

Ratio of X- and Y-Bearing Spermatozoa of Boer Goats Separated Using the Swim-up Method

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Abstract

Background: Spermatozoa sexing is the process of separating X and Y spermatozoa to produce male or female livestock according to production objectives. The aim of this research is to produce offspring of a specific sex in accordance with breeding objectives.

Background: The research materials were Boer buck semen collected using an artificial vagina. The ejaculate was evaluated both macroscopically and microscopically, including semen volume, odor, color, pH, and consistency, as well as progressive motility, viability, abnormalities, and sperm concentration. prepared to separate the X and Y spermatozoa. This study used an experimental method with a completely randomized design (CRD) in three different spermatozoa samples. The unseparated spermatozoa and X and Y spermatozoa resulting from separation were put into different test tubes. A sample of semen solution in each test tube was dripped onto a glass object to make a spermatozoa preparation. The spermatozoa of each preparation were then measured, including the head length and width, tail length, and the length of length of all parts of the spermatozoa. Observation data were analyzed using the Student t-test (Steel and Torrie, 1991).

Results: The results of the study show that the quality of fresh semen used was good, with an average volume of 0.72 ± 0.20 mL; the motility of spermatozoa was $85.80 \pm 2.10\%$; the viability was $87.50 \pm 1.60\%$; the abnormality was $5.40 \pm 0.49\%$; and the concentration was 1.81 ± 0.06 billion. The averaged ratio of X and Y-spermatozoa without separation was $48.57 \pm 0.52\%$ vs $51.43 \pm 0.52\%$. As well as $33.58 \pm 0.52\%$ vs $66.42 \pm 0.48\%$ based on the length of the spermatozoa head; $37.85 \pm 0.92\%$ vs $62.15 \pm 0.71\%$ in head width; $32.88 \pm 0.63\%$ vs $67.12 \pm 0.52\%$ in tail length; and $34.18\% \pm 0.97\%$ vs $65.82 \pm 0.82\%$ based on all parts of the spermatozoa length.

Conclusion: Separation using a swim-up method yielded a range of 60 – 70% Y-bearing spermatozoa in the top layer versus 30 – 40 in the bottom layer. Based on this research, it is expected to produce 60 – 70% of a specific sex, depending on breeding objectives.

Key Words: Boer goat; ratio; sexing; sperm; swim-up

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I. Introduction

Local goats are commonly found in rural areas, but their productivity tends to be low due to their small size, light weight, and relatively low selling price. Therefore, to improve the genetic quality and production of local goats, human intervention is needed to regulate reproduction through cross-breeding programs with superior males (Sumadiasa et al., 2004). Reproduction is one effort to preserve and develop, and simultaneously increase animal productivity to meet human needs throughout life. Without reproduction, sustainable production is impossible; the need for animal protein will also be impossible, and cases of malnutrition, even stunting, will certainly occur.

The local government has taken various steps to meet the need for animal protein, including efforts to increase the number, productivity, and genetic quality of animals through the application of reproductive technologies, such as artificial insemination (AI) and embryo transfer (Sibagariang et al., 2010). Artificial insemination (AI) is a reproductive technology in the livestock sector that allows mating of female livestock without the presence of a male. Semen or sperm collection is carried out using an artificial vagina (Suprianto and Djuliansah, 2006). This technology is systematically designed and programmed to increase the genetic quality of livestock in the future (Setiawan, 2018). It is an effective and efficient technology for improving genetic quality in goats, accelerating genetic progress, and facilitating the application of molecular genetic techniques in selection programs (Arisandi, 2023).

Artificial insemination is an old technology that can be integrated with various biotechnologies in the field of reproduction, including spermatozoa separation or sexing technology. This technology is an alternative method for producing offspring as needed. Principally, the separation of X-chromosome spermatozoa (forming female offspring) from Y-chromosome spermatozoa (forming male offspring) is based on the differences in characteristics of the two types of spermatozoa. Differences in shape, size, weight, density, type, speed of movement, charge, and surface or internal biochemistry are caused by differences in spermatozoa DNA content (Hafez and Hafez, 2008). The X chromosome of spermatozoa is larger and heavier, moves slowly, and has a denser head DNA mass of about 3.8% compared to the Y chromosome (Bintara, 2011). Biologically and economically, the use of separated spermatozoa is quite effective, so that various new techniques have been developed for spermatozoa separation. A modified swim-up procedure was appropriate to increase the number of spermatozoa carrying the Y chromosome (Somarny et al., 2011).

The sex ratio of offspring can be achieved by inseminating a female in heat with separated spermatozoa (X or Y-spermatozoa). In mammals, if spermatozoa X successfully fertilizes an egg, it will produce a female offspring with the XX chromosomes; conversely, if spermatozoa Y fertilizes an egg, it will produce a male offspring with XY chromosomes (Hafez, 2004). The formation of offspring with a certain sex is expected to fulfill the needs or desires of farmers based on the expected offspring. Ultimately, passion for farming increases, livestock population increases, and the income of the farmer's family and local revenue also increase. A study has been conducted on "Ratio of X and Y Spermatozoa of Boer Goats Separated with Swim-up Method". The aim was to produce offspring of a specific sex in accordance with breeding objectives.

II. Material And Methods

This study was conducted from June to August 2025 using an experimental method. The research materials were Boer buck semen collected using an artificial vagina. The ejaculate was evaluated macroscopically and microscopically, including semen volume, odor, color, pH, consistency, as well as progressive motility, viability, abnormalities, and spermatozoa concentration. The evaluation of semen results was used in this research material. The study used a completely randomized design (CRD) in three different spermatozoa sample treatments. The unseparated spermatozoa and X and Y spermatozoa resulting from separation were put into three different test tubes. A sample of semen solution in each test tube was dripped onto a glass object to make a spermatozoa preparation. The spermatozoa of each preparation were then measured, including the head length

Separation of X and Y spermatozoa

The sample of 0.2 mL semen was placed at the bottom of a 10 mL test tube. One milliliter of separation medium containing of 30% egg white albumin in 1.25% NaCl (v/v) was slowly dripped down the side of the tube, then incubated at room temperature ($\pm 28^{\circ}\text{C}$) for 30 min. At the 30th minute, the upper (half top) and the lower (half of low fraction) were carefully pipetted and transferred into two different test tubes for each layer (modified from Volpes et al., 2016; Amano et al., 2024). One milliliter of Tris-egg yolk diluent was added to semen portions. One minute later, the motility was observed. A smear was then prepared to determine the ratio of X and Y spermatozoa in each layer by measuring each spermatozoa.

Data Analysis

The collected data were analyzed with a t-test using SPSS V.20

III. Result

The assessment on the quality and quantity of fresh semen collected must be carried out and evaluated immediately, both macroscopically and microscopically. This assessment is crucial because it is one of the determining factors in the success of an AI program. Semen volume, color, odor, consistency, and pH can be assessed macroscopically without the use of microscopic instruments. Microscopic examination, on the other hand, is used to assess sperm motility, viability, abnormalities, and concentration.

Table 1. The average quality of fresh Boer buck semen used in the research.

No	Variable assessed	Results
1	Volume (mL)	0.72 ± 0.20
2	Color	Milky cream
3	Smell	Specific semen
4	Consistency	thick
5	pH	6.60 ± 0.52
6	Mass motility (%)	(> 90)
7	Individual motility (%)	85.80 ± 2.10
8	Viability (%)	87.50 ± 1.60

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9	Abnormality (%)	5.40 ± 0.49
10	Concentration (10 ⁶ /mL)	1.81 ± 0.06

Generally, semen suitable for processing must have a minimum volume of 0.5–1 ml, a minimum of 70% live and motile spermatozoa, a maximum of 20% abnormalities, and a minimum concentration of 1,000 million/ml of semen (Asadpur et al., 2011; Sumadiasa et al., 2018), as well as Maulana et al. (2022). The data from this study indicate that the fresh semen used is considered very suitable. The volume of Boer goat semen obtained is normal in accordance with several previous researchers, with a range of 6.8 to 7.2, and an average of 7.0 (Sumadiasa, 2018). The volume of collected semen is influenced by variations in goat herds, collection methods, collection frequency, goat age, goat condition, a

The yellowish cream colour of semen indicates good semen quality (Lopes, 2002). Yellowish green semen indicates the presence of *Pseudomonas aeruginosa* bacteria, while red or brownish semen indicates the presence of fresh or decomposed blood (Arianti, 2014; Inonie et al., 2016). Furthermore, the odor, consistency, and pH of the semen, as well as the microscopic examination results presented in Table 1, also meet the eligibility standards for use in artificial insemination (Pamingkas et al., 2014; Rizal et al., 2018). Good semen quality is an absolute requirement for successful artificial insemination (AI), natural mating, or other reproductive biotechnology applications.

The AI technology can now be integrated with various other reproductive biotechnologies, such as estrus synchronization, superovulation for embryo transfer, and sperm separation. The use of separated or sexed sperm in AI programs is to produce offspring of the desired sex, such as female or male. Sperm separation biotechnology is widely sought after in AI technology applications to produce offspring of a specific sex. This biotechnology can also be used as a selection method to obtain high-quality replacement heifers, which is quick and very profitable in dairy farming compared to the use of spermatozoa without separation (Sidel, 2010; De Jarnette, 2010; Cassel, 2010). The use of separated spermatozoa is to produce offspring of a specific sex according to livestock purposes.

The application of livestock productivity, meat consumption, and animal-derived food security. Generally, spermatozoa separation techniques or methods are based on the sedimentation speed, the equality of gravity between spermatozoa and the medium. Albumin column separation is a valid and applicable separation method with results reaching 75-80%, slightly higher than Sephadex filtration, which is 70-75%. The characteristics of Y spermatozoa are smaller, slimmer, and lighter, so they are able to swim upwards through the medium with a certain consistency faster than X spermatozoa. The slenderness of spermatozoa can be seen in the width of their heads, which is several microns below the average head width. The slender and shorter tail size of Y spermatozoa compared to X causes it to be able to penetrate the thicker medium more quickly. Another fairly good method is swim up or swimming upwards (Hafez and Hafez, 2008).

The X-bearing spermatozoa can be separated from the Y-spermatozoa due to the differences in characteristics of both. The difference between X and Y spermatozoa is determined by their average size. If the size is smaller than the average, it is stated as Y-bearing spermatozoa. Conversely, the size is stated as X-bearing spermatozoa. The average spermatozoa size in this research was 8.03 µm for head length, 4.33 µm for head width, 49.12 µm for tail length, and 57.15 µm for the overall length of spermatozoa. The larger and longer sizes of X-bearing spermatozoa are due to higher DNA content, 3.8% (Bintara, 2011). Therefore, the X-bearing spermatozoa are heavier and move more slowly than Y spermatozoa. The parameters of spermatozoa size that were observed in this study include the head length, head width, tail length, and overall length of the spermatozoa, as measured and presented in Table 2.

Table 2. The average sizes of the Boer buck spermatozoa after separation

Part of spermatozoa	Upper layer (µ)		Lower layer (µ)		p
	X	Y	X	Y	
Head length	8.45 ± 1.22	7.31 ± 0.18	8.80 ± 0.72	7.84 ± 0.36	0.16
Head width	4.18 ± 0.20 ^a	3.16 ± 0.11 ^b	4.44 ± 0.35 ^a	4.24 ± 0.06 ^a	0.02*
Tail length	51.67 ± 3.44 ^a	39.57 ± 4.44 ^b	56.62 ± 7.33 ^a	43.64 ± 3.14 ^b	0.05*
Sperm length	60.98 ± 1.15 ^a	47.95 ± 4.55 ^b	65.25 ± 7.74 ^a	44.96 ± 4.82 ^b	0.03*

Biologically and economically, the use of separated spermatozoa is quite effective, so that various new techniques have been developed for spermatozoa separation. The modified swim-up procedure is appropriate for increasing the number of spermatozoa carrying the Y chromosome (Somarny et al., 2011). The sex ratio of offspring can be achieved by inseminating a female in heat with separated spermatozoa (X or Y spermatozoa). In mammals, if the X spermatozoa successfully fertilize the egg, it will produce a female (XX) offspring; conversely, if the Y spermatozoa fertilize the egg, it will produce a male (XY) offspring (Hafez, 2024).

The smaller size of Y-spermatozoa causes them to be light and agile in speed. This motility is important because it is one of the necessary factors that determine whether spermatozoa can reach the ampulla

of the fallopian tube to fertilize the egg. Failure to reach the tube is a failure of fertilization, pregnancy, and reproduction. The overall length of Y spermatozoa is also shorter than that of X spermatozoa because the head and tail length of Y spermatozoa is shorter than that of X. Separation of X-chromosome spermatozoa (female seed carriers) and Y-chromosome spermatozoa (male seeds) is principally based on the differences in characteristics of the two types of spermatozoa. Differences in spermatozoa DNA content influence the size, shape, weight, density, type, and speed of movement, as well as surface or internal biochemistry (Hafez and Hafez, 2008). The X-chromosome spermatozoa are larger, move more slowly, heavier, and have a denser head DNA mass of about 3.8% compared to Y spermatozoa (Bintara, 2011; Seidel and Garner, 2002; Sharma et al., 2023). The differential expression associated with the structural, functional, and molecular features of X and Y

Based on the entire size of the spermatozoa, namely the length of the head, the width of the head, and the length of the tail, will produce characteristics of spermatozoa with X and Y chromosomes or carriers of female and male offspring. This characteristic is determined based on the size of the spermatozoa, where the size is above the average, stated as spermatozoa with X chromosomes, and conversely, those below the average, stated as spermatozoa with Y chromosomes. The proportion of X and Y-spermatozoa will determine the sex of the offspring born. The average percentage of spermatozoa with X- and Y-bearing chromosomes in each fraction or layer of the medium.

Table 3. The average percentage of X and Y-chromosome spermatozoa in the upper and lower fractions/layers of the separation medium based on the size of each part (rep = 7)

Part of spermatozoa	Number of spermatozoa after swim-up separation			
	Upper layer (cells)		Lower layer (cells)	
	X	Y	X	Y
Head length	34.29 ± 0.52	65.71 ± 0.52	67.14 ± 0.48	32.86 ± 0.48
Head width	40.00 ± 0.92	60.00 ± 0.48	64.29 ± 0.71	35.71 ± 0.71
Tail length	31.43 ± 0.63	68.57 ± 0.63	65.71 ± 0.52	34.29 ± 0.52
Sperm length	31.29 ± 0.46	68.71 ± 0.94	66.43 ± 9.3	33.57 ± 4.7

Based on Table 3, the proportion of X and Y-chromosomed spermatozoa separated using the swim-up method reached 66% of Y-chromosomed spermatozoa in the upper fraction and 34% in the lower fraction. Laboratory observations lower than the results of Saili's research (1999), where the egg white concentration of 30%vs 50% in the upper and lower fraction, each produces 73% of X spermatozoa and 73.5% of Y spermatozoa in the egg white albumin column method. The ratio of Y chromosome spermatozoa acquisition is lower than the results of Yuliani's (2000) study, where the upper swimming separation produces a proportion of Y spermatozoa reaching 81% in a 30-minute incubation time. The results of this study are also slightly lower than the results of Afiati's (2004) study. The Y spermatozoa are described as able to penetrate the 30% albumin column, reaching around 80% because of their small size and DNA content, and they can move quickly in a progressive pattern. The Ium resulting from separation using the swim-up method is shown in Table 3.

Generally, post-processed spermatozoa stored at room temperature can only be used for AI for 1 to 2 or 3 hours. In this study, the viability of spermatozoa post-separation reached an average of over 50% after 5 hours of storage. The quality of spermatozoa after separation, which were quite good in terms of progressive motility and viability, can support the success of artificial insemination (AI). Sperm motility early after separation reached an average of $79.2 \pm 1.79\%$ in the upper fraction and $81.8 \pm 3.11\%$ in the lower fraction. Meanwhile, the average viability was $81 \pm 5.24\%$ in the upper fraction and $83.2 \pm 3.27\%$; and abnormalities were only $2.2 \pm 0.84\%$ and $3 \pm 0.71\%$ in the upper and lower fractions, respectively.

After separation, spermatozoa viability in this study was still 30–40% after 9 hours of storage. This means that the Y or X-chromosome spermatozoa obtained from the separation can be used for AI for up to 5 hours after separation storage. The number of abnormal spermatozoa in this study was far below the eligibility standard, averaging only $5.40 \pm 0.49\%$. The percentage of spermatozoa abnormalities permitted for AI purposes is less than 20% (Hafez and Hafez, 2008). Abnormal spermatozoa cannot be used in AI because they cannot reach the egg for fertilization.

IV. Conclusion

The proportion of spermatozoa after sexing by the swim-up method is quite good, up to 66% of Y-spermatozoa in the upper layer, compared to 34% in the lower layer.

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