REDUCED MOTILITY, FERTILITY, AND FERTILE PERIOD OF KAMPUNG ROOSTER SPERM FOLLOWING ROOM-TEMPERATURE STORAGE IN A SKIM MILK–GLUCOSE EXTENDER

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Abstract. Artificial insemination (AI) is a vital reproductive technology for improving poultry fertility and productivity. However, semen quality deteriorates rapidly at tropical ambient temperatures. This study investigated the effects of short-term room-temperature storage in a skim milk–glucose extender on motility, fertility, and fertile period of Kampung rooster sperm. Semen was collected from ten 15-month-old roosters and inseminated into thirty hens following three storage durations: P_0 = 0–10 min, P_1 = 30–40 min, and P_2 = 60–70 min. Sperm motility (%) remained similar between P_0 (85.0 ± 0.0) and P_1 (79.0 ± 3.33) but declined sharply after 60 min at P_2 (60.0 ± 3.33; $P < 0.05$). Fertility (%) showed a comparable pattern—no difference between P_0 (82.25 ± 8.12) and P_1 (76.42 ± 7.63), yet a significant reduction at P_2 (60.25 ± 9.61; $P < 0.05$). The fertile period was shortened only after 60 min of storage. These results indicate that rooster semen can be safely used within 40 min at room temperature, but longer exposure markedly reduces fertility potential.

Keywords: Kampung rooster, semen storage, skim milk–glucose extender, sperm motility, fertility, fertile period

1. Introduction

Artificial insemination (AI) is a key tool to improve genetic progress and reproductive efficiency in poultry. Its success depends primarily on semen quality after collection and handling. In tropical climates, ambient temperatures accelerate sperm metabolism and oxidative stress, causing rapid declines in motility and viability [1,2].

Skim milk-based diluents are widely used due to their buffering capacity and protective proteins that stabilize sperm membranes [3]. Supplementation with glucose supplies an energy source for ATP production and sustains motility [4]. However, prolonged exposure at room temperature leads to ATP depletion and reactive oxygen species (ROS) generation that impair cell membranes and DNA [5,6].

Although cold storage (4–5 °C) is effective for slowing metabolism [7], it requires refrigeration not always available to smallholders. In rural AI practices, semen is often held at ambient temperature for 30–60 minutes before insemination—conditions likely to compromise fertility [8]. Yet, data on the rate of deterioration under such conditions are limited, especially in native breeds.

The Kampung rooster (*Gallus gallus domesticus*) represents an important genetic resource in Indonesia, adapted to tropical conditions but underutilized in reproductive studies. Since breed-specific differences influence sperm physiology [9], understanding its semen preservation behavior is crucial for developing practical AI protocols.

This study evaluated motility, fertility, and fertile period of Kampung rooster semen stored up to 70 minutes at room temperature in a skim milk–glucose extender. The results provide applied insight into time-sensitive management of semen handling in tropical AI programs.

2. Methods

Animals and semen collection

Ten healthy Kampung roosters (15 months old, 2.5–3 kg) and thirty hens were used. Birds were maintained under standard feeding and lighting conditions (14 h light:10 h dark). Semen was collected by the dorso-abdominal massage method [10].

Semen evaluation and dilution

Motility of fresh sperm was measured by observing it under a microscope at 400x magnification. The assessment was carried out by comparing the number of motile sperm to overall sperm, and converting the result into percentage. Semen samples with ≥ 85 % motility were pooled and diluted 1:2 (v/v) in a skim milk–glucose extender (10 % skim milk + 0.4 % glucose + 100 IU/mL penicillin + 100 µg/mL streptomycin) at 25 °C.

Diluted semen was divided into three groups based on storage duration before insemination:

- **P_0:** 0–10 min
- **P_1:** 30–40 min
- **P_2:** 60–70 min

Each of the treatment groups contained 10 hens, hence each treatment groups had 10 replications.

Artificial insemination and fertility assessment

Each hen received 0.2 mL diluted semen (≈ 100 × 10^6 sperm/hen) intravaginally. Eggs were collected daily for 14 days post-AI, incubated for 7 days, and candled to determine fertility. Fertile eggs were confirmed microscopically after egg breaking [11]. The fertile period was defined as the number of consecutive days with fertile eggs after insemination.

Data analysis

All data were expressed as mean ± SD. One-way ANOVA was performed, followed by Duncan's test for mean separation ($P < 0.05$).

3. Results And Discussion

Results

Table 1 presents motility, fertility, and fertile period under different storage durations. Motility remained stable between P_0 and P_1 but decreased significantly at P_2 ($P < 0.05$). Fertility exhibited the same pattern. The fertile period showed a mild decline at P_1 and a significant decrease at P_2.

Table 1. Mean (±SD) values of sperm motility, fertility, and fertile period of Kampung rooster semen during room-temperature storage in a skim milk–glucose extender

Parameter	P_0 (0–10 min)	P_1 (30–40 min)	P_2 (60–70 min)	P-value
Motility (%)	85.0 ± 0.0^{a}	79.0 ± 3.33^{a}	60.0 ± 3.33^{b}	< 0.05
Fertility (%)	82.25 ± 8.12^{a}	76.42 ± 7.63^{a}	60.25 ± 9.61^{b}	< 0.05
Fertile period (days)	12.50 ± 2.10^{a}	10.83 ± 2.42^{ab}	8.76 ± 2.65^{b}	< 0.05

Different superscripts within a row indicate significant differences ($P < 0.05$). Treatments with the same superscript (a) are not significantly different ($P_0 = P_1$), whereas values with different letters differ significantly ($P_1 \neq P_2$; $P_0 \neq P_2$).

Discussion

Short-term storage at room temperature affected sperm quality depending on duration. The absence of significant differences between P_0 and P_1 indicates that spermatozoa remained metabolically stable up to 40 minutes. However, after 60 minutes (P_2), motility and fertility declined significantly, consistent with increased oxidative stress and ATP depletion under tropical conditions [12,13].

Glucose in the extender initially supported motility by sustaining glycolytic energy production [14], but prolonged exposure led to excessive metabolism and ROS accumulation, causing membrane and acrosomal damage [15,16]. The nearest similar findings were reported by Gitayana et al. [17], diluting kampung roster sperm with egg-yolk saline extender, showing that rooster sperm diluted with 10% egg-yolk saline extender stored at 5°C rapidly loses motility from 85.8 to 79.0 percent beyond 45 minutes. This happened because egg yolk gave the sperm a good source of energy and provided a membrane-protection environment.

The fertile period results reflect sperm survival in the female oviduct. Reduced fertility after 60 minutes implies diminished capacity for sperm storage tubule colonization or shortened lifespan after insemination [18,19]. These physiological constraints highlight the need to minimize handling time under field conditions.

Practical Implications

The present study demonstrates that Kampung rooster semen can be safely stored for up to **40 minutes** at room temperature without significant fertility loss. Beyond 60 minutes, both motility and fertility decline sharply. For field applications, insemination should therefore be completed **within 30–40 minutes after collection** when refrigeration is unavailable. This provides a practical reference for village-level AI programs in tropical climates.

4. Conclusion

Room-temperature storage for more than 60 minutes in a skim milk–glucose extender significantly reduced motility, fertility, and fertile period of Kampung rooster semen. Semen handling should be limited to 40 minutes to maintain optimal fertility in tropical AI practices.

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Conflict of Interest

The authors declare no conflict of interest.

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