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Short Communication

Addition of Tomato Juice as Additive in Diluent of Egg Yolk Citrate on the Quality of Pesisir Cattle Semen

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Abstract | This study aims to determine the effect of adding tomato juice as an additive in egg yolk citrate diluent on the quality of Pesisir cattle semen stored at 5°C. The study was conducted on freshly collected Pesisir cattle semen diluted in egg yolk citrate with or without tomato juice viz., P0 (100% egg yolk citrate), P1 (90% egg yolk citrate + 10% tomato juice) and P2 (80% egg yolk citrate + 20% tomato juice). After diluting the semen samples were stored at 5°C for 4 hours. The observed variables included spermatozoa motility, spermatozoa survival, spermatozoa abnormalities and intact plasma membranes of spermatozoa. The obtained data was analyzed using the Analysis of Variance (ANOVA). The results showed that the addition of tomato juice extract into egg yolk citrate diluent exhibited a 20% increase in sperm motility percentage, 26.3% in sperm survival rate and 22.5% in intact plasma membrane percentage as compared to the control group ($P < 0.01$). However, the sperms abnormality data did not show any effect ($P>0.05$) of tomato juice addition. The addition of 20% tomato juice extract in egg yolk citrate diluent showed better results as compared to the 10% tomato juice addition. In conclusion, tomato juice addition in egg yolk citrate diluent is beneficial to improve the sperm quality of Pesisir cattle semen stored at 5°C.

Keywords | Tomato juice, Egg yolk citrate, Pesisir cattle, Semen, Sperm quality

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INTRODUCTION

Pesisir cattle are native Indonesian cattle whose distribution is specifically in the Province of West Sumatra. These cattle have advantages over other cattle because the Pesisir cattle have a very high body resistance and environmental adaptation (Udin et al., 2022). However, every year there is a decline in the population of Pesisir cattle that requires special attention to adopt strategies for saving and increasing the population of Pesisir cattle. Artificial Insemination (AI) is the first reproductive technology in animal husbandry that at first recognized to improve the genetic quality of local cows and further for increasing the population (Afriani et al., 2022; Udin et al., 2022).

The AI process must be carried out using sperm from superior bulls. Budhiyadnya et al. (2021) stated that the criteria for high intensity mating in Pesisir bulls were: 36-48 months age, 348±3.18 kg weight, 134±1.73 cm height, 12.62±3.77 ng/ml testosterone level, 32±0.87 cm scrotal boundary and good semen quality. High-quality semen from the selected bulls is needed for cryopreservation and insemination of the cows (Afriani et al., 2022).

The processing and dilution of semen can reduce the quality of sperm, due to contact with air so that lipid peroxidation reactions occur which can cause damage to the plasma membrane. This damage is caused by the formation of free radicals from spermatozoa metabolism, one of which is the

level of Reactive Oxygen Species (ROS). If the initial ROS reaction is not controlled, a continuous reaction (autocatalytic) will occur (Castleton et al, 2022).

The high levels of ROS can be overcome by adding additives containing antioxidants to the semen diluent. High level of antioxidants can be found in tomatoes (*Solanum lycopersicum*). The antioxidant content in tomatoes is a carotenoid compound called lycopene. According to Babaei et al. (2021), lycopene has a unique chemical structure, namely acyclic beta-carotene without provitamin A activity and is more efficient for scavenging free radicals than other carotenoids. In addition, Li et al. (2021) stated that the juice from tomatoes contains various nutrients such as carbohydrates, protein, vitamin A, vitamin C and lycopene which function as antioxidants.

Carbohydrates and antioxidants found in tomato juice serve as an energy source and scavenge free radicals that can damage cells. Rosmaidar et al. (2013) stated that the addition of 20% tomato juice in 100 ml of egg yolk citrate diluent used to dilute Boer goat semen resulted in the best quality of spermatozoa after 72 hours of storage. The current study describes the effect of adding different doses of tomato juice in egg yolk citrate diluent on the semen quality of Pesisir cattle stored at 5°C.

MATERIALS AND METHODS

Study approval

This research was conducted according to animal ethical guidelines and prior approval at the Laboratory of Animal Biotechnology, Faculty of Animal Husbandry, Andalas University.

Semen collection

The fresh semen were collected from adult Pesisir bulls (n=3, aged 3 years) using a set of artificial vaginal apparatus at 42°C. Two ejaculates were collected from each bull once a week and transferred to the laboratory for initial checks. Fresh semen of Pesisir cattle was tested macroscopically and microscopically immediately after collection. Macroscopic examination includes volume, pH, color, consistency and odor. While microscopic examination includes mass movement, motility, concentration, viable percentage, abnormalities of intact plasma membrane and intact acrosomal hood (Afriani et al., 2022). Qualifying semen samples were pooled to obtain sufficient semen that were divided into the three groups for further study.

Semen processing

This study used a randomized block design (RBD) with 3 treatments and 5 replications. The total number of samples of bull semen were n= 15, and each sample was collected in 2 ml quantity. The egg yolk citrate diluent was prepared according to the procedures of Luna-Orozco et al. (2019). The tomato juice was prepared by using fresh tomatoes that were washed thoroughly, then cut into cubes. These were put into a blender and blended until smooth. After blending, the tomatoes were filtered using a multifold cheese cloth.

The egg yolk citrate diluent was used with or without tomato juice to dilute the semen. P0: 100% egg yolk citrate diluent, P1: 90% egg yolk citrate diluent + 10% tomato juice extract and then homogenized, P2: 80% egg yolk citrate diluent + 20% tomato juice extract and then homogenized.

Statistical analysis

Each treatment was analyzed using the one way ANOVA (analysis of variance) method. If the treatment showed significantly different results ($P < 0.05$), it was further tested using Duncan's Multiple Range Test (DMRT) according to Steel and Torrie (1995). The analysis was carried out using Microsoft Excel program. The mathematical model for the experimental design is as follows:

$Yij = \mu + \tau i + \beta j + \epsilon ij$

Where:
Yij = Observation value
μ = Mean value of observation
τi = Effect of the i-th treatment
Bj = Effect of the j-th group
ϵij = Effect of error (residual)

RESULTS AND DISCUSSION

Quality of Fresh Pesisir Cattle Semen

The quality characteristics of fresh semen of Pesisir cattle were presented in Table 1. The semen quality of Pesisir cattle was examined in ejaculations 1 to 5. The volume of semen of Pesisir cattle ranged from 2 ml to 4 ml. This result is lower than the semen of Munshiganj cattle which ranges up to 4.93 ml (Hossain et al, 2022), however it is higher than the other local cattle such as Bali cattle which has an average of 3.4 ml (Susilawati et al. 2018). Low or high volume does not cause harm, but if the concentration is low, it will cause a limited number of spermatozoa in one ejaculate (Wafa et al., 2010).

The color of the Pesisir cattle semen sample shows the milky white to creamy as the dominant color (Table 1). According to Bearden et al. (2004), the normal color of cow semen is milky white, however about 10% samples have a cream color. These results are also similar to those reported by Dwitya et al. (2019) who reported that Aceh

Table 1: Evaluation of the quality of Pesisir cattle fresh semen

Variables	Quality Characteristics of various ejaculates					
	1	2	3	4	5	Mean
Volume (ml)	4	4	2	2	2	2.8
Color	Milky White	Milky White	Milky White	Cream	Cream	-
Consistency	Thick	Thick	Thick	Medium	Medium	-
pH	7	7	7	7	7	7
Mass Motility	+++	+++	+++	++	++	-
Motility (%)	80	80	80	70	70	76.00
Viability (%)	82.31	84.46	89.60	74.28	76.47	81.42
Abnormality (%)	5.80	5.70	5.06	6.92	5.70	5.84
Intact plasma membrane (%)	81.00	83.80	84.00	78.00	83.02	81.96
Concentration (million/ml)	1140	1190	1365	850	940	1097

cattle semen was a milky white color. The consistency of Pesisir cattle semen ranges from medium to thick (Table 1). The thick consistency, the higher the concentration of spermatozoa (Afriani et al., 2022).

The observed consistency of Pesisir cows from 5 ejaculations had similar results to those reported by Novita et al. (2021), that the consistency of Bali cattle semen ranges from thick to watery. The degree of acidity or pH is a factor that determines the life status of spermatozoa. If the pH is lower than normal, it will cause spermatozoa to die quickly. According to Toelihere (1985), the neutral pH in cattle and sheep semen is around 6.2-7.5. The concentration of spermatozoa obtained ranged from $850x10^6$-$1365x10^6$ /ml of semen with an average of $1097x10^6$ /ml of semen (Table 1). Tolihere (1985) described that the average concentration of spermatozoa cells in bovine semen ranged from 30-$250x10^7$ /ml of semen. Spermatozoa concentration calculations is necessary to determine the amount of diluent to be used.

Mass movement was indicated by the sign (+). This parameter was recorded by observing the mass waves using a microscope. The mass movement of Pesisir cattle samples in this study was ++ to +++ (Table 1). This result indicated that semen samples were fit to carry out further processing because the previous research showed that ++ means semen is good for the AI processing (Partodihardjo, 1992). The individual movement/motility of the Pesisir cow sperms obtained in this study had an average of 76% (Table 1). This result was aligned with a study by Malik (2018) who discovered the 71.83% motility rate in Bali cattle of Indonesia. The little difference in results may be caused by differences in species, age, feed, management, frequency of semen collection and/or collection techniques (Hafez, 2000).

The percentage of live spermatozoa of Pesisir cattle in this study was obtained 81.42% (Table 1). This finding is relatively the same as reported earlier by the Malik (2018) who reported that the average percentage of live spermatozoa in Bali cattle was 81.59% and according to Chung et al., (2019) who reported the 72.25% percentage of live spermatozoa in Jersey cattle. The percentage of live spermatozoa has a higher yield than the percentage of motility because motile spermatozoa are definitely alive, but live spermatozoa are not necessarily motile (Partodihardjo, 1992).

The abnormality of the semen of Pesisir cattle obtained in this study was an average of 5.84% (Table 1). This result was relatively the same as in the Bali cattle reported by Afrianiti (2012) who reported the percentage of abnormality as 6.56%. The integrity of the plasma membrane obtained in this study was 78%-84% with an average of 81.96%. This result was also in line with that reported by the Akhter et al. (2010) in Nili-Ravi buffalo. The percentage of the intact plasma membrane is positively correlated with the level of fertility, the higher the intact plasma membrane, the higher the fertility.

Quality of Semen After Equilibration

After equilibration at 5°C and treated with tomato juice added egg yolk citrate diluent the semen was evaluated for motility, survival percentage, abnormalities and intact plasma membrane (Table 2).

The highest average motility was found in P2 (72.00±4.47%) with the addition of tomato juice extract with a concentration of 0.2 ml into 0.8 ml of egg yolk citrate diluent of. While the lowest percentage was recorded in P0 (60.00±0.00), the group without the addition of tomato juice extract/100% citrate Egg yolk (control) (Table 2).

Based on the results of ANOVA analysis, it was observed that the addition of tomato juice extract to egg yolk citrate diluent significantly ($P<0.01$) increased the percentage of

Table 2: Quality of Pesisir cattle semen with addition of tomato juice in egg yolk citrate diluent after equilibration.

Variables	Treatments		
	P0	P1	P2
Motility (%)	60.00±0.00^{A}	70.00±0.00^{B}	72.00±4.47^{C}
Viability (%)	62.79±4.11^{A}	76.19±4.55^{B}	79.30±4.54^{B}
Abnormality (%)	6.04±1.34	6.98±1.48	6.22±0.93
Intact plasma membrane (%)	65.60±3.71^{A}	78.03±2.33^{B}	80.39±2.29^{B}

Description: Superscripts with different capital letters in the same row showed a very significant difference (P<0.01).
* P0: 100% egg yolk citrate diluent, P1: 90% egg yolk citrate diluent + 10% tomato juice, and P2: 80% egg yolk citrate diluent + 20% tomato juice.

spermatozoa motility in Pesisir cattle semen after equilibration. DMRT test further showed that the treatment was very significantly different between P0, P1 and P2 (P<0.01). The addition of 20% tomato juice extract (P2) was able to maintain better motility than control (P0). These results are relatively similar to the research of Rosmaidar et al. (2013) who used different tomato juice 0%, 20%, 40%, and 80% in egg yolk citrate diluent for boer goat semen diluent, the best result was the addition of 20% tomato juice in egg yolk citrate diluent. This is because the tomato juice extract contains an antioxidant in the form of lycopene which can reduce levels of ROS and peroxide radicals that are responsible to reduce the motility of spermatozoa stored in vitro. Antioxidants are very important to reduce the negative effects of ROS that can cause damage to spermatozoa cells (Pryor et al., 2000). Tvrdá et al. (2016) descibed that, in order to protect cells and tissues from the damaging effects of lipid peroxidation, lycopene can stabilize the molecular element oxygen (O) and stop the effects of peroxide radicals.

Based on the results of Table 2, the percentage of live spermatozoa of Pesisir cattle after the addition of tomato juice extract in egg yolk citrate diluent was highest i.e., 79.30±4.54%. While the lowest result was recorded in control group (P0) i.e., 62.79±4.11%. The results of the statistical analysis showed that the differences were significantly different (P<0.01). Based on the results of the DMRT, the treatment P0 with P1 and P0 with P2 was very significantly different (P<0.01). Meanwhile P1 and P2 treatments were not significantly different (P≥0.05). The low percentage of viable spermatozoa in P0 probably due to chemical damage caused by free radicals during equilibration. Lipid peroxidation occur due to a reaction between reactive oxygen species and unsaturated fatty acids that make up the plasma membrane of spermatozoa cells (Susilowati, 2008). The results obtained are also the same as those reported by Rosmaidar et al. (2013) who reported that the best results were by the addition of 20% tomato juice in egg yolk citrate diluent.

The results of observing the abnormality in Pesisir cattle sperms after equilibration were presented in Table 2. The highest abnormality was found in P1 i.e., 6.98±1.48% and the lowest in P0 i.e., 6.04±1.34%. These results are in accordance as to those described by the Tvrdá et al. (2016) who reported that spermatozoa abnormalities have major reduction with the use of tomato juice in egg yolk citrate diluent. Though this parameter was recoded non significant, however, it is exhibited from the overall study data and results of other studies that the addition of tomato juice extract in egg yolk citrate diluent with different levels does not have any deleterious effects on the spermatozoa. Sperm abnormalities could be divided into two categories, namely primary and secondary abnormalities. Solihati et al. (2008) stated that secondary abnormalities occur during passage through the epididymis, vas deferens and urethra and/or contamination with urine.

The results regarding integrity of the plasma membrane of the equilibrated Pesisir cattle semen showed that the highest yield was in P2 i.e., 80.39±2.29% and the lowest was in P0 i.e., 65.60±3.71%. Based on the statistical analysis results, it was found that the addition of tomato juice extract in egg yolk citrate diluent significantly (P<0.01) improved the intact plasma membranes. This result is lower than that reported by Anwar (2004) who obtained the percentage of intact plasma membranes ranging from 88.88% to 92.74%. The results of Duncan's test showed that the percentage of intact plasma membrane after equilibration was very significantly improved (P<0.01) in P1 and P2 as compared to P0 group, while there was no significant difference (P>0.05) between P1 and P2 groups.

CONCLUSION

Based on the results obtained in the current study it is clear that addition of tomato juice extract (10-20%) in the egg yolk citrate diluent is beneficial to improve the sperm quality including motility, viability, and intact plasma membrane in Pesisir cattle. Meanwhile, it could be helpful to reduce the abnormalities in spermatozoa.

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CONFLICT OF INTEREST

All authors state that there is no conflict of interests.

NOVELTY STATEMENT

The findings of this study could be used as a reference or basic information for further research on Pesisir cattle to improve the genetic quality and fertility in this livestock species.

AUTHORS CONTRIBUTION

Tinda Afriani (TA) and Jaswandi (J) conducted research, Adisti Rastosari (AR), Muhammad Cadillac Al Razzak (MC), and Dwiki Wahyudi (DW) collected data, TA wrote the manuscript, while J and AR revised the manuscript.

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