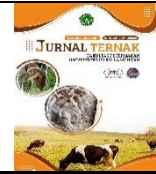




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Preservation of Spermatozoa Sumba Ongole Bulls Using Citrate Yolk Diluent with the Addition of Palmyra Palm Juice

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ABSTRACT

The purpose of this study was to determine the quality of the ability of spermatozoa cells to maintain the use of a yolk citrate (YC) diluent with the addition of palmyra palm juice during preservation. The material used for this research was 2 Sumba Ongole bulls as a source of semen. Semen was collected by using an artificial vagina that is processed twice a week. The obtained semen was macroscopically analyzed, including volume, colour, consistency, pH and smell. Meanwhile, microscopic evaluation includes mass movement, motility, concentration, viability and abnormalities. The semen showed >75% motile sperm divided into four treatments and dilute each with YC (P₀), 95% YC+5% palmyra palm juice (PPJ) (P₁), 90% YC+10% PPJ (P₂), 85% YC+15% PPJ and each was stored at 3-5^oC. The results of statistical analysis showed that P₀, P₁ treatments were significantly different (P<0.05) between P₂, P₃ from day 0 to day 4 of storage. The addition of palmyra palm did not affect the motility of spermatozoa, but had a positive effect on the viability of spermatozoa with the addition of 5% palm sap during storage for 4 days at a temperature of 3-5 ^oC.

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1. Introduction

Spermatozoa preservation is an effort to maintain the quality of spermatozoa Sumba Ongole (SO) bulls. Preservation is carried out to maintain the quality of spermatozoa in order to support the application of technology artificial insemination (AI). The success of AI depends on the quality of semen, inseminator factors, female acceptors and the accuracy of detection of estrus as well as step to maintain semen quality after storage. Fresh semen does not last long in vitro storage, even the use of fresh semen at a temperature of 25-27 C⁰ should not be more than 3 hours [10]. Meanwhile, according to [8], the requirements for semen diluent materials must contain elements similar to the physical, chemical properties of spermatozoa and not be toxic to spermatozoa and the reproductive organs of female livestock. Seeing the requirements of this diluent allows the use of egg yolk citrate diluent as a SO bulls semen diluent which functions as a buffer, to maintain osmotic pressure and electrolyte balance.

Concurrently, palm juice is a source of carbohydrates such as sucrose, glucose and fructose which function as cryoprotectant energy for spermatozoa cells. In Addition, according to [4], reported that palmyra palm juice contains relatively high sugar, namely 10 g/100mL.

According to [14], sugar is a source of extracellular cryoprotectants that protect cells from outside and is used for semen preservation at low temperatures. Conversely, [16] stated that fructose is the basic sugar as an excellent source of energy for spermatozoa through the fructoliasis process. The use of palmyra palm juice as a semen diluent for SO has not been widely reported, whereas palmyra palm juice has the potential to be used as a semen diluent, easy to obtain and relatively inexpensive. This study aims to determine the motility and survival of SO bulls spermatozoa in yellow citrate diluent with the addition of palmyra palm juice with a temperature of 3-5 °C.

2. Results and Discussion

Characteristics of Fresh Semen SO bulls

The characteristics of fresh semen obtained from SO bulls were evaluated macroscopically and microscopically. The results of the semen evaluation can be used as a for dilution according to treatment to determine the motility and viability of the spermatozoa of SO bulls during storage. The data on the characteristics of fresh semen can be seen in table 1 below.

Table 1. Characteristics of Fresh Semen Sumba Ongole bulls

Assessment	Parameters	Mean±SD
Macroscopically	Semen volume (mL)	4.5±0.89
	Consistency	medium category-thick
	Colour	Creamy white
	pH	6.76±0.12
	Smell	Typical bulls SO
Microscopically	Mass activity	+++
	Sperm motility (%)	78.75±3.93
	Sperm concentration (10 ⁶ /cc)	1696.95±525.99
	Live sperm (%)	78.95±3.31
	Sperm abnormalities (%)	14.90±2.63

The results showed that the average volume of semen Sumba Ongole bulls was in the normal category, namely the volume reached 4.05 ± 0.89 ml /ejaculate. The volume obtained in this is lower when compared to the results of the study by [12] which obtained a volume of 5 ml/ejaculate. Simultaneously, the research results reported by [15] showed that the volume of semen was only 2.26 ml/ejaculate. The color of SO bulls semen obtained in this study is creamy white. Semen color is generally milky white or creamy due to the presence of riboflavin which has autosomal recessive properties [16]. Concurrently, the consistency achieved is in the medium category- thick. The average pH obtained was 6.76 ± 0.12 with a typical SO cow odor. This result is low when compared to the results of the study by [2] with an average pH of 6.85. In general, the pH of semen in the normal category ranges from 6.4 to 7.8 [5]. Based on the results of the macroscopic evaluation, cement is suitable for further evaluation at the microscopic evaluation stage.

The results of microscopic evaluation of SO bulls semen showed mass activity ranging from +++, which means that the quality of the cement was good because of the thick waves shaped like thick black clouds, actively and quickly moved. Conversely a study by [12] found that the mass movement

of ongole cattle was +++ . The average motility obtained was $78.75 \pm 3.93\%$. This result is higher than the results of research by [13] with motility reaching 65.46%. Even so, the average motility of the results of the study was in the good category. According to [16] which states that the motility of fresh semen beef cattle ranges between 70-90%. The viability obtained in this study reached $78.95 \pm 3.31\%$. The results of this study are low compared to the results of the study by [12] which obtained a viability of 86.5%, but higher than the results of the study by [15], namely the viability was obtained 73.70%.

The Sperm abnormalities reach $14.90 \pm 2.63\%$. This result is higher when compared to the results of the study by [15] which obtained abnormalities of 7.40%. The level of abnormality obtained in this study is still in the good category. According to [5], the abnormal level of spermatozoa should not exceed 20%. The concentration obtained in this study was 1696.95×10^6 cells / ml. This result is higher when compared to the results of research by [13] which reported an average concentration of 1,079.04 cells / ml. Even so, the semen concentration of the study results was in the normal category. According to [6], the normal average concentration of bovine spermatozoa ranges from 1000-1800 million cells / ml. Based on the results of the macroscopic and microscopic evaluation of fresh semen from SO bulls, it can be continued at the dilution stage according to the predetermined treatment in order to determine the motility of spermatozoa after diluting with yolk citrate and palmyra palm juice.

Effect of Treatment on Motility

Motility is one indicator of the success of spermatozoa cell fertility in Sumba Ongole cattle. The research data can be described in Table 2 below.

Table 2. Percentage of Spermatozoa Motility of SO bulls at a temperature of 3-5 °C

Storage Time (day)	Treatment			
	P ₀	P ₁	P ₂	P ₃
0	78.00±2.74 ^a	79.00±2.24 ^a	73.00±2.74 ^b	72.00±3.94 ^b
1	72.00±4.47 ^a	73.00±2.74 ^a	66.00±4.18 ^b	65.00±5.00 ^b
2	62.00±2.74 ^a	65.00±3.54 ^a	52.00±5.70 ^b	49.00±8.94 ^b
3	55.00±5.00 ^a	57.00±4.47 ^a	46.00±4.18 ^b	42.00±9.08 ^b
4	47.00±4.47 ^a	48.00±7.58 ^a	34.00±8.94 ^b	22.00±10.95 ^c

Different superscript letters show a significant difference ($P < 0.05$)

Table 2 above shows the effect of treatment on spermatozoa motility of SO bulls. The results of statistical analysis showed that the motility of spermatozoa up to the 4th day of storage for treatment P1 reached $48.00 \pm 7.58\%$, the highest compared to treatment P0; P2 and P3 respectively achieved $47.00 \pm 4.47\%$; $34.00 \pm 8.94\%$; $22.00 \pm 10.95\%$. These results indicate that the yolk citrate diluent with the addition of palmyra palm juice was not able to maintain the quality of the cement during the storage process.

Based on the results of ANOVA analysis showed that the treatments were not significantly different ($P > 0.05$) between treatments P0, P1 and P2, P3 from day 0 to day 4 of storage. Nevertheless, SO bulls spermatozoa preservation using yolk citrate diluent with the addition of pamyra palm juice can be used as a SO bulls semen diluent. This is because the composition of the diluent is more complete when compared to other diluents, namely the citrate diluent acts as a buffer source so that the degree of acidity is relatively stable. In addition, palmyra palm diluent is a source of energy for SO spermatozoa so that it is able to maintain the spermatozoa motility of Sumba Ongole cattle until the 4th day of storage.

For the purposes of IB, it is better to use the P1 treatment with the addition of 5% palmyra palm in yellow citrate diluent with the percentage of motility that can only last until the 4 day of storage. In P2 and P3 treatments, 10 and 15% addition of palmyra palm in yolk citrate diluent, respectively, were only able to maintain spermatozoa motility of SO until the 2nd and 1st storage, named spermatozoa motility above 50%. It is suspected that the higher the percentage of lontar sap can reduce the motility of spermatozoa because palmyra palm is acidic so that the role of citrate diluent as a buffer is unable to stabilize pH. According to [7], an increase in pH can result in decreased motility and viability of spermatozoa.

Effect of Treatment on Viability

Viability is the ability of spermatozoa cells to survive during the storage process. Data on the effect of treatment on viability can be described in Table 3 below.

Table 3. Spermatozoa Viability of SO Bulls

Storage Time (day)	Treatment			
	P ₀	P ₁	P ₂	P ₃
0	75,18±3,25 ^a	78,11±3,65 ^a	68,92±2,72 ^b	63.82±2.17 ^c
1	70,58±3,23 ^a	73,52±2,12 ^a	63,45±2,94 ^b	58.11±1.22 ^c
2	64,00±3,05 ^a	67,86±2,57 ^b	53,22±1,46 ^c	48.67±3.34 ^d
3	54,00±5,01 ^a	61,56±5,59 ^b	47.46±0,57 ^c	40.90±4.05 ^d
4	48.19±4,90 ^a	55,98±4,38 ^b	39,15±1,86 ^c	30.32±2.56 ^d

Different superscript letters show a significant difference ($P < 0.05$)

[9] stated that, it takes 50% of the living spermatozoa to be continued at the IB stage. Referring to this provision, only P₁ treatment can continue at the IB stage on the fourth day, while P₀ treatment can be used on the third day, P₂ on the second day and P₃ treatment can only be used on the first day of storage.

The results of statistical analysis showed that the viability of spermatozoa in SO bulls on the 4th day of storage showed a significant difference ($P < 0.05$) between treatment P₀, P₁, P₂, and P₃. Nonetheless, the low value of the viability of the semen of SO in P₂ and P₃ treatments was caused by the possibility that the percentage of palmyra palm juice in the diluent was too high because the palmyra palm has an acidity level if stored for more than 24 hours, as a result it also affects the viability value of SO Bulls. The measurement results of the acidity level decreased from the initial pH of 6.8 to 5.8. Simultaneously, [11] reported that a decrease in viability could occur due to cold temperatures, reduced energy availability in the diluent and decreased pH due to an increase in lactic acid metabolism, damage to the plasma membrane, and acrosome. Conversely, [3] stated that the decrease in the quality of spermatozoa was influenced by the increase in the duration of storage of spermatozoa nutrients in the diluent also decreased.

3. Conclusion

The addition of palmyra palm juice did not affect the motility of spermatozoa, but had a positive effect on the viability of spermatozoa with the addition of 5% palmyra palm juice storage for 4 days at a temperature of 3-5 °C

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