THE APPLICATION OF ARTIFICIAL INSEMINATION USING SEXED SEMEN ON THE REPRODUCTIVE PERFORMANCE OF SAKUB SHEEP

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Abstract. The study aimed to evaluate the reproductive performance of Sakub ewes artificially inseminated using sexed semen from Sakub rams to preserve local genetic resources. Fresh semen was collected from Sakub rams and analyzed for quality and morphometry. The experiment involved 20 Sakub ewes and 2 Sakub rams. All ewes were synchronized by intramuscular injection of 1 ml prostaglandin (PGF2α) in double doses at 11-day intervals. The research used two treatment groups: group I (10 ewes inseminated using the sexed semen) and group II (10 ewes inseminated using unsexed semen as a control). The proportion of Y spermatozoa in sexed semen was significantly ($P<0.01$) higher than in unsexed semen, while the quality of sexed semen was significantly lower ($P<0.05$). The morphometric proportion of Y spermatozoa in sexed and unsexed semen was 71.11 ± 3.27% and 49.58 ± 1.08%, respectively. Motility, viability, abnormality, and spermatozoa concentration in sexed semen were 74 ± 7.75%; 76.29 ± 8.18%; 8.87 ± 3.73%, and 5.44 ± 0.50 million/ml, respectively; while in unsexed semen were 76 ± 6.15%; 79 ± 6.35%; 10.75 ± 3.19%; and 5.87 ± 0.65 million/ml, respectively. Both groups exhibited 100% estrus, with estrus intensity scores of 3.25 ± 0.49 and 2.91 ± 0.67. Conception rates were 80 and 90%, respectively. It was concluded that sexing did not affect reproductive performance; however, further research on the offspring sex ratio is needed.

Keywords: Artificial Insemination, Semen, Sexing, Reproduction, Sakub Sheep

1. Introduction

Sakub sheep, as a regional asset, are a herd or line of sheep whose purity must be preserved and whose population and productivity must be developed. Currently, the Sakub sheep population has reached 16,484 heads, distributed across the western slopes of Mount Slamet, particularly in the Paguyangan, Sirampog, Bumiayu, and Tonjong districts of Brebes Regency (1). Sakub sheep are one of the local sheep that have a large body size at the age of 2 months, reaching 26 kg with an adult body weight of more than 100 kg, so the index and productivity of the ewes are quite good (2). This is because historically, Sakub sheep were the result of crossbreeding among Texel, Merino, Suffolk, and local sheep that developed on the western slopes of Mount Slamet, specifically in Brebes Regency (3). Sakub sheep have been designated as a local genetic resource by Decree of the Ministry of Agriculture No. 882/KPTS/PK.010/M/12/2022, dated 7 December 2022, concerning the designation of the Sakub sheep family.

The purity of Sakub sheep as regional germplasm certainly needs to be preserved, but in the field, there is limited ownership of superior males, so that if breeders want to breed Sakub ewes, they have to take turns borrowing males from the local Service Technical Implementation Unit. To solve this problem, the approach that needs to be taken is to utilize

Artificial Insemination (AI) technology using sexed semen, so that with this technology, a male can mate with several females at the same time. This aligns with the characteristics of Sakub sheep as a meat-type sheep, thereby supporting the sustainability of meat production. Breeders generally expect the birth of ram lamb because they grow faster than ewe lamb (4, 5, 6).

Taking the explanation above into account, a sex separation technology is needed to separate Y-chromosome spermatozoa (male) from X-chromosome spermatozoa (female), thereby obtaining sexed semen for use in artificial insemination (AI). Our team's results have previously carried out sex separation with various Percoll media and bovine serum albumin in Dorper sheep and Saanen goats (7) and Etawah (PE) crossbreed goats (8, 9), as well as research related to the characteristics of Sakub sheep (10, 11) and research on hormonal applications in sheep (12). However, the application of AI using sexing semen in Sakub sheep is still not well documented, so it needs to be carried out in this research.

2. Methods

Research Materials

The research materials included 20 Sakub ewes, sexed semen from a superior Sakub Ram, prostaglandin F2α (PGF2α) for estrus synchronization, and a set of artificial insemination (AI) equipment.

Research methods

The research was conducted using experimental methods, and the Sakub ewes had previously been acclimated to the local environment by being fed a basal diet of forage and given ad libitum access to drinking water. All ewes were synchronized using a double dose of PGF2α administered intramuscularly with an 11-day interval. Next, the ewes that were in heat were divided into 2 (two) treatment groups, namely: group I, consisting of 10 ewes inseminated using sexed semen, and group II, consisting of 10 ewes inseminated using unsexed semen as a control.

Fresh semen was diluted with Tris Egg Yolk Diluent, and semen morphometrics and quality were observed as the initial standard for the research. The process of making sexed semen from liquid semen was carried out using a separating medium of Bovine Serum Albumin (BSA) mixed with Phosphate Buffer Saline, with measurements for a concentration of 5% = 0.3 gr BSA + 6 ml Phosphate Buffer Saline and a concentration of 10% = 0.6 gr BSA + 6 ml Phosphate Buffer Saline. The response observed in sexed and unsexed semen was the proportion of Y spermatozoa, based on spermatozoa head-area morphometrics, as well as semen quality in terms of motility, viability, abnormalities, and spermatozoa concentration.

a) The proportion of Y spermatozoa is determined based on morphometrics of spermatozoa head area (13), with the formula SHA = (0.8988 x L x L) – 1.63, namely SHA = Spermatozoa Head Area µm2; P = Head length of spermatozoa in µm units; L = Width of Spermatozoa Head in µm; 0.8988 = Correction Factor; 1.63 = Regression constant value).

b) Motility is a comparison of the proportion of the number of spermatozoa cells that move forward/progressively with spermatozoa that move around in place or backwards, and dead spermatozoa from one population expressed in percent (14).

c) Viability was assessed by preparing smears with eosin-nigrosin dye, then examining them under a microscope and counting live spermatozoa from a sample of 200 cells (15). The results of the spermatozoa calculation are then entered into the formula:

$$Viability\ (\%) \ = \frac{Number\ of\ live\ Spermatozoa}{The\ number\ of\ Spermatozoa\ observed} x\ 100\%$$

d) Abnormalities were identified on smear preparations and then examined under a microscope at 400x magnification. Abnormalities were observed from a sample of 200 spermatozoa that had an abnormal shape (16), and were calculated based on the formula:

$$Abnormality\ (\%) \ = \frac{Number\ of\ abnormal\ Spermatozoa}{The\ number\ of\ Spermatozoa\ observed}\ x\ 100\%$$

e) Measurement of spermatozoa concentration was carried out based on (17), using a Neubauer counting chamber. The formula is as follows: concentration = Number of spermatozoa calculated X Volume of spermatozoa X 106 spermatozoa/ml

Next, the sexed and unsexed semen was inseminated into ewes that had previously been synchronized using 1 ml of PGF2α per head intramuscularly. Sheep that have been inseminated were observed according to the response of the variables observed as indicators of achievement, namely the percentage of estrus and intensity of estrus (18), as well as the conception rate determined by the sheep rapid pregnancy test.

Data Analysis

The collected data were used to determine differences in the proportion of Y spermatozoa based on morphometric characteristics, and the quality of sexed and unsexed semen was analyzed using the Student's t-test, while reproductive performance data were analyzed using the chi-square test (19).

3. Results And Discussion

3.1. Semen Quality and Spermatozoa Morphometrics

The results of semen quality and spermatozoa morphometrics in Sakub sheep are shown in Table 1.

Table 1. Semen Quality and Morphometrics of Sakub Sheep Spermatozoa

No	Characteristic	Mean ± Standard Deviation	
		Sexing	Unsexing
1.	Proportion of Y Spermatozoa (%)	71.11 ± 3.27	49.58 ± 1.08
2.	Motility (%)	74.00 ± 7.75	76.00 ± 6.15
3.	Viability (%)	76.29 ± 8.18	79.00 ± 6.35
4.	Abnormality (%)	8.87 ± 3.73	10.75 ± 3.19
5.	Concentration (10^6/ml)	5.44 ± 0.50	5.87 ± 0.65

The morphometric calculation results of fresh spermatozoa showed that the spermatozoa head area was 38.98 ± 1.99 μm^2. These results serve as the standard for determining Y spermatozoa, namely those with a smaller average minus fresh SHA (<36.99 μm^2). The results of statistical analysis showed that the proportion of Y spermatozoa in sexed semen was very significantly ($P<0.01$) higher than in unsexed semen. The proportion of Y spermatozoa obtained after sex separation using BSA medium was 71.11 ± 2.39%, while the proportion of unsexed Y spermatozoa was 49.58 ± 1.08%. This result is lower than in local sheep with the same treatment, producing a proportion of Y spermatozoa of 76.45 ± 1.12% (20), in dairy cattle 85 -90% (21), in pigs 90% (22). The proportion of unsexed spermatozoa X : Y is 50 : 50% (23; 24; 25). The results of this study indicate that sex separation of spermatozoa using

BSA medium can be declared successful because more than 50% of Y spermatozoa can be separated. This means that if an ewe in heat is inseminated with sexed semen, she has a 71.11% chance of giving birth to a male Sakub sheep.

The quality of Sakub sheep semen (Table 1) shows that motility, viability, abnormalities and spermatozoa concentration in sexed semen are 74 ± 7.75%; 76.29 ± 8.18 %; 8.87 ± 3.73 % 5.44 ± 0.50 million/ml respectively; while for unsexed semen it is 76 ± 6.15%; 79 ± 6.35 %; 10.75 ± 3.19 %; 5.87 ± 0.65 million/ml, respectively. The results of statistical analysis showed that the quality of sexed semen was significantly lower (P<0.05) than that of unsexed semen. This is because there are physical/chemical processes in separation (sexing) which cause stress and damage to the sperm membrane (26); decreased motility and increased ROS (Reactive Oxygen Species) (27), and reduced viability, which has an impact on abnormalities and spermatozoa concentration (28; 29; 30; 31).

3.2. Reproductive Performance of Sheep

The reproductive performance of Sakub sheep was evaluated in terms of the percentage of oestrus, oestrus intensity, and conception rate, as shown in Table 2.

Table 2. Reproductive Performance of Sakub Sheep

No	Characteristic	Mean ± Standard Deviation	
		Sexing	Unsexing
1.	Oestrus percentage (%)	100	100
2.	Oestrus Intensity Score	3.05 ± 0.49	2.91 ± 0.67
3.	Conception Rate (%)	80	90

The results of Table 2 show that the percentage of ewes inseminated with sexed and unsexed semen all showed 100% heat, with relatively similar heat intensity scores of 3.05 ± 0.49 and 2.91 ± 0.67, respectively. This is because the two groups of ewes received the same treatment to suppress estrus using the hormone prostaglandin. However, the results of the chi-square test on conception rates in ewes inseminated with sexed semen were significantly lower (P<0.05) compared to the group of ewes inseminated with unsexed semen, in accordance with the opinion (Hollinshead and Evans, 2021). This is because the quality of sexed semen is significantly lower than that of unsexed semen (Table 1), so it will affect the conception rate. In addition, the low conception rate is due to a lower sperm count, decreased sperm quality due to the sexing process, as well as increased DNA damage (32), damage to the sperm membrane due to the sexing process (26), and a shorter sperm life time in the female reproductive system (28; 29; 30). Based on this, doubling the doses and using the deep intrauterine insemination techniques are recommended.

4. Conclusion

Sexing spermatozoa using BSA medium in Sakub sheep resulted in a Y spermatozoa proportion of 71.11 ± 2.39%. However, the quality of the cement produced is lower than in the unsexed semen. Therefore, the insemination dose should be doubled when using deep intrauterine insemination techniques. It is recommended that further research be conducted on the sex ratio of lambs born.

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