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Research Article
The Effect of Different Extenders on Some Fertility Properties of Roosters Semen

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Abstract

Background and Objective: Poultry sperm gradually loses its quality within an hour of sample collection. This study aimed to determine the storage effects of different extenders (at 4°C) on rooster sperm fertility properties. **Materials and Methods:** Eighteen seminal fluid samples of a Lohman Brown strain were collected by dorsal-abdominal massage. These were divided into three groups and added to three extenders: a simple medium for assisted reproductive technology (SMART), Tris and milk. Since non-diluted fresh sperm loses its quality within an hour of semen collection, no control group was used. Percentages of mass activity, motility, normality and viability were tested. These were done at 1, 4 and 8 h after semen collection. Completely Random Design (C.R.D) was used in this experiment. **Results:** At the first timepoint, the semen test score of the SMART medium extender was higher than that of the other extenders. In contrast, the milk extender shows a highly significant improvement in sperm parameters post preservation at both the second and the third time tests. The SMART extender was protecting sperm vigor more than Tris at most of the tested timepoints. **Conclusion:** Extenders delay the loss of rooster sperm fertilization ability. Full milk extender was better than other extenders in the protection of sperm fertility.

Key words: Mass activity, milk extender, rooster sperm, semen motility, semen viability

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry semen is highly concentrated (billions of sperms per ml) viscous fluid. Therefore, it needs to be diluted before being used for artificial insemination[1]. Semen dilutions improve the processing of semen tests such as their concentration, motility, abnormality, vitality and pH[2]. Extender solutions maintain the functional proprieties of sperms and increasing the semen volume[3]. The optimal osmotic pressure for rooster semen extender is between 325 and 350 mOsmol kg^{-1} [4]. Artificial insemination success depends on the efficiency of semen collection and storage[5]. Artificial insemination is better than the natural mating for broiler breeder parents stock[6]. Sperms storage of chickens and turkeys without dilution is inefficient processes[7]. As the dilution ratio increase, the negative effect on sperm motility in guinea fowls increases, as well[8]. A dilution rate of 1/10 was shown to affects sperm quality, negatively[9]. Extenders with different ingredient are used to sustain sperm vitality, such as glycerol and egg yolk[10], lactated Ringer's glucose, glucose-Tris-glucose and lactated Ringer's extenders[11], skim milk and Tris-citrate extenders[12], orange juice[13] and vitamins of A, C and E[14]. This study was conducted to determine the efficiency of the understudy extenders in preserving rooster semen under short-term storage (1, 4 and 8 h). These were done by evaluating physical semen proprieties.

MATERIALS AND METHODS

This experiment was conducted in the College of Agriculture-the University of Al-Qadisiyah, Iraq. Eighteen Lohman Brown roosters 1-year-old were used. The roosters were divided randomly into three groups and trained for having semen. It collected by dorsal-abdominal message two weeks in advance. Extenders were assigned randomly to each semen group. Three extenders were used:

SMART extender[15]: A ringer solution composed of 29 mmol L^{-1} bicarbonate, 3.2 g sodium lactate, 6.0 g sodium chloride, 0.4 g potassium chloride and 0.27 g calcium chloride per liter of distilled water. Specific chemicals were applied to this solution, including 0.5 g of red phenol, 0.01 g of sodium pyruvate and human serum albumin (HSA, 20%) and stored in a special non-toxic bottle.

Tris extender[16]: The semen diluent was prepared by dissolving 3.8 g Tris, 2.2 g citric acid and 0.6 g glucose into 100 mL of distilled water.

Full cream milk[17]: Every 100 mL of full cream milk contains 3.2 g total fat (saturated fat of 2.17 g and unsaturated fat of 1.03 g), 7.53 mg cholesterol, 5.4 g lactose, 3.2 g protein, 0.01 g dietary fiber and 0.05 g sodium.

The dilution ratio was 1:3 (1 semen: 3 extenders). Dilutions were sterilized with streptomycin (100 mg) and penicillin (100,000 IU). Data were taken at 1, 4 and 8 h after semen collection.

Evaluation of semen: Mass activity, sperm motility, sperm viability and normality percentage were estimated according to Blom and Christensen[18], Chemineau *et al.*[19], Hancock[20] and Swanson and Bearden[21] methods, respectively.

Statistical analysis was performed using the SPSS18 software according to Completely Random Design (C.R.D). The Duncan multiple ranges test was used to compare differences among means. Significance levels of $p \leq 0.05$ and $p \leq 0.01$ were used.

RESULTS

The result showed the effect of different extenders after an hour of semen collection (Table 1). The SMART extender scored significantly higher in mass activity and sperm viability tests compared to the milk extender ($p \leq 0.05$). Also, the SMART medium outperformed the Tris and milk extenders in the individual motility test. The Tris extender had significantly higher values more than the milk dilution for the same test. This shows non-significant differences among the extenders at $p \leq 0.05$, in accordance with the sperm normality (%) test. Highly significant differences among extenders at ($p \leq 0.01$)

Table 1: The effect of tested extenders on rooster sperm parameters at 1 h after collection

Parameters	SMART medium	Tris	Milk	p-value
Mass activity (%)	92.667 ± 1.45^{A}	90.667 ± 0.67^{AB}	88.333 ± 1.67^{B}	0.1500NS
Individual motility (%)	88.333 ± 1.67^{A}	82.333 ± 1.45^{B}	79.000 ± 1.00^{C}	0.0090**
Sperm normality (%)	93.333 ± 1.67^{A}	92.333 ± 1.45^{A}	91.667 ± 1.67^{A}	0.7684NS
Sperm viability (%)	94.333 ± 0.67^{A}	90.000 ± 2.89^{AB}	87.000 ± 1.53^{B}	0.0910NS

Different letters denote to significant differences at $p \leq 0.05$. Similar letters denote to non-significant differences at $p > 0.05$, **High significant different ($p \leq 0.01$), NS: No significant differences ($p > 0.05$)

Table 2: The effect of tested extenders on rooster sperm parameters at 4 h after collection

Parameters	SMART medium	Tris	Milk	p-value
Mass activity (%)	70.667±0.67^{C}	63.667±0.88^{B}	82.667±1.45^{A}	0.0006**
Individual motility (%)	66.333±0.88^{B}	59.333±0.67^{C}	74.333±2.33^{A}	0.0012**
Sperm normality (%)	83.333±1.67^{B}	74.667±1.45^{C}	88.000±1.53^{A}	0.0025**
Sperm viability (%)	76.667±0.88^{B}	63.000±1.53^{C}	81.000±1.00^{A}	0.0001**

Different letters denote to significant differences at p≤0.05. Similar letters denote to non-significant differences at p>0.05, **High significant different (p≤0.01)

Table 3: The effect of tested extenders on rooster sperm parameters at 8 h after collection

Parameters	SMART medium	Tris	Milk	p-value
Mass activity (%)	62.000±2.00^{A}	41.667±0.88^{B}	66.667±1.67^{A}	0.0001**
Individual motility (%)	57.667±1.45^{B}	37.667±1.45^{C}	62.667±2.67^{A}	0.0002**
Sperm normality (%)	53.333±1.67^{B}	42.000±1.15^{C}	61.667±1.67^{A}	0.0003**
Sperm viability (%)	60.667±0.67^{B}	45.667±1.20^{C}	74.667±1.33^{A}	0.0008**

Different letters denote significant differences at p≤0.05, Similar letters denote non-significant differences at p>0.05. **High significant different (p≤0.01)

only in the individual motility test was observed. After 4 h of semen collection, the milk extender outperforms the other extenders at protecting sperm (Table 2, p≤0.01). At this test time, the SMART outperformed the Tris extender in the individual motility, sperm viability and sperm normality tests. In contrast, the Tris extender outperformed the SMART medium extender in the mass activity test. Full milk dilator had significantly higher values (p≤0.01) as compared to the other extenders. The SMART medium extender significantly outperforms the Tris extender in all tests (Table 3).

DISCUSSION

Extenders delay may cause loss of rooster sperm fertilization ability but the tests values decrease as time increases. This is consistent with the findings of Hudson *et al.*[8], who reported that the storage period at 5°C has a significant influence on sperm motility in guinea fowls. Sperm motility and viability gradually decline after collection[12]. SMART medium performed best at 1 h after collection. This is because the SMART medium includes serum albumin, which acts as an antioxidant and pyruvate, which acts as an energy source[22,23]. However, at 4 or 8 h after collection, the full milk extender outperformed the other extenders. This is in line with the findings of Rahman, who reported that milk dilution significantly increases sperms viability as compared to the Tris extender in both viability (%) and sperm motility (%) tested at different periods in ram[12]. This might be because the sperm contains low cholesterol, phospholipid and low protein phospholipid[24]. Fatty acids affect membrane liquidity[25,26]. The cholesterol and fatty acid contents increase in the plasma membranes that have a low percentage, causes the membranes to become more resistant to oxidative damage that resulted in increasing protection. Milk casein decreases damage to cell membrane lipids and improves sperms motility and viability[27,28].

CONCLUSION

The poultry industry is always searching for new sublimates and extenders to improve the efficiency of prolonging sperms life and activity. This study examined the storage effects of different extenders on roosters sperm fertility properties. Extenders delay the deficiency of roosters sperms fertilization ability. Birds semen dilutions make work with poultry breeding much easier and allow for the insemination a large number of females. The inclusion of milk dilator better protects sperm fertility compared to the SMART and Tris extenders.

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