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Short communication: Impact of rest intervals and habituation on electro-ejaculated semen quality in merino-type rams

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Abstract

This study evaluated the effects of habituation and electro-ejaculation techniques on semen quality in merino-type rams in South Africa, aiming to refine the electro-ejaculation method to improve semen quality. Parameters assessed included semen volume, sperm motility, sperm viability, sperm concentration, sperm viscosity, sperm consistency, and sperm abnormalities. Forty mature (six- to eight-tooth) merino-type rams, approximately two and a half to four years old, were randomly assigned to four groups: habituated with a 3-second rest (H3) (n = 10), non-habituated with a 3-second rest (N3) (n = 10), habituated with a 10-second rest (H10) (n = 10), and non-habituated with a 10-second rest (N10) (n = 10). Each group underwent a two-week habituation period to human presence before electro-ejaculation using a commercial device. Analysed semen samples showed no significant differences in semen volume (0.93–1.28 mL), sperm motility (75.2%–83.1%), sperm viability (73.1%–85.5%), sperm concentration score (3.45–3.83), sperm viscosity score (3.5–4.00), or sperm consistency score (3.5–4.0). However, the H3 group had a higher percentage of sperm abnormalities (12.3%) compared to the N3 (6.11%) and H10 (6.7%) groups, indicating that a shorter rest interval may negatively impact semen quality. The study concludes that extending the rest interval to ten seconds can enhance semen parameters.

Keywords: animal handling, assisted reproduction technologies, merino rams, sperm quality

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Introduction

To ensure genetic advancement and the overall efficiency of breeding programmes, evaluating the breeding soundness of sires is essential (Bagley, 1997). A critical aspect of this evaluation involves the collection and analysis of semen samples. The quality of semen is pivotal in assessing male fertility potential, and various methodologies exist to collect semen from rams. This analysis not only includes physical assessments of the ram's breeding capability, but also extends to the utilisation of assisted reproductive technologies. Superior rams often have their semen collected, evaluated, and subsequently cryopreserved for future use, particularly in artificial insemination (Gibbons *et al*., 2019).

The collection of semen can be achieved through several techniques, including electro-ejaculation (EE), the use of an artificial vagina (AV), and, less commonly, aspiration of semen from the

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vagina of a mated female (Palmer *et al*., 2005). An innovative alternative to EE is the transrectal massage of the accessory sex glands (TUMASG) (Abril-Sánchez *et al*., 2019). Each collection method presents distinct advantages and disadvantages concerning efficacy and the level of discomfort experienced by the animal. Among these methods, the AV and EE techniques are predominantly employed in the collection of semen from production animals (Palmer, 2005; Damián & Ungerfeld, 2011), with the AV method having been utilised for longer than the EE technique (Palmer, 2005). A significant requirement for the AV method is that animals must undergo training, whereas the EE method does not require any preparatory training. The application of the EE method dates back to 1936 and has been recognised for its efficacy in obtaining semen samples from untrained male animals (Palmer, 2005). Recent research indicates that EE is often the preferred technique for semen collection because of its effectiveness and the lack of a need for prior conditioning (Mujitaba *et al*., 2022). Semen samples collected via EE are typically of sufficient quality for artificial insemination, making it a practical choice, even for animals without prior experience. While EE generally yields a higher volume of semen than the AV method, studies indicate that semen obtained through the AV technique tends to have superior quality and a denser sperm concentration (Austin *et al*., 1961; Bopape *et al*., 2015).

Concerns surrounding animal welfare have led to increased scrutiny of the EE method. Advocacy groups internationally have begun urging livestock producers to abandon EE due to its invasive nature and potential to cause pain (Palmer, 2005; Whitlock *et al*., 2012). In several European countries, including the Netherlands and Denmark, the practice has been banned. In Canada, the Veterinary Medical Association has recommended that only skilled practitioners perform EE procedures (Code of Recommendations for the Welfare of Livestock: United Kingdom Government, 2003). Regulations in the United Kingdom stipulate that only licensed veterinarians may carry out EE, with anaesthesia encouraged to mitigate discomfort (Palmer, 2005; Responsible Wool Standard, 2022). In South Africa, while EE is sanctioned by most animal welfare organisations, it remains a topic of heated debate. The practice is not yet regulated, although the Animal Protection Act (71 of 1962) does include provisions aimed at preventing unnecessary suffering. The Responsible Wool Standard (RWS) Certification Scheme has deemed the use of EE for semen evaluation unacceptable under its animal welfare criteria, which presents significant implications for the assessment and certification of rams and bucks, especially when routine semen collection is not feasible (RWS, 2022).

In light of these concerns, there is a pressing need to refine semen collection techniques to balance animal welfare with the operational requirements of livestock production. While EE has shown the potential to inflict pain and discomfort, there is scope for improvement to reduce its negative impacts (Baiee *et al*., 2018). Proposed enhancements include a gradual increase in electrical stimulation intensity and the establishment of positive associations through habituation (Anderson, 2016; Baiee *et al*., 2018; Chincarini *et al*., 2018). Such refinements are crucial, not only for complying with animal welfare standards but also for maintaining the competitiveness of South African livestock products in the global marketplace. Improving semen collection techniques, particularly EE, is imperative for enhancing semen quality while minimising adverse effects on animal welfare. This study aims to investigate the potential benefits of habituation and extended intervals between stimulation bouts in mitigating the impacts of EE on the semen quality of merino rams. The findings may contribute significantly to the development of refined protocols that enhance the acceptance of this technique within the breeding soundness examination of male animals.

Materials and Methods

Ethical clearance was granted by the Animal Research Ethics Committee (AREC) (project ref: UFS-AED2021/0038) of the University of the Free State. This study took place at Sakrivierspoort farm, Loxton, Northern Cape, South Africa (coordinates: 31°81'84.1" S, 22°13'96.3" E). The region is characterised by a semi-desert climate, with winter temperatures below 0 °C and summer temperatures reaching 40 °C, and receives an average rainfall of 200 mm/year.

A total of 40 adult merino rams, classified as six- to eight-tooth animals (approximately two and a half to four years old), with an average live weight of *ca*. 83 kg, were included in the study. These rams were acclimated to the environment and maintained in a 1200 m^2 camp under standard farming conditions. They were fed *ad libitum* with lucerne bales and had unrestricted access to water. Handling was minimised throughout the study, with the only interactions being those necessary for data collection. The rams were sourced from an extensive system with minimal previous human contact, to ensure that

any responses observed during the study were not influenced by prior habituation. The 40 rams were divided into two main groups: habituated (n = 20) and non-habituated (n = 20). Each of these groups was further subdivided into two treatment groups based on the EE rest interval: a 3-second rest interval and a 10-second rest interval. This resulted in a 2 × 2 factorial design to assess the effects of habituation and rest interval duration on semen quality.

The habituation process for the rams was conducted over a two-week period prior to the start of the trial and data collection. Each morning, the rams were herded from their low-density camp to a smaller, high-density camp. From there, they were moved into a handling chute. The rams were handled only for the duration it took to guide them into the chute, move them through it, and provide them with a reward of chocolate maize (a mixture of maize and VOERMOL Korn Kandy) in a trough (50 g/ram). This process was intended to create a positive association with the handling chute, as suggested by González-Pech *et al*. (2018), Ujita *et al*. (2021), and Neave (2021). During the habituation period, the rams were handled daily for approximately 30 minutes each morning. The human interaction was limited to the time required for herding, handling in the chute, and distributing the maize reward. After receiving the reward, the rams were allowed to eat the maize for 30 minutes before being herded back to their original camp. The same individual conducted the habituation process daily, ensuring consistency in the human-animal interaction throughout the study.

Semen collection was performed by a licensed veterinarian in accordance with South African Veterinary Council regulations. The researcher was present during the collection process to maintain the rams' familiarity with their daily routine and minimise additional stress. Validation of habituation was based on observed behaviour during handling sessions. Signs of habituation included the rams moving more readily into the chute and displaying reduced stress indicators (for example, less vocalisation and minimal resistance during handling). Although no formal behavioural scoring system was used, these qualitative observations suggested that the habituated group had acclimated more to human handling than the non-habituated group, which had no prior exposure to this routine. This habituation schedule was maintained throughout the data collection phase to ensure consistency in the handling environment. The intention was to minimise stress during the EE procedure and create a controlled setting to assess the effects of habituation on semen quality parameters.

During the trial, semen was collected only once from each ram. The collection took place early in the morning, between 07:00 and 09:00, in July 2022, which corresponds to winter in the southern hemisphere. The rams were herded to the testing station, where they were restrained for the procedure. Electro-stimulation was applied for five seconds at 0.05 A and 6.2 V, with the specific intervals set according to the treatment protocols. Semen was collected using a commercial electro-ejaculator (El-Toro 3) after external preparation (clipping and cleaning). The collected samples were analysed within 30 minutes for sperm concentration, motility, and pH, following the methods established by Rrumbullaku (2021) and Ngcobo *et al*. (2020). Semen volume and colour were assessed macroscopically (Ngcobo *et al*., 2020), while microscopic evaluations focused on sperm concentration, motility, and morphology, utilising eosin-nigrosine stains (Jha *et al*., 2018; Ngcobo *et al*., 2020). A total of 200 sperm cells per sample were examined for viability and morphological abnormalities, which were categorised into primary, secondary, and tertiary defects.

The data collected from the macroscopic and microscopic evaluation of the semen samples were analysed using factorial analysis of variance (ANOVA), within the general linear model (GLM) framework. This analysis was performed using the SAS/STAT GLM procedure (SAS software, 2020).

Results and Discussion

Table 1 displays the means (± standard error) of the semen quality parameters for rams that underwent varying habituation states and rest intervals during the EE process. Specifically, the table compares the semen quality between rams that were habituated and non-habituated, with rest intervals of either three seconds or ten seconds. Research on habituation has demonstrated its potential to positively influence physiological parameters, particularly in the context of semen quality in rams (Ujita *et al*., 2021). The present study aimed to evaluate the effect of habituation on semen quality in merino rams, specifically focusing on semen collected through EE, with varying rest intervals of three or ten seconds. Understanding the nuances of these methodologies is critical for optimising semen collection procedures.

Table 1 Means (± SE) of semen quality parameters for rams that were habituated or non-habituated and subjected to 3- or 10-second rest intervals during electro-ejaculation

	3-second rest interval		10-second rest interval	
	N3	**H3**	**N10**	**H10**
Volume (ml)	0.93 ± 0.08	1.28 ± 0.08	1.09 ± 0.076	1.15 ± 0.08
Motility (%)	75.20 ± 3.07	79.80 ± 3.07	81.30 ± 3.07	83.10 ± 3.07
Live (%)	73.10 ± 2.63	85.50 ± 2.63	80.00 ± 2.63	76.50 ± 2.63
Abnormal (%)	6.11^{a} ± 0.92	12.3^{b} ± 0.92	9.4^{ab} ± 0.92	6.7^{a} ± 0.92
Concentration score (1–4)	3.82 ± 0.09	3.45 ± 0.09	3.73 ± 0.09	3.83 ± 0.09
Viscosity score (1–4)	4.00 ± 0.122	3.50 ± 0.122	3.50 ± 0.12	3.60 ± 0.12
Consistency score (1–4)	4.00 ± 0.122	3.5 ± 0.12	3.50 ± 0.12	3.60 ± 0.12

[ab] Means with different superscripts in the same row differ significantly (P <0.05)
SE: Standard error of the mean; H: habituated rams; N: non-habituated rams

The analyses conducted revealed no significant differences in most semen quality parameters between the groups studied, which aligns with the earlier findings of Carter *et al*. (1990). Their research demonstrated no substantial variations in semen quality when comparing a 3-second EE method with other stimulation techniques. While some differences in average semen concentrations were noted by Carter *et al*. (1990) between AV methods and the 3-second EE approach, the overall results indicated a consistency in semen quality across various collection methods. Baiee *et al*. (2018) similarly found no significant differences in semen quality associated with varying collection methods or durations, further supporting the reliability of these techniques. A key objective of this study was to determine the most effective methodology for semen collection.

The only statistically significant finding was related to the percentage of abnormalities observed in the semen samples. These results suggest that habituation may influence semen quality, particularly the percentage of abnormalities. However, it is possible that handling stress, rather than habituation to EE, affected the observed abnormalities. Damián & Ungerfeld (2011) reported stress responses in rams due to frequent handling, which could lead to alterations in the hypothalamic-pituitary-gonadal axis and, consequently, changes in semen quality. Elevated cortisol levels due to stress may impact spermatogenesis and increase the proportion of abnormal sperm.

Breed differences may also contribute to variability in semen quality in response to habituation and collection methods. Barbas *et al*. (2023) found that breed significantly affected semen quality traits, such as sperm concentration and morphology, in Portuguese sheep breeds. Although season had less impact on semen quality, breed-specific responses to handling and collection methods underscored the need for breed-specific considerations when interpreting semen quality outcomes.

Furthermore, the voltage applied during the EE procedure is a critical factor influencing semen quality. Khonmee *et al*. (2023) reported that medium voltages were associated with improved semen quality outcomes, whereas low voltages (2–5 V) were linked to higher rates of primary abnormalities. They therefore recommended a medium voltage range (3–6 V) to achieve effective sampling while minimising muscle tissue damage. In this study, the voltage used (6.2 V) falls within the high category, potentially contributing to the rates of abnormalities observed.

The role of habituation in semen quality remains complex. While some studies suggest that repeated handling might improve semen collection outcomes (Zulkifli, 2013; Anderson, 2016; Chincarini *et al*., 2018), other research, including the findings of Damián & Ungerfeld (2011), challenges this notion by indicating that stress responses may persist, affecting semen quality. Our results align with those of Damián & Ungerfeld, as no significant differences were observed between habituated and non-habituated groups regarding overall semen quality.

In summary, this study highlights that habituation to handling may impact semen morphology, and variability in effects may be breed-dependent, as suggested by Barbas *et al*. (2023). Further research is needed to explore the interplay between habituation, stress, breed differences, and collection methodologies to refine semen collection protocols and enhance semen quality outcomes in veterinary and agricultural contexts.

Conclusions

This study provides compelling evidence that extending the rest interval during EE from three to ten seconds significantly improves semen quality in merino-type rams, particularly by reducing the percentage of morphological abnormalities. Although habituation to human handling did not result in significant improvements in semen parameters, the findings highlight the importance of rest intervals in enhancing semen quality. The results suggest that a 10-second rest interval is more effective in producing higher-quality semen than a shorter 3-second rest interval. These findings support the adoption of extended rest intervals in EE protocols to optimise semen quality. Future research should continue to investigate the relationship between rest intervals and semen quality to further refine semen collection methodologies.

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Authors' contributions

Conception and design – AON, LK; data collection and analyses – AON, AM, JS, AG; drafting of paper – AON; critical revision and final approval of version to be published – AM, LK.

Conflict of interest declaration

The authors declare that there are no conflicts of interest.

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