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# Comparative Analysis of Various Extenders for Chicken Semen Preservation and its Effects on Hatchability and Post-Hatch Performance of Fayoumi Chicken

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**Key words**
**Extenders, Fayoumi, Hatchability, Post hatch performance, Semen**

## ABSTRACT

This study investigated the effects of different extenders on chicken semen preservation and their impact on hatchability and post-hatch performance in Fayoumi chickens. Twenty-five males and two hundred fifty females were divided into five treatment groups: T1 (Fresh), T2 (GP extender for 15 min), T3 (AD2E for 24, 48, and 72 h), T4 (AD2E+Pomegranate juice for 24, 48, and 72 h), and T5 (Garlic extract for 1, 2, and 3 h). Initial assessments of fresh semen revealed promising parameters: semen volume (0.43ml), semen state (fresh), semen pH (7.44), sperm motility (75.63%), wave motion (4+), sperm viability (80.44%), membrane integrity (82.39%), morphology (82.82%) acrosomal integrity (90.64%), and sperm concentration (2990 ± 99 M/ml). Furthermore, fertility rates ranged from 21.06% to 78.44%, chick hatchability from 17.76% to 78.51%, and embryonic mortality from 21.71% to 81.27% in respective treated groups at various intervals, all showing significant differences among treatment groups. Post-hatch performance parameters such as chick abnormalities, day-old chick weight (ranging from 42.20g to 39.32g), weekly relative body weight (ranging from 210.00g to 180.00g), chick length (ranging from 18.00cm to 20.34cm), mortality (ranging from 3.10% to 3.80%), morbidity (ranging from 7.20% to 8.40%), and chick welfare were also evaluated in various treated groups. Results showed variations across treatment groups and storage durations, highlighting the importance of selecting appropriate semen extenders and storage methods to preserve reproductive success and post-hatch chick welfare in the Fayomi breed.



## INTRODUCTION

Avian semen preservation is essential for maintaining fertility in poultry production, requiring effective extenders to sustain sperm viability (Long, 2006). While extensive research exists on chicken semen extenders, there is limited information regarding semen extenders for other poultry species, highlighting a critical gap in knowledge (Blesbois *et al*., 2007). Avian sperm, characterized by a unique morphology, are susceptible to damage during cryopreservation due to their filiform structure and low intracytoplasmic water content, posing challenges for semen preservation (Iaffaldano *et al*., 2005). The poultry industry's increasing demand for high-quality semen, driven by the adoption of artificial insemination for enhanced production efficiency, underscores the importance of investigating and optimizing semen preservation methods to ensure reproductive success (Siudzinska and Lukaszewicz, 2008).

Artificial insemination (AI) serves as a cornerstone in poultry production, facilitating the widespread use of genetically superior roosters to boost productivity and genetic enhancement (Getachew, 2016). However, challenges such as declining fertility rates and the unclear significance of prolonged sperm storage in birds persist, impacting the effectiveness of AI techniques (Khillare *et al*., 2018). Factors influencing AI success, including breeder stock management, semen quality, and dosage, necessitate comprehensive research to optimize breeding strategies and ensure optimal reproductive outcomes

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(Saleh *et al*., 2012).

The utilization of preserved semen in poultry production has garnered significant interest due to its potential impact on reproductive efficiency and post-hatch performance of chicks (Smith *et al*., 2018). Preservation techniques such as cryopreservation and refrigeration offer opportunities for extending storage life, facilitating AI, and enabling genetic improvement programs (Jones *et al*., 2020). However, the effects of preserved semen on embryo quality, chick viability, and subsequent performance remain under investigation, highlighting the need for further research in this area to optimize semen preservation techniques (Rashid *et al*., 2005).

This study aims to address these gaps by investigating the impact of preserved semen on post-hatch chick performance, including hatchability, chick quality, growth performance, and mortality rates. By analyzing the outcomes of artificial insemination using preserved semen and comparing them with those of fresh semen, valuable insights into the benefits and challenges associated with semen preservation techniques in poultry production will be provided. This research is crucial for improving overall flock health, maximizing productivity, and ensuring sustainability in poultry production systems.

## MATERIALS AND METHODS

*Experimental design*

The experimental trial was conducted at the Department of Poultry Husbandry, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam with the approval of supervisory committee. Local Fayoumi breed chickens were individually housed for two weeks before semen collection and insemination. Twenty-five males and two hundred fifty females were divided into five treatment groups i.e. T1= Fresh semen with no extender, T2= Grand pharma (GP) extender (10%) use for 15 min, T3= Aldaraji diluents 2 (AD2E) (Sodium citrate, 2.94 g/L; glucose, 0.5 g/L; egg yolk, 20%; penicillin, 100,000 IU/L; streptomycin, 100,000 IU/L), T4= Aldaraji diluents 2 (AD2E with pomegranate juice, 10%) and T5= Garlic extender (% garlic) with each group consisting of five males and fifty females.

*Processing, preservation, and thawing of semen*

Semen was collected from 20 to 30-week old Fayoumi cocks using the abdominal massage method. The collected semen was immediately evaluated for quality parameters, including motility and absence of fecal contamination. Only ejaculates with at least 60% motility were used for further processing.

For groups requiring thawing, such as T3 and T4 (stored for 24, 48, and 72 h), the semen samples were thawed at 37°C for a few minutes before insemination. The thawing process was conducted with gentle handling to preserve sperm viability and motility.

*Insemination and egg collection*

Thawed semen samples were gently inseminated into the oviducts of healthy hens using gentle pressure. The inseminated hens were then managed under standard conditions.

Eggs were collected daily post-insemination, stored at 18-20°C for five days, and then transferred to the hatchery for incubation. Eggs were incubated under appropriate temperature (98.5°F) and humidity (50-55%) conditions. After hatching, day-old chicks were transferred to a brooding facility with suitable temperature, nutrition, water, space, and management practices.

*Parameters of study*

Semen volume, semen state, semen pH, sperm motility, wave motion, sperm viability, membrane integrity, morphology, acrosomal integrity, sperm concentration, fertility rates, chick hatchability, embryonic mortality, chick abnormalities (reflex, appearance, eyes, gait score, naval area, yolk sac absorption), weight of day old chick, weekly relative body weight, chick length, mortality, morbidity, and welfare indicators, were assessed.

*Statistical analysis*

The study used statistical analysis, employing ANOVA to compare means across multiple groups. A post-hoc LSD test was then applied to identify significant differences between individual group.

## RESULTS

*Qualitative and quantitative parameters of fresh Fayoumi semen*

Observations of various parameters in fresh semen were analysed. Each bird ejaculated an average semen volume of 0.43 ± 0.10 ml, with a pH of 7.44 ± 0.45 and sperm motility rate of 75.63 ± 0.89%. The wave motion score for semen was recorded as 4+, and the viability of fresh semen was 80.44 ± 0.78%. Additionally, the average acrosomal integrity was 90.64 ± 0.60%, the membrane integrity was 82.39 ± 0.67%, sperm concentration was 2990 ± 99 M/ml, and sperm morphology was observed at 82.82 ± 0.23%.

Figure 1 shows the effect of various extenders on the pH, motility, sperm viability, plasma membrane integrity,

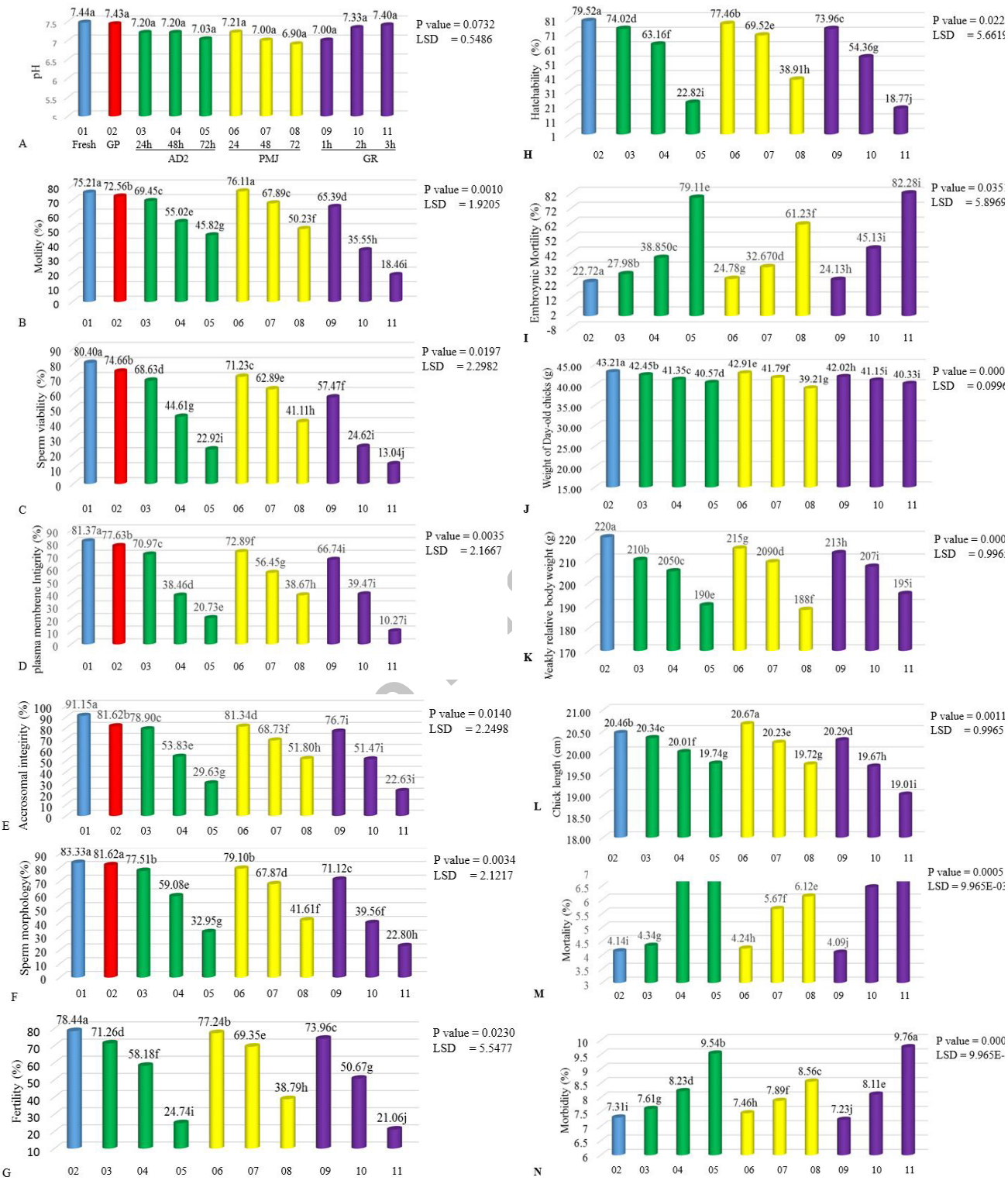


Fig. 1. Effect of various extenders on the pH (A), motility (B), sperm viability (C), plasma membrane integrity (D), acrosomal integrity (E), sperm morphology (F), fertility (G), chick hachbility (H), embryonic mortality (I), weight of day old chick (J), weekly relative body weight (K), chick length (L), mortality (M), and morbidity (N) of semen of the Fayomi rooster breed.

acrosomal integrity, sperm morphology, fertility, chick hachbility, embryonic mortality, weight of day old chick, weekly relative body weight, chick length, mortality, and morbidity of semen of the Fayoumi rooster breed. The pH of fresh semen was 7.47. With the GP extender, it remained similar at 7.43. For the AD2 (alone) extender, pH decreased to 7.20, 7.20, and 7.03 at 24, 48, and 72 h, respectively. For the AD2 (PMJ) extender, pH decreased to 7.21, 7.00, and 6.90 at 24, 48, and 72 h, respectively. In the garlic extract group, pH increased over time to 7.00, 7.33, and 7.40 at 1, 2, and 3 h, respectively. These changes were statistically non-significant ($P>0.05$).

Fresh semen had a motility rate of 75.21%, which decreased to 72.56% with the GP extender. With the AD2 (alone) extender, motility dropped significantly to 69.45%, 55.02%, and 45.82% at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 76.11%, 67.89%, and 50.23% at the same time points. In the garlic extract group, motility dropped significantly to 65.82%, 35.55%, and 18.46% at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

Fresh semen viability was 80.40%, decreasing to 74.66% with the GP extender. With AD2 (alone), viability declined significantly to 68.63%, 44.61%, and 22.92% at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 71.23%, 62.89%, and 41.11% at the same time points. The garlic extract group saw viability drop to 57.47%, 24.62%, and 13.04% at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

Plasma membrane integrity in fresh semen was 81.37%, which decreased to 77.63% with the GP extender. For AD2 (alone), it decreased significantly to 70.97%, 38.46%, and 20.73% at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 72.89%, 56.45%, and 38.67% at the same time points. The garlic extract group showed a significant decline to 66.74%, 39.47%, and 10.27% at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

Acrosomal integrity in fresh semen was 91.15%, which decreased to 81.62% with the GP extender. For AD2 (alone), it decreased significantly to 78.90%, 53.83%, and 29.63% at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 81.34%, 68.73%, and 51.80% at the same time points. In the garlic extract group, it dropped significantly to 76.70%, 51.47%, and 22.63% at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

Sperm morphology in fresh semen was 83.33%, which decreased to 81.62% with the GP extender. With AD2 (alone), it declined significantly to 77.51%, 59.08%, and 32.95% at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 79.10%, 67.87%, and 41.61% at the same time points. The garlic extract group showed a significant decline to 71.12%, 39.56%, and 22.80% at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

*Embryonic mortality*

Embryonic mortality with the GP extender was 22.72%. For AD2 (alone), mortality increased significantly to 28.11%, 38.85%, and 79.21% at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed an increase to 24.78%, 32.67%, and 61.23% at the same time points. The garlic extract group showed significant increases to 24.13%, 45.13%, and 82.28% at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

*Egg fertility*

Fertility with the GP extender was 78.44%. For AD2 (alone), fertility decreased significantly to 71.26%, 58.18%, and 24.74% at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 77.24%, 69.35%, and 38.79% at the same time points. The garlic extract group showed significant decreases to 73.96%, 50.67%, and 21.06% at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

*Chick hatchability and abnormalities*

Chick hatchability with the GP extender was 79.52%. For AD2 (alone), hatchability decreased significantly to 74.02%, 63.16%, and 22.82% at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 77.46%, 69.52%, and 38.81% at the same time points. The garlic extract group showed significant decreases to 73.96%, 54.36%, and 18.77% at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

The effect of various extenders on post hatch performance was observed and various abnormalities in chick were observed as presented. In chick abnormalities, chick reflex, appearance, eyes, gait, naval area and yolk sac were observed in all treated groups. It was further observed that in AD2 (alone) extender group, chick reflex, appearance, eyes, gait, naval area and yolk sac were active, normal complete normal, completely hell and completely absorbed respectably on 24 h, 48 h and 72 h, respectively. Similar results were observed in GP extender, AD2 (PMJ) extender and garlic extract treated group at various intervals.

*Chick body weight and dimensions*

Day-old chick weight with the GP extender was 43.21g. For AD2 (alone), weight decreased significantly to 42.45g, 41.35g, and 40.57g at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 42.91g, 41.79g, and 39.21g at the same time points. The garlic extract group showed significant decreases to 42.02g, 41.15g, and 40.33g at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

Weekly relative body weight with the GP extender was 220.00g. For AD2 (alone), weight decreased significantly to 210.00g, 205.00g, and 190.00g at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 215.00g, 209.00g, and 188.00g at the same time points. The garlic extract group showed significant decreases to 213.00g, 207.00g, and 195.00g at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

Chick length with the GP extender was 20.34cm. For AD2 (alone), length decreased significantly to 20.46cm, 20.01cm, and 19.74cm at 24, 48, and 72 h, respectively. The AD2 (PMJ) extender showed a decrease to 20.30cm, 20.15cm, and 19.56cm at the same time points. The garlic extract group showed significant decreases to 20.08cm, 20.01cm, and 19.83cm at 1, 2, and 3 h, respectively. Significant differences ($P<0.05$) were observed among treatment groups.

*Chick mortality and morbidity rates*

GP extender had a mortality rate of 4.14%. Mortality rates in AD2 (alone) extenders were 4.34%, 6.78%, and 7.25% at 24, 48, and 72 h, respectively. For AD2 (PMJ), the rates were 4.24%, 5.67%, and 6.12% at the same time points. Garlic extract showed mortality rates of 4.09%, 6.45%, and 7.77% at 1, 2, and 3 h, respectively. A significant increase in mortality was observed with extended storage in all treatments ($P < 0.05$).

GP extender resulted in a morbidity rate of 7.31%. In AD2 (alone), morbidity rates were 7.61%, 8.23%, and 9.54% at 24, 48, and 72 h, respectively. AD2 (PMJ) extenders had rates of 7.46%, 7.89%, and 8.56%. Garlic extract resulted in morbidity rates of 7.42%, 8.11%, and 9.76% at 1, 2, and 3 h, respectively. Morbidity increased with storage duration across all treatments ($P < 0.05$).

*Chick welfare*

The effect of various extenders on post hatch performance was observed and chick welfare was observed as presented. In chick welfare, ruffled feather, lameness and cannibalism were observed in all treated groups. It was further observed that in AD2 (alone) extender, in ruffled feather, lameness and cannibalism, no lesion, minor lesion and ulcerative lesion were observed on 24 h, 48 h and 72 h, respectively. Similar results were observed in GP extender, AD2 (PMJ) extender and garlic extract at various intervals.

## DISCUSSION

Extenders play a crucial role in preserving the quality of semen by providing an optimal environment for sperm cells during storage. The choice of extender can significantly influence both qualitative and quantitative traits of Fayoumi cock semen, including volume, pH, motility, viability, membrane integrity, acrosomal integrity, and morphology. Extenders often include buffering agents to maintain the pH within a range that supports sperm viability and function. For instance, tris-citrate and phosphate buffers are commonly used to stabilize pH and prevent shifts that could harm sperm cells (Amann and Katz, 2014). Extenders containing antioxidants, such as vitamin C and E, can improve sperm motility by reducing oxidative stress. The addition of energy sources like glucose or fructose provides the necessary substrates for sperm metabolism, enhancing motility (Chalah *et al*., 2019). The inclusion of cryoprotectants like glycerol or dimethyl sulfoxide (DMSO) in extenders helps to protect sperm cells from damage during the freezing and thawing processes, thus maintaining higher viability rates (Kumaresan *et al*., 2017). Lecithin-based extenders have been shown to protect the sperm plasma membrane from damage caused by cold shock and osmotic stress. Additionally, extenders with added cholesterol can stabilize the sperm membrane, reducing the risk of damage (Jeyendran *et al*., 2019). The acrosomal membrane can be protected by extenders that contain specific proteins or amino acids, such as egg yolk or casein, which act as membrane stabilizers and protect against enzymatic degradation (Kumaresan *et al*., 2017). Extenders with balanced osmolarity and appropriate ionic composition prevent morphological abnormalities by maintaining the structural integrity of sperm cells. For example, extenders that include egg yolk or soy lecithin can mitigate the detrimental effects of osmotic changes and mechanical damage during semen handling (Amann and Katz, 2014). The decrease in fertility with preserved semen is in line with previous findings (Zong *et al*., 2023), highlighting the sensitivity of sperm quality to preservation techniques. Reduced chick hatchability observed in this study is consistent with prior research (Suwimonteerabutr *et al*., 2023), emphasizing challenges in maintaining optimal hatchability with preserved semen. These declines in fertility and hatchability with increased storage duration are also supported by previous studies (Wolc *et al*., 2010),

underlining the vulnerability of stored chicken semen to decreased reproductive success. The significant increase in chick embryonic mortality highlights the vulnerability of developing embryos to semen preservation techniques (Nakage *et al*., 2003).

The evaluation of chick abnormalities is crucial for assessing post-hatch performance and chick health. Chick abnormalities, including reflex, appearance, eyes, gait score, naval area, and yolk sac absorption, serve as indicators of overall chick health and development. Our findings indicate that the choice of extender did not significantly influence these parameters immediately after hatching (Smith *et al*., 2018). These studies suggest that extenders primarily affect semen quality and fertility, rather than directly influencing chick abnormalities. However, factors such as breeder age, egg storage conditions, and incubation parameters may contribute to the occurrence of abnormalities (Reis *et al*., 2019). The weight of day-old chicks and relative body weight are crucial indicators of chick growth and development. Our study revealed a decrease in both parameters with prolonged storage periods across all treatment groups, consistent with previous research (Brown *et al*., 2019; Zhang *et al*., 2020). Prolonged storage may lead to reduced fertility and hatchability rates, resulting in developmental delays or growth impairments in chicks hatched from stored semen. Mortality and morbidity rates serve as critical indicators of chick health and welfare. Our findings revealed an increase in both parameters with prolonged storage periods, suggesting potential health challenges faced by chicks hatched from stored semen (Smith *et al*., 2018). Chick welfare encompasses various behavioral and physiological indicators, reflecting the overall health and well-being of chicks. Our study observed occurrences of welfare issues across all treatment groups, indicating potential challenges associated with semen preservation methods (Guibert *et al*., 2020). Understanding the welfare implications of semen preservation methods is crucial for promoting responsible and sustainable poultry production practices.

## CONCLUSION

This study highlights extender play's role in preserving poultry semen quality during storage. AD2 and garlic extract extenders displayed promise, albeit with varying effectiveness. Additionally, the study underscores semen preservation methods' significant impact on fertility, hatchability, and embryonic development in Fayoumi breed chickens. Variations were observed in fertility rates, chick hatchability, and embryonic mortality among treatment groups. Longer storage periods and specific extenders correlated with reduced fertility, lower chick hatchability, and increased embryonic mortality. In post hatch performance and chick welfare, long-term storage leads to decreased chick weight, relative body weight, and length, along with higher mortality and morbidity rates. Short-term storage also impacts chick performance and welfare, albeit less severely, while on-farm storage has minimal effects.

## DECLARATIONS

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*IRB approval*

This study was approved by the Department Board and the Directorate of Advanced Studies and Research, Sindh Agriculture University (SAU), Tandojam.

*Ethical approval*

This study was conducted in accordance with the ethical guidelines for the use of animals in research. Ethical approval was obtained from the Ethical Committee at SAU Tandojam, ensuring that all procedures involving birds were carried out humanely and in compliance with relevant regulations and standards.

*Statement of conflict of interest*

The authors have declared no conflict of interest.



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