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# LIVE ESTROUS FEMALE VERSUS PHEROMONAL STIMULATION: A COMPARATIVE ASSESSMENT OF CANINE SEMEN COLLECTION OUTCOMES

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***Abstract***

*This pilot descriptive study compared manual semen collection outcomes in sexually experienced adult male dogs collected either in the presence of a live intact female in estrus (n = 3) or using isolated estrous pheromones sampled from an estrous female (n = 3). Outcome measures included ejaculation latency (time from stimulus exposure to ejaculation), ejaculate volume, semen pH, and macroscopic appearance (color, odor). Inferential statistical analysis was not performed because of the small sample size; results are presented descriptively. Median (range) ejaculation latency was shorter in the live - female group at 3:00 min (2:52–3:37) than in the pheromone group at 4:35 min (4:18–5:02). Mean ejaculate volumes were similar between groups - 3.93 mL (SD 2.70) for live - female stimulation and 4.03 mL (SD 2.85) for pheromone - only stimulation. Semen pH values were broadly comparable between groups, and all ejaculates were milky - white and odorless. These findings indicate that pheromone - only stimulation can induce ejaculation and produce grossly comparable ejaculates under low - stress conditions, although ejaculation latency was longer. Given the small parallel - group design, use of a single pheromone donor, and absence of objective sperm - quality parameters, results are preliminary. Further research using larger crossover designs and comprehensive fertility endpoints is warranted.*

***Key words****: dogs, ejaculate, pheromones, semen collection.*

## INTRODUCTION

Assisted reproductive techniques (ART), including artificial insemination and semen cryopreservation, are increasingly important in canine breeding, particularly when natural mating is impractical because of anatomical, behavioral, geographic, or management constraints (Concannon, 2011; Luvoni, 2013). Reliable, low-risk semen collection methods are therefore essential for preserving genetic resources and supporting fertility management in domestic dogs. Manual semen collection is commonly facilitated by the presence of an estrous female, which provides multisensory cues: visual, behavioral, tactile, and olfactory, that promote sexual arousal and ejaculation. However, the use of live estrous females presents logistical, welfare, and safety challenges, including the need for suitable donors, coordinated timing, and potential stress or injury to animals and handlers (Micșa et al., 2024).

An alternative approach is the use of estrous pheromones sampled from females in estrus, providing primarily olfactory stimulation while avoiding direct contact. Pheromones are chemical signals that influence conspecific physiology and behavior and contribute to mating attraction and reproductive readiness in many mammalian species (Robertson & Martin, 2022; Patra, Barman & Kumar, 2026). Semio-chemical signaling is among the oldest forms of communication across taxa and plays a key role in initiating and modulating sexual behavior (Dzięcioł et al., 2016). In dogs, studies of female-derived cues have examined behavioral and endocrine responses, yet evidence supporting pheromone-only stimulation for reliable semen collection remains limited (Woszczyło et al., 2023).

Evaluating whether isolated pheromonal cues can produce ejaculates comparable, on gross characteristics, to those obtained in the presence of live-females may help simplify semen collection protocols and reduce risks

associated with direct male-female interaction. Semen evaluation is central to assisted reproduction, as ejaculate volume alone does not predict fertility; standardized assessment methods, including concentration and motility analysis, are essential for interpreting suitability for artificial insemination and cryopreservation (Johnson & Johnston, 2018). Semen analysis in dogs also allows repeated assessment across time and supports evaluation of functional impacts on male reproduction (Dostal, Juneau & Rothwell, 2001).

This study presents a pilot, descriptive comparison of manual semen collection outcomes in sexually experienced male dogs collected with either a live estrous female or pheromone-only olfactory stimulation. Ejaculation latency, ejaculate volume, semen pH, and macroscopic appearance (color, odor) were assessed. Owing to the small sample size and parallel-group design, results are presented descriptively without inferential statistics. Animals were washed and sanitized prior to collection to minimize bacterial contamination during sampling (Darie et al., 2020).

## MATERIALS AND METHODS

This was a pilot, parallel-group, descriptive study. Six sexually experienced adult male dogs were enrolled and assigned to one of two groups (n = 3 per group) matched approximately by body size and weight to reduce breed/size-related confounding. Each dog contributed a single semen collection under the assigned condition.

Animals and grouping (see Table 1): Group 1 (live-female stimulus): Rottweiler (6 y 2 m; 49 kg), Boston Terrier (8 y 4 m; 11 kg), Wire-haired Dachshund (4 y 9 m; 9 kg). Group 2 (pheromone stimulus): Rottweiler (6 y 2 m; 52 kg), French Bulldog (7 y 7 m; 14 kg), Wire-haired Dachshund (3 y 1 m; 10 kg). Breed names are standardized.

Table 1. Breed, age and body weight of male dogs allocated to the two experimental groups

| Group 1 | | | Group 2 | | |
|---|---|---|---|---|---|
| **Breed** | **Age** | **Weight** | **Breed** | **Age** | **Weight** |
| Rottweiler | 6y 2m | 49 kg | Rottweiler | 6y 2m | 52 kg |
| Boston Terrier | 8y 4m | 11 kg | French Bulldog | 7y 7m | 14 kg |
| Wire haired Dachshund | 4y 9m | 9 kg | Wire haired Dachshund | 3y 1m | 10 kg |

All males were sexually experienced and previously used for manual semen collection and natural mating. Dog semen is typically collected manually from conscious animals (Christensen et al., 2011).

Collections were performed in familiar, low-stress environments with owners present. Animals were washed and sanitized prior to handling to reduce contamination risk (Darie et al., 2020). Owner consent was obtained for participation and for use of photographs.

Female estrous status was confirmed by serum progesterone measurement performed by an external diagnostic laboratory (Figure 1), and only females with progesterone concentrations consistent with the peri-ovulatory/estrus period were used. For live stimuli, a clinically healthy intact female in estrus was positioned near the male during collection.

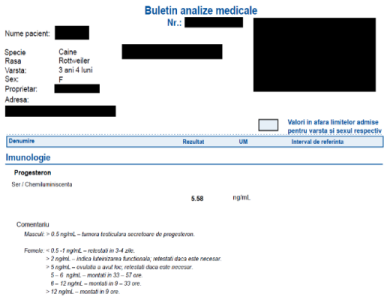
Buletin analize medicale
Nr.: [redacted]
Nume pacient: [redacted]
Specie Caine
Rasa Rottweiler
Varsta: 3 ani 4 luni
Sex: F
Proprietar: [redacted]
Adresa: [redacted]
Valori in afara limitelor admise pentru varsta si sexul respectiv
Denumire | Rezultat | UM | Interval de referinta
Imunologie
Progesteron
Ser / Chemiluminiscenta
5.58 ng/mL
Comentariu
Masculi: > 0.5 ng/mL – tumora testiculara secretoare de progesteron.
Femele: < 0.5 -1 ng/mL – retestati in 3-4 zile.
> 2 ng/mL – indica luteinizarea functionala; retestati daca este necesar.
> 5 ng/mL – ovulatia a avut loc; retestati daca este necesar.
5 – 6 ng/mL – montati in 33 – 57 ore.
6 – 12 ng/mL – montati in 9 – 33 ore.
> 12 ng/mL – montati in 9 ore.

Figure 1. Positive test bulletin for ovulation (own source)

For pheromone stimuli, a sterile cotton pad was gently applied to the vulvar region of an estrous donor female (progesterone 5.58 ng/mL for the donor used), then placed in a sterile container; during collection the container was opened and the pad positioned near the male's muzzle to provide olfactory stimulation only (See Figure 2).



Figure 2. Cotton pad previously applied to the vulvar region of an estrous female (own source)

**Semen collection and evaluation**

Manual semen collection was carried out by experienced personnel using standard manual stimulation techniques (Figure 3). Collections were performed once per male under assigned conditions.



Figure 3. Manual collection of the seminal material (own source)

Semen was evaluated immediately in fresh state. Parameters recorded were:

- Ejaculation latency: time from onset of exposure to stimulus (live-female present or pheromone container opened) to ejaculation (minutes: seconds).
- Ejaculate volume (mL).
- Semen pH (measured on fresh ejaculate).
- Macroscopic appearance: color and odor (descriptive). Erection latency was not measured and is not reported.

Rationale for not cryopreserving: the study focused on immediate ejaculatory and gross semen characteristics measured on fresh semen. While cryopreservation is important for long-term storage and ART, freeze-thaw processes can alter cellular structures and motility; therefore, cryopreservation was not performed for this pilot comparison (Bălan et al., 2020; Bradu et al., 2025).

## RESULTS AND DISCUSSIONS

Because of small sample sizes, no inferential statistical testing was performed. Results are presented descriptively as individual values and as group summaries (means ± standard deviation, medians, and ranges). All original numeric values from the study dataset are preserved in this report (Tables 2 and 3).

Table 2. Ejaculation latency and macroscopic semen characteristics in Group 1 (live female stimuli)

| Dog | Interval (min: s) | Volume (ml) | pH | Color | Smell |
|---|---|---|---|---|---|
| R1 | 3:37 | 7.1 | 6.4 | milky-white | odorless |
| B1 | 2:52 | 2.3 | 6.2 | milky-white | odorless |
| D1 | 2:59 | 2.4 | 6.3 | milky-white | odorless |

R1 - Rottweiler,
B1 - Boston Terrier
D1 - re-haired Dachshund

Table 3. Ejaculation latency and macroscopic semen characteristics in Group 2 (Pheromone stimuli)

| Dog | Interval (min: s) | Volume (ml) | pH | Color | Smell |
|---|---|---|---|---|---|
| R2 | 4:18 | 7.3 | 6.4 | milky-white | odorless |
| F2 | 5:02 | 2.2 | 6.9 | milky-white | odorless |
| D2 | 4:35 | 2.6 | 6.4 | milky-white | odorless |

R2 - Rottweiler,
F2 - French Bulldog
D2 - Wire-haired Dachshund

Group descriptive summaries Ejaculation latency (converted to seconds)

- Group 1 (n = 3): Mean 185 s (3:05), SD 27 s; median 179 s (2:59); range 172-217 s (2:52-3:37).
- Group 2 (n = 3): Mean 277 s (4:37), SD 35 s; median 275 s (4:35); range 258-302 s (4:18-5:02).

Ejaculate volume

- Group 1: Mean 3.93 mL, SD 2.70; median 2.4 mL; range 2.3-7.1 mL.
- Group 2: Mean 4.03 mL, SD 2.85; median 2.6 mL; range 2.2-7.3 mL.

Semen pH

- Group 1: Mean 6.3, SD 0.1; median 6.3; range 6.2-6.4.
- Group 2: Mean 6.57, SD 0.35; median 6.4; range 6.4-6.9.

Macroscopic appearance: all ejaculates were described as milky-white and odorless in both groups; no gross discoloration, hemato-spermia, or malodor suggestive of contamination or infection was observed.

Discussion: summary of findings In this small pilot series, manual semen collection was successful under both stimulatory conditions:

live estrous females and isolated estrous pheromones. Ejaculation latency was consistently shorter when a live female was present (median 2:59) than when only pheromonal cues were used (median 4:35). Despite the latency difference, pH, and macroscopic characteristics (ejaculate volumes, color, odor) were broadly similar between groups.

Longer ejaculation latency during pheromone-only stimulation is plausibly explained by the reduced multisensory input: live females provide visual and behavioral cues (e.g., lordosis-like behaviors, attention-seeking) and potential tactile stimulation that may accelerate arousal and ejaculation (Robertson & Martin, 2022).

Olfactory cues alone may be sufficient to elicit sexual behavior and ejaculation in many males but may require more time or higher stimulus concentration to achieve equivalent arousal levels. Woszczyło et al. (2023) observed hormonal changes associated with female presence during manual collection, demonstrating that multisensory exposure can alter endocrine markers (cortisol, oxytocin, prolactin, testosterone) and potentially influence sexual behavior and semen parameters. Our descriptive results align with the idea that male arousal dynamics differ between full multisensory stimulation and olfactory-only stimulation, but do not provide data on endocrine correlates or objective sperm function. The similarity in ejaculate volumes between groups suggests that once ejaculation occurs, the quantitative output may not be markedly affected by the presence versus absence of a live female - at least in sexually experienced males under low-stress conditions. However, ejaculate volume alone is an incomplete indicator of semen quality: concentration and progressive motility are critical to fertility outcomes (Concannon, 2011; Luvoni, 2013). Standardized semen collection and evaluation methods - including careful manual collection techniques, objective motility assessment (preferably by CASA), and morphology and viability staining - are necessary to assess reproductive potential accurately (Ainsworth & Linde-Forsberg, 2009; Johnson & Johnston, 2018). The absence of motility, morphology, concentration, and viability data in the present study is a major limitation for interpreting reproductive utility.

Strengths and limitations Strengths of the study include standardized handling in familiar environments, use of progesterone-confirmed estrous females for pheromone sampling, and preservation of original numeric data for transparent descriptive reporting. The study also addresses a practical question of interest to clinicians and breeders: whether pheromone-only stimulation can provide a lower-contact alternative for collecting ejaculates.

Limitations are substantial: the small sample size (n = 6 total, 3 per group) and parallel-group design prevent separation of individual- or breed-related effects from treatment effects. Each male was collected once, so repeatability and intra-individual variability were not assessed. The pheromone stimulus was derived from a single donor female; pheromone composition and concentration likely vary among females and across estrous stages, so generalizability is limited. Importantly, objective sperm-quality parameters and fertility outcomes were not measured; without concentration and motility data, it is not possible to infer semen suitability for artificial insemination or cryopreservation. Finally, because no inferential statistics were performed, the results should be interpreted as descriptive observations rather than evidence of equivalence or superiority of either method.

Practical implications and recommendations For practitioners and breeders, pheromone-based stimulation may be a feasible lower-contact option to obtain ejaculates from sexually experienced males when live females are unavailable or pose logistic/welfare/safety concerns. However, given current evidence, pheromone-assisted collection should be used cautiously and validated on a case-by-case basis—for example by performing paired collections comparing methods in a subset of males and by assessing critical semen quality metrics (concentration, motility, morphology) before adopting pheromone-only protocols for artificial insemination or cryopreservation. For researchers, we recommend future studies that:

- Employ larger, adequately powered cohorts across multiple breeds.
- Use crossover designs (each male exposed to both conditions in randomized order with washout) to control for individual variation.

- Standardize and quantify pheromone sampling and presentation (e.g., consistent pad mass, solvent extraction and GC-MS profiling, or pooled/synthetic pheromone preparations).
- Include comprehensive semen analyses (sperm concentration, CASA-motility parameters, morphology, viability, membrane and acrosome integrity).
- Measure behavioral and endocrine correlates (arousal scoring, cortisol, testosterone, oxytocin) and safety/welfare metrics.
- Assess repeatability across multiple collections per male and across pheromone donors.

## CONCLUSIONS

This pilot descriptive study showed that pheromone-only stimulation can elicit ejaculation in sexually experienced male dogs and yield ejaculates with macroscopic similar characteristics (volume, pH, color, odor) to those obtained in the presence of a live estrous female, while ejaculation latency was longer with pheromone-only stimulation. These observations suggest potential practical utility for pheromone-based semen collection as a lower-contact alternative in certain settings. However, due to the study's small sample size, parallel-group design, single pheromone donor, and absence of objective sperm-quality and fertility endpoints, the results are preliminary and insufficient to support routine adoption of pheromone-only collection for assisted reproduction. Rigorous follow-up studies incorporating the design and measurement recommendations above are required to establish whether pheromone-based protocols can reliably replace or complement live-female stimulation in clinical and breeding practice.

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