Fertilizing ability and survivability of rooster sperm diluted with a novel semen extender supplemented with serine for practical use on smallholder farms

Vibuntita Chankitisakul, Wuttigrai Boonkum, Theerapat Kaewkanha, Maruay Pimprasert, Ruthaiporn Ratchamak, Supakorn Authaida, and Pachara Thananurak

Department of Animal Science, Faculty of Agricultural, Khon Kaen University, Khon Kaen, 40002, Thailand; The Research and Development Network Center of Animal Breeding and Omics, Khon Kaen University, Khon Kaen, 40002, Thailand; and Division of Animal Science, Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi, Pathum Thani, 12130, Thailand

ABSTRACT Semen extenders are essential for maintaining the quality of sperm during storage and assuring the success of fertility after insemination. The objective of this study was to examine the effect of a new rooster semen extender (hereinafter, NCAB) supplemented with serine on the survivability and fertilizing ability of sperm following storage. NaCl solution, the NCAB extender, and IGGKPh extender were used as treatments. In Experiment 1, different storage temperatures (5°C and 25°C) and durations of storage (0, 12, and 24 h) were used to compare the semen quality and determine the suitable storage temperature. The fertility test was performed in in-station tests and on-farm experimental trials in Experiments 2 and 3, respectively. The results indicated that the interaction effect of duration, treatment, and storage temperature on all sperm parameters was highly significant. The NCAB extender significantly improved rooster semen quality during storage for 24 h at 5°C (P < 0.01). The sperm diluted in saline solution could not survive 24 h of storage at 25°C. The fertility and hatchability rates obtained for sperm diluted with the NCAB extender were higher than those diluted with other extenders. In addition, the fertilizing capacity of the NCAB extender-diluted sperm under field conditions was significantly higher than that of the saline solution-treated sperm. In conclusion, the NCAB extender supplemented with serine and stored at a low temperature (5°C) positively affects sperm quality and fertilization.

Key words: liquid semen, artificial insemination, chickens, sperm motility

INTRODUCTION

Chicken semen is highly concentrated, containing 4 to 6 billion sperm/mL (Santiago-Moreno and Blesbois, 2020). It is viscous with a low volume, and therefore, there is the risk of sperm being killed by dehydration due to water evaporation from the seminal plasma (Lake and Stewart, 1978). The sperm motility and fertilizing capacity of undiluted raw fowl semen stored in vitro usually decrease within 1 h of collection (Dumpala et al., 2006). Dilution of rooster semen immediately after semen collection is necessary to maintain sperm viability. This practice also provides an optimum environment to ensure viability, thereby increasing the number of insemination doses from each collection and ensuring the uniform distribution of sperm in diluents (Vašiček et al., 2015).

For making AI economically viable to local farmers, semen dilution should be performed under conditions that are practical for farmers. Although there have been reports of practical methods for semen storage in poultry using different extenders, such as Schramm (Sonseeda et al., 2013), IGGKPh (Surai and Wishart, 1996; Lukaszewicz, 2001), BPSE (Sexton, 1978), and Ringer’s solution (Karl and Hyre, 1960; Adebisi and Ewuola, 2019; Telnoni et al., 2021), they are not practical for use by local farmers. This is because these semen extenders are not commercially sold and are therefore not easily available. Additionally, farmers must prepare semen extenders by themselves and adjust the pH and osmotic pressure of these extenders. Therefore, almost all local Thai farmers use NaCl (saline) solutions as semen extenders because of their low cost and ease of use (Bootwalla and Miles, 1992). However, the farmers need to artificially inseminate twice a week when NaCl is used as a diluent, and even though the resulting

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1Corresponding author: p.thananurk@gmail.com, pachara_th@rmutt.ac.th (PT)
fertility rates are acceptable, they are not outstanding and can be improved.

Lipid peroxidation is an important aspect of oxidative stress that shortens the life span of sperm in vitro, while also affecting the preservation of semen for AI (Jones et al., 1979). Therefore, antioxidants, which act as free radical scavengers, have been proposed as semen additives to extenders for improving semen preservation. Serine is a conditionally nonessential amino acid that plays important biological roles ranging from protein synthesis to cell signaling (Metcalf et al., 2008; Hunter, 2012). The addition of serine can reduce oxidative stress and increase the activity of antioxidant enzymes (Zhou et al., 2017; Zhou et al., 2018a). According to our previous study, the addition of serine-containing semen cryopreservation diluents decreased peroxidation and improved the efficiency of semen freezing (Thananurak et al., 2020). However, to the best of our knowledge, there are no data available on the influence of serine supplement being used as an antioxidant for up to 24 h of rooster semen storage. Therefore, the present study was conducted to examine a new rooster semen extender (NCAB), supplemented with serine on the survivability of sperm and fertilization ability following storage for up to 24 h. To achieve this, NaCl solution and IGGKPh extender, commonly used on farms, were used as treatments. Different temperatures (5°C and 25°C) and durations (0, 12, and 24 h) of storage were used to compare the semen quality and determine the ideal storage temperature in Experiment 1 before additional fertility tests performed in Experiments 2 and 3.

**MATERIALS AND METHODS**

The present study was approved by the Institutional Animal Care and Use Committee based on the Ethics of Animal Experimentation of the National Research Council of Thailand (record no. IACUC-KKU-28/65). The farmers’ on-farm experimental trials were performed in a rural community in Thung Pong Distinct, Khon Kaen Province. These trials were supported by the career development initiative under the "Development of indigenous chicken farming for sustainability" project (reference no. 007/2562) through the collaboration between the Royal Initiative Discovery Foundation and the Research and Development Network Center of Animal Breeding and Omics, Khon Kaen University.

**Animals and Management**

Experiments 1 and 2 were performed in-station, while Experiment 3 was an on-farm experimental trial.

In-station research: Sixty Thai native chickens (Pradu Hang Dum; 1-yr-old male breeder roosters) were managed intensively in a battery cage system with dimensions 60 cm × 45 cm × 45 cm and one rooster per cage in an open environment system. Each rooster received approximately 130 g of commercial breeder feed for male chickens per day, and water was given ad libitum. One hundred and five commercial laying hens (48 wk of age), with egg production >85%, were used for the fertility test in Experiment 2. The hens were housed individually, fed approximately 110 g of layer feed per day, and were given water ad libitum.

On-farm experimental trials: Twenty-five roosters and 250 hens of the Thai native chickens (Pradu Hang Dum) from 2 farms were used to verify the performance of a new extender under conditions typically experienced by farmers. The animals were 42 wk old at the start of the experiment and continued to collect data for 20 wk. They were managed in a manner similar to that followed for the in-station research.

**Rooster Sperm Preparation and Semen Dilution**

Semen samples were routinely collected twice a week using the dorsoabdominal massage method. The samples from individual roosters were collected in a 1.5-mL microtube containing 0.1 mL of the 3 semen extenders: 1) 0.9% sodium chloride solution, 2) IGGKPh extender, and 3) NCAB extender. All three extenders warmed at a temperature range of 22°C to 25°C before dilution, and the diluted semen was kept in the transportation box at that temperature during transport to the laboratory for further analyses (Thananurak et al., 2020). Transport occurred within 20 min after collection. Semen volume, mass movement score, sperm concentration using the SYBR-14 and propidium iodide staining method were determined. Only the semen samples with a mass movement score ≥4, sperm concentration ≥3 × 10⁹ sperm/mL, and sperm viability ≥90% were used in the experiment.

After evaluation, the semen samples from each rooster were pooled and extended in different semen extenders (1:3 v/v) according to the experimental design. The final concentration of extended semen was approximately 100-150 × 10⁶ spz/dose. The composition of each extender is presented in Table 1.

| Constituents          | NaCl (g) | IGGKPh (g) | NCAB (g) |
|-----------------------|----------|------------|----------|
| NaCl                  | 0.90     | 0.10       | 0.10     |
| Potassium citrate monohydrate | 0.14     | 0.50       |          |
| Sodium glutamate      | 1.40     | 1.50       |          |
| Sodium hydrogen phosphate | 0.98     | 0.05       |          |
| Sodium dihydrogen phosphate | 0.21     | 0.07       |          |
| Magnesium acetate     | 0.12     | 0.17       |          |
| Sodium acetate        | 0.90     | 1.00       |          |
| Glucose               | 0.90     | 1.00       |          |
| Inositol              | 0.90     |            | 0.04     |
| Fructose              | 1.00     |            |          |
| D-Serine              |          |            |          |
| mOsm                  | 308      | 396        | 410      |
| pH                    | 5.5      | 6.9        | 6.9      |
| Distilled water (mL)  | 100      | 100        | 100      |

Table 1. Composition of the three semen extenders.
Experimental Design

Experiment 1: Effects of Storage Temperature (at 5°C and 25°C) and Different Semen Extenders on Sperm Quality During Storage for Up To 24 h

To assess the most suitable storage temperature, liquid semen diluted with the three extenders (0.9% sodium chloride, IGGKPh, and NCAB) was stored at 5°C and 25°C for 24 h. Sperm motility (total motility and progressive motility) and sperm viability were evaluated immediately after the semen was diluted (T0) or after storage at 12 and 24 h (T12 and T24, respectively). The experiment was repeated six times.

The storage temperature that most positively affected sperm quality was used in the fertility test in Experiment 2.

Experiment 2: Effects of Different Extenders on Fertility

To determine the fertilizing ability of semen diluted with the 3 extenders, the hens in each group (n = 35 per group per time) were artificially inseminated once at 15-d intervals (d 0 as insemination day). Eggs were collected for 14 d during d 2 to 15 after insemination. The fertility and hatchability were determined. The experiment was replicated 3 times.

The best treatment group from this experiment was selected to examine the fertility under practical use by farmers at smallholder farms in Experiment 3.

Experiment 3: Effects of the Best Extender on Fertility in Practical Use

To verify the fertility of the new technology (extender) under conditions typically experienced by farmers at smallholder farms, the best performing semen extender from Experiment 2 was delivered to the farmers. The farmers had to dilute the semen with a NaCl solution and our extender (powder form), as described by our researcher, who was present to guide the experiment. Briefly, water was added to the powder, about half a final volume, then shook the bottle until all the powder was thoroughly mixed. Then filled the water to a final volume. The diluted semen was stored at 5°C during the insemination procedure. The period of semen collection, dilution, and insemination were within 2 to 3 h after semen collection. The hens were inseminated once a week. To reduce variable fertility factors from human error, the insemination of chickens from both treatment groups was performed by the same person simultaneously. The fertility and hatchability were recorded. The data continued to be collected for 20 wk of insemination.

Sperm Evaluation

Sperm Concentration

Sperm concentration was assessed using a Neubauer hemocytometer after diluting the semen with 4% NaCl to 1:200 (v/v). The concentration was then evaluated using phase contrast microscopy at 400 × magnification. The sperm concentration was expressed as sperm × 10^6 sperm/mL.

Sperm Viability

The sperm viability was evaluated via dual fluorescent staining using SYBR-14 and propidium iodide kits (Live/dead sperm viability kit L7011; Invitrogen, Thermo Fisher Scientific, Waltham, MA). Staining was performed according to the manufacturer’s instructions. Briefly, each sample was diluted to a concentration of 150 million sperm/mL, and 300 µL of diluted semen was mixed with 5 µL of SYBR-14 solution diluted in sterile water (at a ratio of 1:49) in a cytomteric tube. This was then incubated at 24 to 27°C for 10 min, followed by staining with 5 µL propidium iodide for 5 min. The cells were then fixed with 30 mL of 10% formaldehyde. For assessment, at least 200 sperm cells were analyzed under a fluorescence microscope IX71 (Olympus, Tokyo, Japan) at 400 × magnification. The nucleus of live sperm with an intact plasma membrane is stained bright green with SYBR-14, while that of dead sperm or sperm with a damaged plasma membrane is stained red with propidium iodide. Sperm viability was expressed as the percentage of live sperm with intact plasma membranes.

Sperm Motility

Total motility and progressive motility (PMOT) were assessed using a computer-assisted semen analysis (CASA) system (Hamilton Thorne Biosciences, version 10 HIM-IVOS, Beverly, MA). This system was set up as described by Chauychu-noo et al. (2021) using the following settings: frames per second, 60 Hz; minimum contrast, 25; minimum cell size, 4 pix. A sperm was defined as non-motile at the average path velocity was less than 5 µm/s, and sperm was considered progressively motile when the average path velocity was greater than 20 µm/s, and the straightness index was 80. For each assessment, pooled semen samples were diluted at 1:10 (v/v) as required for each extender. Thereafter, 3 µL of the diluted sperm solution was loaded into a prewarmed (38°C) counting chamber. At least 5 fields were evaluated, with a minimum of 300 sperm cells per sample. The total motility and progressive motility were recorded.

Fertility

The fertilizing ability in Experiment 2 was tested using single intravaginal insemination of the layer hens at d 0 with 0.1 mL of extended semen from each group. Insemination was performed between 3:00 pm and 5:00 pm. Three replicates of the fertility test were performed at 15-d intervals, and the females underwent different treatments for each replication. Eggs were collected daily since d 2 after insemination. They were stored on paper trays at a temperature range of 22 to 25°C. They were incubated weekly to determine fertility and hatchability on d 7 (by candling) and 21 of incubation, respectively. Fertility rates were calculated using the formula: (total number of fertile eggs/total number of incubated eggs) × 100. The percentage of hatchability was calculated using the following formula: (total number of chicks/total number of incubated eggs) × 100.

For Experiment 3, the fertility on smallholder farms and the fertilizing ability of the semen were determined as mentioned above, except that all hens were inseminated once every 7 d. The data continued to be collected for 20 wk of insemination.
Statistical Analysis

Experiment 1 had a split-plot design that considered a 3 x 2 factorial design in a completely randomized design (CRD). The treatments consisted of 3 factors and 6 replicates each. Factor A consists of a1 = NaCl, a2 = IGGKPh, and a3 = NCAB; factor B consists of b1 = 5°C and b2 = 25°C; and factor C consists of c1 = 0 h, c2 = 12 h, and c3 = 24 h of semen storage. The observation values consisted of total motility, progressive motility, and sperm viability were recorded. Statistical analysis was performed using ANOVA (Proc ANOVA). Tukey’s test was used to determine the differences in semen quality parameters, and P < 0.01 was considered statistically significant. The results were analyzed using the statistical software program SAS version 9.1.

Experiments 2 and 3 were CRD, and the treatment mean differences were compared using Tukey’s test with a significance level of 0.05 (P < 0.05).

RESULTS

Experiment 1: Effects of Storage Temperature (at 5°C and 25°C) and Different Semen Extenders on Sperm Quality During Storage for Up To 24 h

The interaction effect of duration, treatment, and storage temperature on all sperm parameters was highly significant (P < 0.01; Table 2).

Table 2 shows the total motility, progressive motility, and viability parameters of rooster semen diluted with 3 extenders and stored at 5°C and 25°C for up to 24 h. At T0 of storage, the high total motility and progressive motility were observed in semen diluted with all extenders when stored at 5°C. Meanwhile, the highest viability was found only in the NCAB group after storage at both 5°C and 25°C (P < 0.01). In contrast, semen diluted with NaCl solution and stored at 25°C exhibited the lowest total motility, progressive motility, and viability rates (80.7, 59.9, and 75.6%, respectively; P < 0.01).

At T12 of storage, the best motility parameters were observed in semen diluted with IGGKPh and NCAB extenders and stored at 5°C (P < 0.01), whereas the progressive motility was <50% for semen diluted with IGGKPh and NaCl and stored at 25°C (40.0% vs. 28.7%). The viability of the NCAB group was higher than that of the IGGKPh group at both 5°C and 25°C (90.6% and 87.9% vs. 79.0% and 75.9%, respectively; P < 0.01). The lowest viability was observed in NaCl stored at 25°C (60.7%; P < 0.01).

At T24 of storage, slight decreases in progressive motility and viability were observed at 5°C, except in the NaCl group which all considered parameters decreased significantly (P < 0.01). Meanwhile, all sperm cells in the NaCl group stored at 25°C were dead after 24 h of storage. Therefore, data at that level were not included in any of the tables. Interestingly, all sperm parameters in the NCAB group stored at 5°C at T12 and T24 were not different from those observed at T0 (P > 0.01).

As there was an interaction among treatments, temperature, and durations of storage, semen samples diluted with the 3 extenders and stored at 5°C were selected to study the fertility test in Experiment 2.

Experiment 2: Effects of Different Extenders on Fertility

Figure 1 shows the fertility in the 3 treatment groups for 14 d after each act of insemination. During the first 2 d after AI, the fertility rates between NCAB and IGGKPh groups were not different (P > 0.05) while the fertility rates in the NaCl group were lower compared with the others (P < 0.05). The highest fertility rate from day 3 onward was observed in the NCAB group (P < 0.05), whereas the lowest fertility rate was found in the NaCl group (P < 0.05).

Table 2. Mean values of total motility, progressive motility (PMOT), and viability of semen diluted with three different extenders and stored for 24 h (T0, T12, and T24) at 5°C and 25°C.

| Extenders | 5°C | 25°C | 5°C | 25°C | 5°C | 25°C | SEM | Time of storage | Treatment | Temperature | Time of storage x Treatment x Temperature |
|-----------|-----|-----|-----|-----|-----|-----|-----|----------------|-----------|------------|----------------------------------------|
| NaCl      |     |     |     |     |     |     |     |                |           |            |                                        |
| 0         | 95.3<sup>a</sup> | 80.7<sup>b</sup> | 95.8<sup>a</sup> | 89.8<sup>c</sup> | 96.7<sup>a</sup> | 93.6<sup>a</sup> | 0.24 | <0.01          | <0.01     | <0.01      | <0.01                                   |
| 12        | 81.8<sup>c</sup> | 61.4<sup>d</sup> | 87.2<sup>c</sup> | 75.1<sup>e</sup> | 89.8<sup>c</sup> | 89.0<sup>c</sup> |     |                |           |            |                                        |
| 24        | 68.5<sup>e</sup> | N/A     | 83.7<sup>c</sup> | 71.6<sup>d</sup> | 88.8<sup>c</sup> | 86.0<sup>c</sup> |     |                |           |            |                                        |
| IGGKPh    |     |     |     |     |     |     |     |                |           |            |                                        |
| 0         | 65.4<sup>c</sup> | 59.9<sup>d</sup> | 64.9<sup>c</sup> | 65.4<sup>c</sup> | 65.0<sup>c</sup> | 63.4<sup>c</sup> | 0.36 | <0.01          | <0.01     | <0.01      | <0.01                                   |
| 12        | 59.3<sup>d</sup> | 28.7<sup>e</sup> | 64.1<sup>c</sup> | 40.0<sup>d</sup> | 66.4<sup>c</sup> | 58.6<sup>c</sup> |     |                |           |            |                                        |
| 24        | 48.1<sup>e</sup> | N/A     | 57.8<sup>c</sup> | 33.3<sup>d</sup> | 64.5<sup>c</sup> | 53.9<sup>c</sup> |     |                |           |            |                                        |
| NCAB      |     |     |     |     |     |     |     |                |           |            |                                        |
| 0         | 80.2<sup>c</sup> | 75.6<sup>d</sup> | 82.4<sup>c</sup> | 79.4<sup>ac</sup>| 90.5<sup>c</sup> | 92.9<sup>c</sup> | 0.30 | <0.01          | <0.01     | <0.01      | <0.01                                   |
| 12        | 74.6<sup>c</sup> | 60.7<sup>d</sup> | 79.0<sup>c</sup> | 75.9<sup>c</sup> | 90.6<sup>c</sup> | 87.9<sup>c</sup> |     |                |           |            |                                        |
| 24        | 65.6<sup>c</sup> | N/A     | 81.7<sup>c</sup> | 73.5<sup>d</sup> | 88.7<sup>c</sup> | 86.0<sup>c</sup> |     |                |           |            |                                        |

<sup>a,b,c</sup>Different letters within a row indicate significant differences (P < 0.01). N/A: data unavailable as sperm was considered dead.
The effects of different extenders on fertility and hatchability for 2 periods following AI (d 1−7 and 8−14) are presented in Table 3. The results showed that both the fertility and hatchability rates of semen samples diluted with NCAB were the highest for both periods. In the first period, the fertility rates did not differ between the NaCl and IGGKPh groups (P > 0.05), but the fertility rates were lower in the NaCl group in the second period (P < 0.05), and the hatchability rates did not differ between the 2 periods (P > 0.05).

**Experiment 3: Effects of the Best Extender on Fertility in Practical Use**

The results of the fertility tests between semen diluted with NaCl and NCAB are shown in Table 4. The fertility and hatchability rates were better for the group that was inseminated with semen diluted with NCAB than those of the group inseminated with semen diluted with NaCl (P < 0.01).

**DISCUSSION**

In the present study, we attempted to identify a novel semen extender that can help preserve rooster semen for up to 24 h (with minimal impairment of sperm quality and fertilizing capacity) and is suitable to be used by smallholder farms under field conditions. Our results showed that the interaction effect of duration, treatment, and storage temperature on all sperm parameters was highly significant. The NCAB extender improved rooster semen quality during storage for 24 h at 5°C. However, the sperm diluted in saline solution could not survive 24 h of storage at 25°C. The fertility and hatchability rates of semen samples diluted with the NCAB extender were higher than those diluted with other extenders. In addition, the fertilizing capacity of semen samples diluted with the NCAB extender under field conditions was satisfactory.

Dilution of rooster semen immediately after semen collection is necessary to maintain sperm viability; otherwise, the sperm would die from dehydration (Lake and Stewart, 1978). However, this procedure results in increased sperm respiration due to the high availability of nutrients in semen extenders (Clarke et al., 1982). Hence, a reduction in sperm metabolism by storing them...
at low temperatures, without seriously decreasing semen quality, is required for semen storage. Even the low temperature of a refrigerator (2–5°C) is highly recommended to store rooster semen for up to 24 h (Mohan et al., 2013; Slanina et al., 2015). However, refrigeration might not be available to local farmers with no electricity. Therefore, insemination after semen collection and processing under practical conditions (ambient temperature; 25°C) are now being conducted (Adebisi and Ewula, 2019). In the present study, different storage temperatures (5°C and 25°C) were examined for additional advantages under different conditions. As in other reports, the average motility and viability rates at the same durations during storage at 5°C were higher than those at 25°C, except when the NCAB extender was used for dilution (Table 2).

In the present study, the live sperm cells of the saline solution group were fewer than those in the other groups and were found dead when stored at 25°C for 24 h. This is because the NaCl solution did not contain any nutrients that could have aided the respiration process of the sperm, and the osmolarity and pH were not balanced. Generally, semen extenders should have a balanced osmolarity in the range of 320 to 450 milliosmoles and a pH range of 6.8 to 7.4; however, the pH of saline solution is 5.5. In addition, energy substrates, such as sugar, are necessary to prolong sperm viability during sperm storage (Gibb and Aitken, 2016). Adebisi and Ewula (2019) reported that the sperm motility of semen diluted with saline was <50% after 4 h of storage at ambient temperature. Vasiček et al. (2015) also suggested the suitability of saline solution for semen dilution for 4 h of storage at low temperatures. This result indicates that the use of saline solution is not sufficient to prolong the survival of stored semen. In addition, the energy for metabolism and the buffering capacity provided by semen diluents is necessary to prevent an increase in the metabolic activity of the spermatozoa and enhance their motility (Tvrdá et al., 2013).

During sperm storage, sperm are exposed to several sources of oxidative stress from dilution and cooling incubation. Furthermore, the high concentration of polyunsaturated fatty acids in sperm cells readily undergo lipid peroxidation in the presence of reactive oxygen species (ROS) (Cerolini et al., 2006; Partyka et al., 2012), which is a consequence of plasma membrane dysfunction, and finally leads to decreased fertilizing ability (Alvarez and Storey, 1982). Therefore, sperm plasma membranes must be protected from free radicals. The addition of antioxidants, such as vitamin E (Tabatabaei et al., 2011) and glutathione (Shamiah et al., 2017), can positively affect ROS production during liquid storage. In the present study, we found that sperm motility and viability with the NCAB extender were more significant than those with the other extenders for at least 24 h at different storage temperatures. Moreover, the sperm quality, in terms of progressive motility and viability, with the NCAB extender after storage at 5°C did not differ among the groups at different durations. Furthermore, the semen quality was maintained similarly at 12 and 24 h after storage at 25°C. This might be highly beneficial as the antioxidant of serine (Thananurak et al., 2020), even in sperm cells stored for longer durations and at higher temperatures, results in increased ROS production. Serine improves glutathione peroxidase activity (Wang et al., 2016) by supporting glutathione synthesis and the methionine cycle, mostly by condensing with homocysteine to synthesize cysteine and providing one-carbon units for homocysteine remethylation (Zhou et al., 2017a). This process can alleviate hepatic oxidative stress by upregulating the expression of glutathione synthesis-related genes, increasing glutathione concentration (Zhou et al., 2018b), decreasing malondialdehyde concentration, and increasing superoxide dismutase, GSH-Px, and catalase concentrations (Zhou et al., 2018a).

The fertilizing ability is related to the quality of diluted and stored semen. Once sperm cells are introduced into the female’s cloaca, they are transported to the uterine junction of the oviduct and stored for a longer period in the sperm storage tubes (SSTs), depending on the species (Holt and Fazeli, 2016) or the characterization of these regions (Kheawkanha et al., 2021). Generally, SSTs hold a high number of sperm for 24 to 48 h after insemination with less than 2% of an inseminated dose of 100 to 200 million sperm (Brillard, 1992). Once sperm are in the SSTs, they must maintain their position against the fluid current generated by the SST epithelial cells. Motility is also involved in their release to fertilize the egg (Froman, 2003). Therefore, motility is the key to the success of sperm reaching the SSTs (Bakst et al., 1994). However, because rooster sperm contain little intracellular energy reserves, extracellular energy is important for their metabolism and mobility (Blesbois, 2012). This might be a reasonable explanation for the lower fertility of the NaCl group sperm. The IGGKPh and NCAB groups contained energy substances that could promote sperm motility in vivo; therefore, their fertilizing ability was higher than that of the saline solution. However, sperm survival in the female reproductive tract seemed longer in the NCAB group than in the IGGKPh group, resulting in the highest fertility rate after a single act of AI (Table 3). Therefore, we infer that free radicals and peroxides from sperm metabolism during storage in the SSTs were scavenged by serine.

In the present study, a new semen extender that prepare as easy to use was introduced to local farmers. When used by farmers under field conditions, the fertilizing capacity of sperm diluted with the NCAB extender was better than that of sperm diluted with NaCl (Table 4). Moreover, the farmers were satisfied, with less time required once weekly instead of twice weekly for the insemination procedure. The results of our on-farm experimental trials imply that farmers can effectively utilize the novel extender.

CONCLUSIONS

Our findings showed that the NCAB extender supplemented with serine and stored at a low temperature
(5°C) maintained rooster sperm quality for up to 24 h. The fertility and hatchability rates of semen samples diluted with the NCAB extender were higher than those diluted with other extenders. In addition, the fertilizing capacity of semen samples diluted with the NCAB extender under field conditions was satisfactory. Nevertheless, further studies are required to examine the fertilizing capacity of semen diluted with the NCAB extender and stored at ambient temperature (25°C) for up to 24 h; this is because minor decreases in total motility and viability within 24 h of storage at 25°C were observed when compared with quality parameters of sperm stored at 5°C.

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DISCLOSURES

This manuscript has not been published or submitted for publication elsewhere. The content does not impose any conflict and all the authors are agreeable in the manuscript content.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102188.
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