this boar stud would still need to improve a noncompliance issue for a fundamental item: contamination of water submitted to the reverse osmosis process. Additionally, stud C could also be considered a reference since it achieved a similar median score (4) and presented the greatest production of semen doses/boar/month, likely due to its large boar inventory.

The quality control audits in 11 Brazilian boar studs over 24 months revealed that factors mainly related to semen collection were associated with

increased odds of reduced semen quality. In contrast, the risk of water and semen contamination was increased by deficient cleaning and disinfection of equipment. Given these data, constant monitoring, appropriate hygiene of the facilities and equipment, and periodical staff training can be highlighted as non-negotiable points for boar studs. Out of all the boar studs evaluated, three could qualify as potential benchmarks due to their higher frequency of compliance with the checklist items. Nevertheless, we do recommend implementing the quality control approach in a higher number of boar studs in Brazil, making possible further characterization and monitoring of the semen process in the Brazilian swine industry, consequently guaranteeing the production of high-quality boar semen doses.

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