Effects of Semen Dosage and Insemination Frequency on Fertility and Hatchability in Horasi Chicken Ecotype

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ABSTRACT

The objective of this study was to assess the effect of Horasi chicken ecotype semen dilution, insemination dosage, and frequency of insemination on spermatozoa fertility potentials (hatchability and fertility). Twenty adult hens and four cockerels were used in a 2x2 factorial experiment. The factors were semen type (undiluted versus diluted semen), insemination volume (0.5 versus 0.1 ml), and insemination frequency (once versus twice inseminations per week). Egg fertility and hatchability among semen type, semen volume and insemination frequency varied from 68.35 ± 2.10 to 82.20 ± 2.29, and 59.38 ± 2.15 to 86.87 ± 2.57; 70.99 ± 2.45 to 79.56 ± 2.62, and 69.33 ± 3.88 to 76.92 ± 3.83; 67.41 ± 1.99 to 83.15 ± 2.06, and 68.53 ± 4.16 to 77.73 ± 3.40 respectively. The type of semen significantly (P <0.05) affected both fertility and hatchability. Higher fertility was recorded in hens inseminated with diluted fresh semen than those inseminated using undiluted fresh semen in all semen volumes and insemination frequencies. Insemination volume and insemination frequency had a significant (P <0.05) effect only on fertility. Higher egg fertility was observed in hens inseminated with 0.5mL of semen compared to those inseminated using 0.1mL of semen irrespective of semen type and insemination frequency. Also, hens inseminated twice a week had higher egg fertility in comparison to those inseminated once a week irrespective of semen volume and semen type. From the results obtained, it is concluded that the BPSE improved the fertilizing potential of fresh stored sperm and subsequent hatchability, while inseminating dose and insemination frequency only affected fertility. Thus, twice weekly insemination with diluted semen using 0.5mL of semen will maximize fertility and hatchability of Horasi chicken ecotype eggs.

Keywords: Diluted semen, fertilizing ability, hatching rates, Horasi chicken ecotype eggs.

I. INTRODUCTION

The indigenous chickens in Tanzania have been bred naturally over centuries for hardiness and survival. In the most developed countries, characterized by low egg production ranging from 40 to 60 eggs/hen/year and body weight of 1.2 kg at maturity, compared with improved tropical adapted breeds producing about 200 eggs/hen/year and 2.0 kg body weight at 16 weeks of age [1]. Poultry producers over the years have used genetic selection and improving management practices which have resulted into increased growth rate in poultry [2], [3] but with some unfavourable effects on reproduction [4]. Alternatively, assisted reproduction technology (ART’s), such as artificial insemination is widely used for genetic improvement to increase poultry as it uses genetically superior cockerels with a high productive performance [5]. If AI technology is effectively used in the poultry industry, will assist in addressing a number of difficulties including the increasing demand for poultry products and food insecurity resulting from a changing climate and growing population.

Artificial insemination technology is utilized in the animal industry to improve breeding efficiency and genetic progress [6]. It entails the injection of sperm into the female bird's reproductive system using specialized equipment. Artificial insemination in poultry is used not only to manage breeding activities but also to boost chicken production by allowing large-scale adoption and utilization of cockerels with higher genetic efficiency [7]. That is, sperm taken from a few genetically superior males is utilized to inseminate a wide group of females, lowering production costs by reducing the number of males in a flock, and so contributing to the fast spread of genetic material from a few outstanding cockerels [8]. For instance, a single ejaculate of a cock can be used to inseminate up to twenty (20) hens [9], [10]. This is due to the fact that it enables the dilution, storage, and transportation of poultry sperm to faraway fields in order to successfully inseminate a significant number of hens for efficient usage of superior male’s sperm [11]. AI offers a more efficient and controlled method of breeding than natural mating, leading to higher rates of genetic progress. In addition to its breeding value, AI plays a major role in the prevention of venereal diseases [10].

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Furthermore, it has been documented that excellent fertility and fecundity in poultry can be achieved through artificial insemination, which is superior in several ways to natural mating [8], [12], [13], [14]. However, enhancing fertility and hatchability necessitates technical aid for sperm collection, preservation, and subsequent use in breeding animals [15]. That is, the methods of managing the females and males, the semen gathering technique, the processes for managing sperm in vitro, and the insemination techniques need to be thoroughly understood and skillfully applied in order to achieve effective fertilization [12], [16]. This emphasizes that all the procedures from semen collection to insemination cannot be conducted by anybody, and accurate protocols should be observed in order to get the highest feasible quality of sperm, fertility, and subsequent hatchability.

Artificial insemination in avian is often limited due to poor survival of cryopreserved avian sperm. Thus, AI in poultry is mainly performed using extended fresh and frozen semen [17]. However, in Tanzania, AI in poultry particularly in chicken has not been fully performed. Horasi is an important native chicken ecotype in Tanzania with native traits [18]. It is chosen for its superiority in terms of meat and egg production, fast growth rate, and high adaptability as [19] and [20] described. Therefore, the current study was carried out to assess and compare the fertilizing ability and hatchability of diluted fresh stored and undiluted Horasi chicken ecotype semen when inseminated at different volumes (0.1mL versus 0.5mL) and different insemination frequency per week (once versus twice). Results from this study will contribute to genetic improvement and increasing productivity of indigenous chickens in the country using superior Horasi cockerels as sources of semen for AI, and thus inevitably will contribute to the rural and peri-urban poultry farmers to improve production and their livelihood.

II. MATERIALS AND METHODS

A. Study Location

Sokoine University of Agriculture's (SUAs) experimental poultry farm served as the site of the investigation. Morogoro Municipality, which is roughly 200 km west of Dar es Salaam, has Sokoine University of Agriculture located 3.0 km from its center. Morogoro Municipality is located in eastern Tanzania, at latitude 6°49′15″ S and longitude 37°39′40″ E. The elevation above sea level is 504 m, with a mean yearly temperature of 24.3 °C and 935 mm of precipitation.

B. Experimental Design

To accomplish this objective, a completely randomized design (CRD), with a 2×2×2 factorial experiment involving two semen types, two insemination volumes, and two insemination frequencies was used. Undiluted and diluted semen were the two types of semen that were employed. Insemination volumes were 0.1 mL and 0.5 mL. While insemination frequencies were once a week and twice a week.

C. Management of Experimental Birds

Four Horasi cocks and twenty Horasi hens aged 6 months were used in this experiment. The birds were housed individually in cages measuring 40 cm ×40 cm × 60 cm in an open-sided building receiving 12 hours of natural light. Individual identification was done by fitting a numbered wing band on each bird. Ample food, water, and other managerial tasks such as vaccination against common viral diseases (Gumboro, Newcastle Disease, and Fowl pox), deworming, and general cleanliness were carried out regularly.

D. Ethical Clearance

The Sokoine University of Agriculture Ethical Committee approved the use of birds and gave their approval with reference number SUA/DPRTC/R/186 VOL III in the directorate of research, technology transfer, and consultation.

E. Semen Collection, Dilution, and Storage

According to [21], semen was extracted from cocks utilizing an abdominal massaging technique and pooled. In order to express the semen, the cloaca is massaged first, and then the surrounding area is compressed [22]. Semen was collected in graduated tubes and immediately transported to the laboratory for quality assessment (concentration, motility, viability, and morphological normalcy) while maintained at 4 °C. Semen samples with good quality (> 5.5×10^6 sperms per mL, >70% motility, >80% viability, and > 80% normalcy) were pooled and divided into two groups; one was diluted at a ratio of 1:2 in commercial extender (BPSE) to get about 300 million spermatozoa per mL of semen, and another part was left undiluted. Diluted semen sample was subsequently stored under refrigeration temperature (4 °C) for 48 hours before being used for insemination [23], whereas undiluted semen was inseminated immediately after the quality assessment.

F. Chemical Composition of BPSE Extender

The BPSE was prepared by adding potassium diphasphate (trihydrate) (1.27 g), sodium glutamate (monohydrate) (0.867 g), fructose (0.5 g), sodium acetate (anhydrous) (0.43 g), tris (0.195g), potassium monophosphate (0.065 g), potassium citrate (monohydrate) (0.064 g), magnesium chloride (0.034g), and double distilled water (100.00ml). The pH of this extender was 7.5 with osmotic pressure 333.00 mOsmol/kg [24].

G. Chicken Insemination

Totally 40 hens were used for the AI experiment that was replicated twice (between January and April 2023). Hens were first divided into two insemination groups; the first group (20 hens) was inseminated using undiluted semen and the second group of hens (n=20) was inseminated utilizing BPSE-diluted semen. From the first insemination group (using undiluted semen), hens were further divided into sub-groups, the first sub-group (5 hens) was inseminated using 0.10mL of semen once per week, the second sub-group (5hens) was inseminated using 0.1 mL of semen twice per week, the third sub-group (5hens) received 0.5 mL of semen once per week and the fourth sub-group (5hens) received 0.5 mL of semen twice per week. After four weeks of insemination, hens were allowed to rest without insemination for two weeks, and the insemination was resumed again using diluted fresh stored semen in respective sub-groups. In all hens, AI procedure was performed by two trained and experienced technicians using intravaginal method [25]. Throughout the experiment, AI was performed in the afternoon (between 15:00 and 16:00).

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**H. Egg Collection, Incubation, Candling and Hatching**

In the hen, the day of artificial insemination (AI) was recorded as day 0 of fertilization. After single insemination with both fresh and preserved sperm, eggs were gathered every day beginning the second day after AI and lasting 7 days and proceeded for four weeks of insemination. To distinguish between semen used (undiluted semen versus extended semen), semen volume (0.1mL versus 0.5mL), insemination frequency (once versus twice per week) and the collection day, the eggs were labeled. All eggs were collected in the afternoons (between 13:00 and 15:00) and stored at room temperature (25-28 °C) and relative humidity of 75% prior to incubation for 21 days. Fertility or embryonic development assessment was done through the candling technique on the 7th day of incubation. Fertilized eggs were returned to the incubator to complete 21 days of incubation, and analysis was done on the candled cleavers. After 21 days of incubation, the hatching rates of fertilized eggs were determined, and an investigation of unhatched eggs was performed. Fertility was calculated based on the total eggs set while hatchability was calculated on the basis of the total fertile eggs set. Percentage of fertility and hatchability were calculated employing formulae previously described by [18] whereby:

\[ \text{Percentage Fertility} = \left( \frac{\text{Fertile Egg}}{\text{Total egg set}} \right) \times 100 \]

\[ \text{Percentage Hatchability} = \left( \frac{\text{Eggs that have hatched}}{\text{Eggs that are fertile set}} \right) \times 100 \]

**I. Statistical Investigation**

The data was statistically analyzed using the software Statistical Package for the Social Sciences 25.0. One-way analysis of variance (ANOVA) and the independent-sample T-test were used to examine the fertility and hatching rates between semen type, semen volume, and insemination frequency. Variations in fertility and hatching rates between semen type, semen volume, and insemination frequency were regarded as significant at the level P<0.05.

**III. RESULTS**

**A. The Effect of Sperm Type: Undiluted Versus Diluted, on Fertility and Hatchability**

The fertility and hatching rates of Horasi chicken eggs in undiluted and diluted semen ranged from 50.00 to 87.50%, 40.00 to 77.78%; 62.50 to 100.00%, and 60.00 to 100.00% respectively as presented in Table I. The type of semen inseminated significantly affected both the fertility and hatchability of Horasi chicken eggs (P<0.05, Table I). Higher fertility and hatching rates were observed in hens that received diluted semen compared to those that received undiluted semen as shown in Table I.

**B. The Effect of Semen Volume: 0.1 ml vs 0.5 ml, on Fertility and Hatchability**

The fertility and hatching rates of Horasi chicken eggs in 0.1 mL and 0.5 mL of semen ranged from 50.00 to 88.89%, and 40.00 to 100.00%; 62.50 to 100.00%, and 50.00 to 100.00%, respectively as indicated in Table I. Irrespective of semen type and insemination frequency, significant variations were recorded in egg fertility between the two semen volumes (P<0.05, Table I). Hens inseminated using 0.5 mL of semen; their eggs had higher fertility in comparison to those inseminated using 0.1 mL of semen (Table I). Also, there were differences in hatching rates of Horasi eggs among the two semen volumes used, however, the differences were not significant (Table I).

**C. The Effect of Insemination Frequency: Once vs Twice Per Week, on Fertility and Hatchability**

Fertility and hatching rates of Horasi chicken eggs in hens inseminated once per week and twice per week ranged from 50.00 to 83.33%, and 40.00 to 100.00%; 70.00 to 100.00, and 57.14 to 100.00% respectively as presented in Table I. The fertility of Horasi eggs was significantly affected by the insemination frequency (P<0.05, Table I). Higher fertility rates were observed in eggs produced by hens inseminated twice a week than those produced by hens inseminated once a week (Table I). However, the hatchability of Horasi chicken eggs was not significantly affected by the insemination frequency, although differences existed as indicated in Table I.

**TABLE I: EFFECTS OF SEMEN AMOUNT, TYPE, AND FREQUENCY OF INSEMINATION ON THE FERTILITY AND HATCHABILITY (MEAN ± SEM) OF HORASI CHICKEN ECTYPE EGGS**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
<th>Parameters</th>
<th>Fertility (%)</th>
<th>Hatchability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen type</td>
<td>Unidiluted</td>
<td>68.33 ± 2.10a</td>
<td>59.38 ± 2.15b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diluted</td>
<td>82.20 ± 2.29b</td>
<td>86.87 ± 2.57b</td>
<td></td>
</tr>
<tr>
<td>Semen volume</td>
<td>0.1 mL</td>
<td>70.99 ± 2.45a</td>
<td>69.33 ± 3.88a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 mL</td>
<td>79.56 ± 2.62a</td>
<td>76.92 ± 3.83a</td>
<td></td>
</tr>
<tr>
<td>Insemination frequency</td>
<td>One/week</td>
<td>67.41 ± 1.99a</td>
<td>68.53 ± 4.16a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Twice/week</td>
<td>83.15±2.06bc</td>
<td>77.7±3.40bc</td>
<td></td>
</tr>
</tbody>
</table>

a,b Values in the same column with distinct letters differ significantly from one another.

**D. Performance of Undiluted and Diluted Semen on Fertility at Different Semen Volumes**

In both undiluted and diluted semen, fertility was observed to increase with an increase in semen volume. Semen volume of 0.1 mL resulted to lower egg fertility compared to 0.5 mL in all semen types (Fig. 1). Higher egg fertility rates 77.41% and 86.99% were observed in eggs produced by hens inseminated using diluted semen in both 0.1 mL and 0.5 mL semen volumes compared to eggs produced by hens inseminated using undiluted semen in 0.1 mL and 0.5 mL which were 64.58% and 72.13%, respectively (Fig. 1). Maximum fertility (86.99%) was recorded in eggs produced by hens inseminated using diluted semen with 0.5 mL of semen (Fig. 1).

**E. Performance of Undiluted and Diluted Semen on Fertility at Different Insemination Frequencies**

As the insemination frequency increased, egg fertility was also increasing in both undiluted and diluted semen. Hens that were inseminated with diluted semen both once and twice a week produced eggs with higher fertility rates 74.10% and 90.30% respectively than those inseminated using undiluted semen once (60.72%) or twice (75.99%) a week (Fig. 2). Eggs produced by hens inseminated using diluted semen twice a week had higher fertility (90.30%), while lower fertility (60.72%) was observed in eggs produced by
hens inseminated using undiluted semen once a week (Fig. 2).

![Image](57x449 to 301x592)

Fig. 1. Performance of undiluted and diluted semen on fertility of Horasi chicken ecotype eggs at different semen volumes.

![Image](57x624 to 301x767)

Fig. 2. Performance of undiluted and diluted semen on fertility of Horasi chicken ecotype eggs at different insemination frequencies.

**F. Interaction of Dilution, Semen Volume and Insemination Frequency on Egg Fertility**

The interaction effects between semen type, inseminating volume, and insemination frequency on the fertility of Horasi chicken eggs are presented in Table II. It was observed that egg fertility had an increasing trend as the semen volume and insemination frequency increased in both undiluted and diluted semen as shown in Table II. Fertility was higher in eggs produced by both hens inseminated using undiluted or diluted semen, once or twice a week using inseminating volume of 0.5 mL than eggs produced by hens inseminated using undiluted or diluted semen once or twice a week using inseminating volume of 0.1 mL (Table II). Also, an insemination frequency of twice a week resulted in higher fertility rates in both semen types and semen volumes than that of once a week. Maximum egg fertility (94.85 ± 4.71) for Horasi chicken ecotype was found in diluted semen, inseminated twice a week using 0.5 mL semen volume, and minimum egg fertility (57.04 ± 4.75) was observed in undiluted semen inseminated once a week using 0.1 mL semen volume as displayed in Table II.

**TABLE II: PERFORMANCE OF UNDILUTED AND DILUTED SEMEN ON FERTILITY OF HORASI CHICKEN ECOTYPE EGGS (MEAN ± SD) AT DIFFERENT SEMEN VOLUMES AND INSEMINATION FREQUENCIES**

<table>
<thead>
<tr>
<th>Semen type</th>
<th>Insemination frequency</th>
<th>0.1mL</th>
<th>0.5mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>One/week</td>
<td>57.04 ± 4.75</td>
<td>64.40 ± 2.13</td>
</tr>
<tr>
<td></td>
<td>Twice/week</td>
<td>72.12 ± 1.88</td>
<td>79.86 ± 5.23</td>
</tr>
<tr>
<td>Diluted</td>
<td>One/week</td>
<td>69.07 ± 3.74</td>
<td>79.13 ± 4.14</td>
</tr>
<tr>
<td></td>
<td>Twice/week</td>
<td>85.75 ± 2.48</td>
<td>94.85 ± 4.71</td>
</tr>
</tbody>
</table>

**G. Performance of Undiluted and Diluted Semen on Hatchability at Different Semen Volumes**

As the volume of semen increased, Hatching rates also increased in all types of semen (diluted and undiluted) (Fig. 3). In both undiluted and diluted semen higher hatching rates recorded in hens receiving 0.5 mL of semen compared to those received 0.1 mL of semen (Fig. 3). However, in all inseminating volumes (0.1 mL and 0.5 mL), eggs produced by hens inseminated using diluted semen had superior hatching rates (81.94% and 91.80%) compared to eggs produced by hens inseminates using undiluted semen (56.73% and 62.04%) respectively as presented in Fig. 3. The highest hatchability (91.80%) was recorded in eggs produced by hens inseminated using diluted semen with 0.5 mL of semen, and the lowest hatchability (56.73%) was observed in eggs produced by hens inseminated using undiluted semen with 0.1 mL of semen as presented in Fig. 3.

![Image](305x81 to 548x224)

Fig. 3. Performance of undiluted and diluted semen on hatchability different semen volumes.

**H. Performance of Undiluted and Diluted Semen on Hatchability at Different Insemination Frequencies**

The hatchability of eggs was increasing as the insemination frequency increased in both undiluted and diluted semen (Figure 4). Hens that were inseminated with diluted semen both once and twice a week produced eggs with higher hatching rates 83.42% and 90.32% respectively than those inseminated using undiluted semen once (53.63%) or twice (65.14%) a week (Fig. 4). Maximum hatchability (90.32%) was observed in eggs produced by hens inseminated using diluted semen twice a week had, and the minimum hatchability (53.63%) was observed in eggs produced by hens inseminated using undiluted semen once a week (Fig. 4).

![Image](315x429 to 559x572)

Fig. 4. Performance of undiluted and diluted semen on hatchability of Horasi chicken ecotype eggs at different insemination frequencies.
I. Effects of Dilutions, Semen Volume, and Insemination Frequencies on Egg Hatchability

The percentage hatching rates of Horasi chicken eggs produced by hens using undiluted and diluted semen with different semen volumes and insemination frequencies are shown in Table III. As the inseminating volumes and insemination frequencies were increasing, hatching rates were also increasing in both undiluted and diluted semen (Table III). Higher hatching eggs were generated by hens that were inseminated with diluted semen compared to those produced by hens inseminated using undiluted semen, in terms of both insemination volumes and frequencies (Table III). The highest hatchability (96.18 ± 5.24) was found in eggs produced by hens inseminated using diluted semen with 0.5 mL of semen twice a week, and the lowest hatchability (49.33 ± 10.90) was seen in eggs produced by hens inseminated using undiluted semen with 0.1 mL of semen once a week as shown in Table III.

<table>
<thead>
<tr>
<th>Semen type</th>
<th>Insemination frequency</th>
<th>Hatchability %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1mL</td>
<td>0.5mL</td>
</tr>
<tr>
<td>Undiluted</td>
<td>Once/week</td>
<td>49.33 ± 10.90</td>
</tr>
<tr>
<td></td>
<td>64.12 ± 4.91</td>
<td>66.15 ± 7.59</td>
</tr>
<tr>
<td>Diluted</td>
<td>Once/week</td>
<td>79.43 ± 15.04</td>
</tr>
<tr>
<td></td>
<td>84.45 ± 10.51</td>
<td>96.18 ± 5.24</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

The effectiveness of undiluted and diluted fresh stored semen, semen volume, and frequency of insemination on egg fertility and hatchability in the Horasi chicken ecotype was assessed in the current study. Both undiluted and diluted semen had good performance, but diluted semen had superior results on fertility and hatchability compared to undiluted semen. Complimentary observations were reported by [26], where the percentage fertility and hatchability were found to be higher in eggs produced by hens inseminated using diluted (dextrose + watermelon juice diluted) semen than those inseminated using undiluted semen. Furthermore, higher fertility rates were obtained in turkey semen diluted using BPSE extender, inseminated after 24 hours of storage [27]. High fertility rates (87 to 97%) were reported in hens inseminated using semen stored for 24 h at 4 °C [28], [29]. Similarly, higher fertility of about 90% was obtained in hens inseminated using 0.1 mL of diluted semen [30]. Also, [24] reported higher fertility when using semen diluted with BPSE at a dilution rate of 1:1. These observations agree with those reported elsewhere [31]-[34]. In contrast to the current findings [35] found similar fertility rates between diluted (BPSE) semen and undiluted semen. It is known that poultry semen extenders are composed of seminal plasma [36]. Fresh diluted semen, particularly extremely diluted semen, has been shown to retain its fertility when treated with seminal plasma [33], [37]. Furthermore, according to studies by [38], [39], [40], seminal plasma enhances the movement and respiration of avian sperm cells. Irrespective of semen type fertility and hatchability may also be influenced by insemination dosage and insemination frequency. In this study, semen volume and insemination frequency affected fertility as well as hatching rates of Horasi eggs. Insemination twice a week using semen volume of 0.1 mL and 0.5 mL improved fertility compared to once a week using semen volume of 0.1 mL and 0.5 mL. These findings are in line with those reported by [41], whereby the best fertility was obtained in eggs produced by broiler breeder pullets inseminated twice a week using semen volume of 0.05 mL than those inseminated weekly using semen volume of 0.1 mL. Furthermore, the author discovered that utilizing semen volumes of 0.1 and 0.05 mL when inseminating chickens twice weekly led to higher hatching rates. Also, [30] and [42] found that fertility was higher in 3 days insemination interval compared with 6 days interval. [43] reported fertility of about 80% in pullets inseminated using 0.03 mL of semen once a week compared to 92-95% fertility when inseminated twice a week. Adding to that, for diluted semen the best performance is reported to be obtained at 0.10mL semen inseminated twice per week, compared to 0.05mL in fertility [44]. Moreover, a higher proportion of fertile eggs (97%) can be produced when chickens are inseminated using a semen volume of 0.1cc weekly as opposed to semen volumes below 0.05 mL [25].

The influence of insemination frequency and insemination dosage on fertility differs between poultry breeds/lines [34], [45]. Hence, improving fertility by just raising the insemination dose is not always a guarantee [42]. However, it is important to appreciate that theoretically a single sperm cell is needed for fertilization of the ovum, but in practice, it is unlikely that a single sperm cell will produce such a result [44].

V. CONCLUSION

In conclusion, extended fresh stored Horasi cockerel’s semen resulted into superior fertility and hatchability inseminated with 0.5 mL of semen twice weekly compared to undiluted fresh semen. This indicates the feasibility of the BPSE extender in preserving and improving the fertilizing ability of Horasi cockerel’s semen under liquid storage at 4 °C.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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