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Instant extender for buck semen (Inexb): Impact on semen quality and fertility

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ABSTRACT

The preservation of buck semen plays a crucial role in enhancing reproductive biotechnology in small ruminants. This study evaluated the effectiveness of the Instant Extender for Buck Semen (Inexb) supplemented with different concentrations of organic α-tocopherol on semen quality and in vivo fertility. A completely randomized design with ten replications was applied, consisting of four treatments: Tris egg yolk without α-tocopherol (TFF0) and Tris egg yolk supplemented with 5% (TFF5), 7% (TFF7), and 9% (TFF9) α-tocopherol (v/v). Fresh semen was diluted to a final concentration of 150 × 106 spermatozoa/mL and stored at 5°C for 120 hours. Semen quality parameters, including total motility, progressive motility, plasma membrane integrity, and viability, were evaluated at 24-hour intervals. Data were analyzed using ANOVA followed by Duncan's multiple range test. Results demonstrated that TFF5 and TFF7 maintained significantly higher total motility (44.0 ± 2.2% and 47.2 ± 2.0%) compared with TFF0 and TFF9 after 120 hours ($P < 0.05$). Similar trends were observed in progressive motility, plasma membrane integrity, and viability, indicating that α-tocopherol at moderate concentrations effectively reduced oxidative damage during liquid storage. In vivo fertility trials revealed that semen extended with TFF7 achieved the highest pregnancy rate (33%) after 120 hours of storage at 5°C. These findings indicate that the inclusion of 5–7% α-tocopherol in Inexb provides optimal antioxidant protection, preserves sperm functionality, and sustains fertility during extended storage. Further optimization of extender formulation is recommended to enhance reproductive efficiency and facilitate wider application in goat artificial insemination programs.

Introduction

The quality of liquid and frozen semen is a crucial determinant of artificial insemination (AI) success in livestock production (Leboeuf *et al*., 2000; Barbas *et al*., 2024). Cryopreservation of semen routinely leads to reduced sperm motility and viability, largely as a result of cold shock and excessive reactive oxygen species (ROS) generation during the freeze–thaw cycle (Zhang *et al*., 2021; Yimer and Rosnina., 2019; Bodu *et al*., 2025). In buck semen, oxidative stress arising from excessive reactive oxygen species (ROS) generation can lead to lipid peroxidation, disruption of sperm plasma membranes, and impaired mitochondrial activity, thereby reducing semen quality and fertility potential (An *et al*., 2025; Assar *et al*., 2023). Improving semen extender formulations with antioxidant supplementation has become a primary research focus to mitigate cryodamage and enhance post-thaw sperm quality in various livestock species (Bang *et al*., 2021; Amrozi *et al*., 2024; Ruiz *et al*., 2024).

Various extenders, such as citrate-egg yolk, skim milk, or Tris-based solutions, have been optimized by incorporating natural antioxidants to protect sperm cells during storage (Dorado *et al*., 2007; Bodu *et al*., 2025). The Tris-egg yolk diluent remains the most effective for maintaining sperm viability at low temperatures (Barbas *et al*., 2024; Falchi *et al*., 2022). Its protective capacity is limited when oxidative stress exceeds physiological thresholds, necessitating the addition of antioxidant compounds Pintus, 2021; Basuki and Devitasari, 2022).

Vitamin E, particularly α-tocopherol, is one of the most potent lipophilic antioxidants in biological membranes (Bansal and Bilaspuri, 2008; Zhang *et al*., 2021). It prevents lipid peroxidation by donating a hydrogen atom from its hydroxyl group to neutralize free radicals, forming a stable tocopheroxyl radical (Shahidi *et al*., 2021). This mechanism maintains membrane fluidity and sperm motility, thereby enhancing fertility (Bustani and Baiee, 2021; El-Sheshtawy *et al*., 2020). Several studies have confirmed that supplementing semen extenders with α-tocopherol significantly improves post-thaw motility and membrane integrity in bucks (Astrini *et al*., 2017; Barbas *et al*., 2024; Falchi *et al*., 2022).

Numerous plant-based materials rich in antioxidants have been evaluated for semen preservation, including tomato extract (Situmorang *et al*., 2024), green tea extract (Susilowati *et al*., 2022), resveratrol (Falchi *et al*., 2022), flavonoids (Batool *et al*., 2024), and cysteamine (Mokhtari and Khodaei-Motlagh, 2024). These bioactive compounds possess phenolic, flavonoid, and polyphenol groups that scavenge ROS and protect sperm membranes from oxidative degradation (Siger and Górnaś, 2023). Recent findings demonstrated that natural antioxidants often outperform synthetic ones due to their synergistic effects and biocompatibility (Sharma and Arangasamy, 2021).

The use of fig (*Ficus carica* L.) filtrate has emerged as a promising alternative due to its high content of polyphenols, flavonoids, and vitamin E analogs (Zaenuri *et al*., 2017; Triyani *et al*., 2024; Zaenuri and Rodiah, 2025). Zaenuri and Yuliani (2025) First reported that adding fig filtrate to Tris-egg yolk extender effectively maintained Kacang buck sperm motility for up to 179 hours at 5°C. Triyani *et al*. (2024) found that Boer Cross buck semen diluted in Tris-albumin supplemented with fig filtrate showed significantly improved viability and plasma membrane integrity. The freeze-dried figit-enriched extender developed by Zaenuri and Rodiah, (2025) demonstrated high stability, suggesting potential use as an instant extender for buck semen.

The need for practical and effective AI technologies in goat breeding is increasing (Lukman *et al*., 2023; Zaenuri *et al*., 2023). Developing a ready-to-use extender that maintains sperm quality during storage and transportation is highly desirable. The Instant Extender for Buck Semen (Inexb), formulated with fig fruit filtrate and vitamin E derivatives, may offer an innovative solution to improve sperm motility, viability, and fertility potential (Sharma and Arangasamy, 2021).

This study aimed to evaluate the effects of a freeze-dried Tris-egg yolk extender supplemented with fig fruit filtrate (*Ficus carica* Linn) on sperm motility, progressive motility, plasma membrane integrity, and viability at 5°C. The findings are expected to contribute to the development of an efficient, antioxidant-based, instant extender formulation for buck semen cryopreservation, supporting the sustainability of small ru-

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minant reproductive biotechnology (Yimer and Rosnina, 2019; Barbas and Simões, 2022).

Materials and methods

This study was conducted using an experimental approach to evaluate the effect of fig fruit filtrate (*Ficus carica* L.) as an antioxidant supplement in a freeze-dried Tris-egg yolk extender for Kacang buck semen preservation. The tools used included an artificial vagina, collection tube, Styrofoam box, test tubes, Erlenmeyer flask, pH meter, microscope (Olympus CX43, Japan), centrifuge, syringe, hot plate, magnetic stirrer, and Ohaus scale, while the materials consisted of warm water, Vaseline, tissue, fresh egg yolk, Tris aminomethane ($C_4H_{11}NO_3$), fig filtrate, distilled water, fructose ($C_6H_{12}O_6$), citric acid monohydrate ($C_6H_8O_7$), streptomycin (Wonder, Japan), penicillin G (Wonder, Japan), eosin, nigrosin, sodium citrate, and 70% alcohol (Zaenuri *et al*., 2024; Triyani *et al*., 2024). The semen extender was prepared in three stages following Zaenuri and Rodiah (2025). A mixture of 3.786 g Tris and 2.172 g citric acid was dissolved in 100 mL of distilled water, heated until boiling, and cooled to 37°C before adding 0.06 g penicillin, 0.1 g streptomycin, and 0.625 g fructose. The solution was homogenized using a magnetic stirrer and stored at 5°C until use. The fig fruit filtrate was prepared as described by Zaenuri *et al*. (2014), by washing ripe figs, cutting them into quarters, and blending with distilled water at a 1:1 ratio. The mixture was centrifuged at 3,500 rpm for 30 minutes, and the supernatant was re-centrifuged before filtration through a 0.20 µm membrane and pasteurization at 70°C for 2 minutes. The filtrate was stored at 5°C and used as an extender supplement.

The equivalence of α-tocopherol concentration in fig filtrate to the required concentration in the extender was calculated according to Heidari *et al*. (2022) and (Zaenuri and Yuliani, 2025). The molecular weight of α-tocopherol is 430.7 g/mol, corresponding to 1 mM = 0.4307 mg/mL. The average α-tocopherol content in figs is 0.081 mg per 100 g of fruit, with a water content of 35.91 mL per 100 g. After dilution with distilled water (1:1), the concentration was estimated at 0.05695 mg per 100 mL. To achieve the optimal antioxidant concentration of 0.04 mg α-tocopherol per 100 mL extender, 7 mL of fig filtrate was added per 100 mL extender. The freeze-dried extender was formulated by mixing Tris buffer with 2.5% (v/v) fresh egg yolk to form the control extender (TFF0), while treatment extenders (TFF5, TFF7, TFF9) were supplemented with 5%, 7%, and 9% (v/v) fig filtrate, respectively. All extenders were freeze-dried for 48 hours and stored at 5°C until use, forming the Instant Extender for Buck Semen (Inexb)(Zaenuri and Rodiah, 2025).

A healthy, sexually mature 2-year-old Kacang buck weighing approximately 45 kg was used as a semen donor. Ejaculates were collected every six days using an artificial vagina and immediately evaluated for volume, concentration, and motility. Sperm concentration was determined using a hemocytometer and only ejaculates with ≥2×10^9 spermatozoa/mL, mass motility ≥3 (+++), and individual motility ≥80% were used (Zaenuri *et al*., 2023). The freeze-dried extenders (TFF0, TFF5, TFF7, and TFF9) were reconstituted with distilled water before use. Each semen sample was diluted to 150×10^6 spermatozoa/mL and stored at 5°C. Evaluations were conducted at 0, 24, 48, 72, 96, and 120 hours for total motility (TM), progressive motility (PM), plasma membrane integrity (PMI), and viability (V) following the method of Sharma and Arangasamy (2021).

Fifteen healthy does were used for estrus synchronization and artificial insemination (AI). Estrus was synchronized using controlled internal drug release (CIDR) devices containing 45 mg fluorogestone acetate for 12 days. AI was performed 48–50 hours after CIDR withdrawal using intra-cervical insemination with 200×10^6 spermatozoa per 0.25 mL dose. Pregnancy was confirmed by ultrasonography on day 47 post-insemination (Lukman *et al*., 2023). All animal handling and experimental procedures were conducted following the ethical principles for animal research and approved by the Ethical Committee for Animal Research, University of Mataram (Approval No. 12/UN18.F2/EC/2024). Data were analyzed using one-way ANOVA in a completely randomized design (CRD) with CoStat software version 6.303, and results were expressed as Mean ± SE. Differences among treatments were considered statistically significant at $P < 0.05$ ((Steel and Torrie, 1993).

Results

The evaluation of fresh semen from Kacang goats is summarized in (Table 1). The macroscopic and microscopic assessments revealed that the average semen volume was 0.97 ± 0.04 mL, with a pH of 6.98 ± 0.04, mass motility of +++, individual motility of 89.6 ± 3.4%, and a sperm concentration of 2.67 × 10^6 cells/mL. These values confirmed that the fresh semen met the minimum quality requirements for further dilution and cryopreservation.

Table 1. Macroscopic and microscopic characteristics of the Kacang goat semen.

Evaluation	Average
Volume (ml)	0.97±0.04
Consistency	Medium
Color	Cream
Acidity (pH)	6.98±0.04
Mass Motility	+++
Total motility (%)	89.6 ± 3.4
Concentration/ml ×10^9	2.97

Table 1. Macroscopic and microscopic characteristics of the Kacang goat semen.

Treatments[1]	Stored Duration at 5°C (hours)					
	0	24	48	72	96	120
	Total Sperm Motility					
TFF0	80.6±1.8^a	68,4±1.7^a	60.6±2,6^a	46.4±1.1^a	44.4±1,3^a	38.4±1.2^a
TFF5	82.8±3.7^a	68.8±1.6^a	61.4±1.1^a	49.0±2.1^a	48.0±2.3^{ab}	44.0±2.2^b
TFF7	82.0±1.6^a	72.8±1.3^a	66.0±3.1^b	57.2±1.1^b	55.2±2.1^b	47.2±2.1^b
TFF9	80.2±3.5^a	72.8±2.7^a	63.8±1.3^{ab}	47.4±1.5^a	44.2±1.2^a	39.1±1.1^a
	Progressively Motile Sperm					
TFF0	78.6±1.3^a	70.4±1.5^a	58.6±1.4^a	44.4±1.1^a	38.2±1.1^a	32.4±0.4^a
TFF5	80.8±2.7^a	68.8±1.3^a	59.4±1.3^a	46.0±1.3^a	44.0±2.1^b	38.0±1.5^b
TFF7	80.0±1.2^a	70.8±1.5^a	62.0±2.2^a	48.2±2.1^a	45.2±1.1^b	41.2±1.1^b
TFF9	78.2±2.5^a	67.8±1.7^a	60.8±2.5^a	45.4±1.8^a	39.4±0.8^a	30.4±0.8^a

[1]Tris egg yolk without organic alpha-tocopherol supplementation (TFF0), and TFF0 supplemented with 5% (TFF5), 7% (TFF7), and 9% (TFF9).

Table 3. The effect of instant extender for Buck (Inexb) semen on the sperm plasma membrane integrity and sperm viability.

Treatments[1]	Stored Duration at 5°C (hours)					
	0	24	48	72	96	120
	Sperm Plasma Membrane Integrity					
TFF0	82.2±3.7[a]	76.2±1.7[b]	65.2±1.6[a]	64.1±1.1[a]	51.3±5.3[a]	48.3±1.3[a]
TFF5	86.4±4.7[a]	74.2±2.2[a]	69.0±1.7[a]	65.4±1.4[b]	60.6±4.7[b]	55.6±4.7[b]
TFF7	86.0±2.6[a]	74.2±1.6[b]	70.12±1.7[b]	63.6±1.5[b]	61.8±5.4[b]	54.4±3.1[b]
TFF9	89.4±1.2[a]	72.0±3.4[a]	68.0±2.0[b]	57.2±3.1[a]	52.1±5.1[a]	45.1±5.1[a]
	Sperm Viability					
TFF0	85.2±3.7[a]	78.2±3.7[a]	64.2±1.6[a]	56.0±2.4[a]	52.3±5.3[a]	42.3±5.3[a]
TFF5	87.4±4.7[a]	79.2±7.2[a]	77.0±1.7[a]	58.4±3.4[a]	54.6±4.7[a]	49.6±4.7[b]
TFF7	85.0±2.6[a]	78.2±6.3[a]	74.2±1.4[a]	59.6±1.5[a]	55.8±5.4[a]	48.8±5.4[b]
TFF9	85.4±1.2[a]	76.0±5.4[a]	66.0±2.0[a]	54.8±3.1[a]	53.1±5.1[a]	42.1±5.1[a]

[1]Tris egg yolk without organic alpha-tocopherol supplementation (TFF0), and TFF0 supplemented with 5% (TFF5), 7% (TFF7), and 9% (TFF9).

Following dilution with Tris-fig filtrate (TFF) extenders and storage at 5°C, a gradual decline in total and progressive motility was observed over the 120-hour preservation period (Table 2). However, semen treated with TFF5 and TFF7 maintained significantly higher ($P < 0.05$) total motility after 120 hours (44.0 ± 2.2% and 47.2 ± 2.1%, respectively) compared to TFF0 (38.4 ± 1.2%) and TFF9 (39.1 ± 1.1%). Progressive motility also showed a similar trend, where TFF7 preserved the highest values at 96 hours (45.2 ± 1.1%) and 120 hours (41.2 ± 1.1%), followed by TFF5 and TFF9.

The plasma membrane integrity (PMI) results (Table 3) indicated that TFF5 (35.6 ± 4.7%) and TFF7 (34.4 ± 3.1%) exhibited significantly greater ($P < 0.05$) membrane integrity at 120 hours compared with TFF0 (28.3 ± 1.3%) and TFF9 (25.1 ± 5.1%). The pattern was consistent with sperm viability, where extenders containing moderate concentrations of fig filtrate (TFF5 and TFF7) maintained higher viable sperm percentages than the control and the highest supplementation level.

Furthermore, the in vivo fertility test supported these in vitro findings. Semen extended with TFF7 yielded the highest pregnancy rate (33%, n = 15) after 120 hours of cold storage, outperforming the other treatments. These results indicate that moderate supplementation of fig filtrate (5–7%) in Tris-albumin extender effectively preserves sperm motility, membrane integrity, and fertility potential during short-term storage at 5°C.

Discussion

The fresh Kacang goat semen examined in this study showed normal characteristics for volume, pH, motility, and sperm concentration, which indicates that it is appropriate for further preservation processes. Similar physiological semen ranges in goats have been noted by Astrini *et al.* (2017), who highlighted the influence of genetics, nutrition, and environmental conditions. Comparative studies also show that semen quality of local Indonesian goats varies among breeds, with Sapera goats demonstrating comparable motility and viability characteristics (Prastiya *et al*., 2021), while Peranakan Etawah goats often display slightly higher reproductive performance (Ardiansyah *et al*., 2022). Findings from Boer goats preserved under different antioxidant systems also provide supportive evidence for the biological consistency of goat semen quality (Dorado *et al*., 2007; Bang *et al*., 2021). These patterns align with broader evaluations of semen traits in small ruminants, including those reported by Yimer and Rosnina (2019) and Leboeuf *et al*. (2000), suggesting that Kacang goat semen maintains a reproductive profile comparable to both regional and international breeds.

A decrease in total motility during storage at 5°C is a natural consequence of metabolic slowdown and increasing oxidative stress within sperm cells. Reduced mitochondrial activity and the accumulation of reactive oxygen species during cold storage have been documented in broader cryobiology studies, including those addressing cooled semen physiology (Zhang *et al*., 2021). Supplementation of extenders with α-tocopherol at 5 percent (TFF5) and 7 percent (TFF7) in the present work helped reduce this motility decline. This pattern is consistent with (Basuki and Devitasari, 2022), who reported that antioxidant-rich media stabilize sperm membranes and improve preservation outcomes. Similar benefits of vitamin E have been described in various species, where α-tocopherol enhances motility and viability by suppressing oxidative damage (Bansal and Bilaspuri, 2008; Astrini *et al*., 2017).

The role of α-tocopherol in protecting the sperm plasma membrane is strongly tied to its function as a lipid-soluble antioxidant. The higher membrane integrity observed in TFF5 and TFF7 is consistent with previous findings showing that vitamin E prevents oxidation of polyunsaturated fatty acids, which make up a substantial portion of sperm membrane lipids (Shahidi *et al*., 2021; Siger and Górnaś, 2023). Comparable improvements in structural stability and reductions in lipid peroxidation were noted by El-Sheshtawy *et al.* (2020), who demonstrated that vitamin E, particularly when combined with minerals such as selenium, can prolong the functional lifespan of preserved semen.

The concentration of α-tocopherol plays a decisive role in determining extender performance. The reduced effectiveness observed in TFF9 suggests that excessively high antioxidant concentrations may shift toward pro-oxidant behavior, a mechanism previously described by Zaenuri *et al.* (2017) in natural antioxidant systems. Batool *et al.* (2024) also highlighted that imbalance in antioxidant levels can disturb redox homeostasis and damage sperm structures. Similarly, Falchi *et al.* (2022) reported a decline in sperm quality when antioxidant concentrations exceed optimal thresholds. These findings demonstrate that α-tocopherol must be used within an optimal dosage range to maintain its protective benefits.

Higher sperm viability in TFF5 and TFF7 treatments indicates that α-tocopherol helps suppress cellular injury during storage by controlling reactive oxygen species. Protective effects of natural antioxidants on sperm viability are also reflected in studies utilizing plant-derived bioactive compounds, such as tomato extract, which improved sperm membrane stability under storage (Situmorang *et al*., 2024). This trend aligns with reports showing that antioxidant-rich extenders generally enhance sperm survival (Susilowati *et al*., 2022). Additional support comes from Mokhtari and Khodaei-Motlagh (2024), who found that cysteamine sourced from plant-based systems also improved post-thaw fertility in buck semen.

The biological effectiveness of TFF7 was further supported by a 33 percent pregnancy rate. Although this value is lower than conception rates achieved using optimized antioxidant-based extenders in other studies (Díaz Ruiz *et al*., 2024), it still demonstrates functional fertility after extended storage. The ability of semen preserved under Inexb-based conditions to maintain acrosome and motility characteristics for long storage durations has also been documented by Sharma and Arangasamy, (2021), reinforcing the practical potential of this extender system

under field conditions.

Positive effects of natural antioxidants on semen quality are further reflected in the use of fig fruit filtrate. Zaenuri *et al*. (2017) demonstrated that fig filtrate, which contains natural α-tocopherol, supported motility and viability of preserved buck sperm. Synergistic antioxidant effects were also noted by Susilowati *et al*. (2022), who demonstrated improved sperm endurance from combinations of hydrophilic and lipophilic antioxidants. Tomato extract, rich in lycopene, showed similar benefits, further emphasizing the importance of natural antioxidants in reproductive preservation systems (Situmorang *et al*., 2024).

The suitability of Inexb as a semen preservation medium is strengthened by its balanced physiological composition, which maintains energy supply, osmotic stability, and pH balance. Barbas and Simões (2022) emphasized that extenders must maintain equilibrium between buffering systems, energy substrates, and antioxidants to prevent rapid cellular decline. Comparable success has been observed with fig-filtrate–enriched Tris-based extenders that maintained sperm quality during storage (Triyani *et al*., 2024). The effectiveness of cooled-semen extender formulations is also supported by Lukman *et al*. (2023), who demonstrated promising artificial insemination outcomes using preserved semen under field conditions.

Overall, α-tocopherol supplementation in Inexb extender effectively slows cellular deterioration, protects membrane integrity, and helps sustain fertilization potential. Evidence from vitamin E–enhanced reproductive systems indicates its central role in reducing oxidative damage and supporting membrane function (Bustani and Baiee, 2021). Studies by Falchi *et al*., (2022) and Zaenuri and Yuliani, (2025) further demonstrated that preserved sperm viability is strongly associated with fertilization success. The present findings support the development of α-tocopherol–enriched Inexb extender as a practical alternative for improving artificial insemination efficiency in local goat production.

Conclusion

Tris-egg yolk extender with Fig Fruit Filtrate and freeze-dried maintained its effectiveness in preserving goat sperm quality, with conception rates consistent with prior studies. This research encourages further exploration to find the best formula, possibly as an instant extender (Inexb).

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Conflict of interest

The authors have no financial or personal conflicts of interest related to this paper.

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