# Spermatozoa separation method with different percoll composition on motility, abnormalities, and mortality of local goat sperm

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**Abstract.** This study aimed to determine the effect of spermatozoa separation on local goats using different percoll compositions for abnormalities, mortality, and motility. Percoll is a tool for efficient in biochemistry. It is used for the isolation of cells, organelles, or viruses by density centrifugation. The material in this study was four local goats, aged 1.5 years. The treatment in this study consisted of: P1 = Percentage of Percoll: percentage of M-199 + aquabidestillata + Bicarbonate [10:90], P2 = [20:80], P3 = [30:70] and P4 = [40:60]. The experiment was carried out six times and the semen collection interval every two days. The design of this study uses a random block design to check motility, mortality, and abnormalities. The result of this study is the separation of goat spermatozoa using different percoll compositions affecting motility, mortality, abnormalities, but no effect on sperm abnormalities. Percoll can increase the dominance of Y sperm with a high proportion in $P_3$ and $P_4$ 90.3 ± 5.8 and 87 ± 5.2 present, respectively. Additional percoll composition can reduce motility and increase mortality, but still good enough for Artificial Insemination [AI] implementation

## 1. Introduction

Sex chromosome separation is needed for the cost efficiency and maintenance time of the local goat. The effort to maintain the quality of semen after the collection must be carried out so that the good post-separation cement condition. The condition of cement after separation can be evaluated if the method is used to maintain the quality of the goat's cement. The percoll is often used in research, but more research is needed on the use of different compositions on the quality of goat semen. Considering this, how to apply separately and how much effect is the composition of sperm abnormalities, mortality, and motility in local goats. Previous results from Percoll have been used to separate spermatozoa in humans, FH cattle and sheep with quite high yields, but goats have not been made so problems arise, namely how much influence the spermatozoa separation technique uses Percoll with M-199 + bicarbonate + aquabidestillata on abnormalities, mortality, and motility of spermatozoa of goat cattle.

The use of the Percoll method on goat spermatozoa to separate the sex chromosomes X and Y are expected to produce a goat with the desired sex. Spermatozoa with the sex chromosome X can produce female goats that can be used as superior seeds. The Percoll method is also expected to maintain the motility, mortality, and abnormalities of the separation spermatozoa. Results of research that percoll is a good medium for cell centrifugation, viruses, and subcellular particle gradients. Percoll consists of colloidal silica with a diameter of 15-30 nm which is coated with polyvinylpyrrolidone [8].

Percoll can be used at pH 5.5 to 10 without changing the composition of the substance. M-199 powder is the ingredients that must be mixed so that Percoll can become a gel. M-199 + bicarbonate powder and aquabidestillata mixed first before Percoll is inserted into the tube. Different composition of Percoll can affect the success rate of spermatozoa with sex chromosomes X and Y. The high concentration of Percoll, the more tightly the filter is formed from spermatozoa X falls on the upper layer and the spermatozoa Y is in the lower layer. Spermatozoa X is mostly found in the lower layer because it has a weight and size greater than Y spermatozoa [3]. The greater weight and size of spermatozoa X is caused by the presence of DNA in the head of spermatozoa X as much as 3-4% larger than the DNA contained in the head of the Y spermatozoa, so centrifugation causes spermatozoa X to form deposits faster than Y spermatozoa.

## 2. Materials and Methods

The material in the study included fresh semen from 4 local 18-month-old goats, Percoll, egg yolk, Physiological NaCl, 3 present NaCl, M-199 powder, bicarbonate, tris-aminomethane, fructose, gum Arabic, aquabidestillata, 70 present alcohol, citric acid, eosin and nigrosin, ice cubes, warm water, yellow eggs. The equipment consists of cemented tube, centrifuges, ice box, electric microscope, ordinary microscope, autoclave, pipette, egg separator, measuring cup, watch glass, object glass, glass cover, Erlenmeyer, haemocytometer, pH meter, Thomas counting chamber, injection syringe, Bunsen heater, cuvette, cone test tube, clean towel, aluminium foil, artificial vagina, cotton, counter check, paper label, paper tissue.

The method used in the study is experimental. Variables measured for each treatment are Motility, mortality, and abnormalities of spermatozoa with a dominance of the Y chromosome. A design used is a Randomized Block Design [RBD] to test the motility, mortality, and abnormalities of chromosome X domination spermatozoa goats. The treatment used in this study consisted of: $P_1$: Percentage of Percoll: percentage of M-199 + Aquabidestilata + Bicarbonate [10:90]; $P_2$: Percentage of Percoll: percentage of M-199+ Aquabidestilata +Bicarbonate [20:80]; $P_3$: Percentage of Percoll: percentage of M-199 + Aquabidestilata +Bicarbonate [30:70]; $P_4$: Percentage of Percoll: percentage of M-199 + Aquabidestilata +Bicarbonate [40:60]. Replication is done six times and the cement collection interval is once every two days.

## 3. Results and Discussion

Quality of fresh goat semen from the research results can be seen in Table 1.

**Table 1.** Fresh semen of Local Goats

| Observation | Results |
|---|---|
| Colour | Beige |
| Consistency | Thick |
| Volume | 0.8±0.24 |
| pH | 6.8 ± 0.20 |
| Motility (%) | 91.67 ± 4.08 |
| Mortality (%) | 5.17 ± 1.83 |
| Abnormality (%) | 6.17 ± 0.75 |
| Concentration | $4.53 \times 10^9 \pm 0.40$ |

*3.1 Effect of percoll composition on spermatozoa motility*

The results of local goat sperm motility with different compositions showed a gradual decrease in motility [Table 2]. The average percentage of sperm motility is 72.9 ± 8.30. The average is still within the normal range [7] that motility above 50 present is still considered good for AI. Effect of Percoll's composition different on motility can be seen in Table 2.

**Table 2**. Average motility of Y spermatozoa dominance after separation

| Percoll Composition | Motility (%) |
|---|---|
| P1 [10 : 90] | 80 ± 3.2 [b] |
| P2 [20 : 80] | 77.5 ± 2.7 [b] |
| P3 [30 : 70] | 68.3 ± 93 [a] |
| P4 [40 : 60] | 65.8 ± 66 [a] |

Progressive spermatozoa were 65.69% in a study [12]. Motile spermatozoa will move in a straight line following the centrifugal force, moving progressively and more easily transferred to the bottom of the centrifugation tube. [8] In their research on the separation of spermatozoa with percoll gradient using Balinese cattle obtained spermatozoa motility values of 50-70% or an average of 63 ± 4.36%.

The results of variance analysis showed that percoll was very significant [P <0.01] sperm motility. Further test results with the LSD test showed that [$P_1$] was significantly different [P <0.01] with [$P_2$] and [$P_4$]. Similarly [$P_1$] and [$P_3$] showed very significant differences [P <0.01] while [$P_2$] and [$P_3$] showed significant differences [P <0.05]. This means that a higher composition is used that significantly reduces sperm motility. A decrease in motility can occur if the metabolic process anaerobic conditions. [9] This condition will reduce pH and consequently sperm motility can decrease.

*3.2 Effect of percoll composition on spermatozoa mortality*

Mortality is the level of dead spermatozoa or the percentage of dead spermatozoa. Death can be caused by external factors such as sunlight, pH, electrolyte effects, germs, and temperature. The results of the evaluation of local goat spermatozoa mortality after separation were listed in Table 3.

**Table 3.** Mortality of spermatozoa domination after separation

| Percoll Composition | Mortality [%] |
|---|---|
| $P_1$ [10 : 90] | 10.2 ± 3.2[a] |
| $P_2$ [20 : 80] | 11.3 ± 1.9[a] |
| $P_3$ [30 : 70] | 19.0 ± 5.1[b] |
| $P_4$ [40 : 60] | 23.8 ± 6.9[b] |

Percoll doses will significantly increase sperm mortality and tend to increase mortality. Damage to the plasma membrane during the separation of the X and Y chromosomes so that the metabolism does not function properly. [7] Stated that enzymes require high pH to achieve maximum activity. Damage in metabolic activity causes higher mortality followed by a decrease in pH and contamination of germs that form ammonia.

The percentage of deaths in this study was 16.2 ± 7.2. The average percentage of deaths was 23.81 ± 0.12 [6]. Separation of X and Y chromosome using percoll of two layers resulting from the gradient

in the percentage of spermatozoa life of 76.9 ± 13.3 present and the percentage of spermatozoa mortality of 23.1% [13].

*3.3 Effect of percoll composition on spermatozoa abnormality*

Abnormalities are deformities in the spermatozoa. Assessment of abnormalities is done by calculating the percentage of abnormal morphological spermatozoa.

**Table 4.** Abnormality of spermatozoa domination after separation

| Percoll Composition | Abnormality [%] |
|---|---|
| $P_1$ [10 : 90] | 10.2 ± 3.2 |
| $P_2$ [20 : 80] | 11.3 ± 1.9 |
| $P_3$ [30 : 70] | 19.0 ± 5.1 |
| $P_4$ [40 : 60] | 23.8 ± 6.9 |

Average sperm abnormalities in this study have a normal range. Abnormalities are 20% or more, semen quality is considered bad. Total average abnormalities in this study were 13.0 ± 7.3% the result was still in the normal range [5]. [6] In his research on density 2, the average abnormality of 18.00 ± 1.37%, the results were higher than the results of research conducted. Separating spermatozoa using percoll, found that the percentage of normal spermatozoa was 77% and abnormal was 23% [12]. Abnormal spermatozoa may be caused by the centrifugation process. [14] Abnormalities in Fresh semen can be reduced by about 10%.

Length of centrifugation also plays a role in the separation so that there is the possibility that abnormal sperm can reach the bottom of the tube [12]. The results of the analysis of variance obtained information that the composition of percoll was not significant [P>0.05] in abnormalities. This means that different percoll compositions do not affect the spermatozoa abnormalities of local goats. So centrifugation and other factors do not affect spermatozoa abnormalities.

*3.4 Effect of percoll composition on spermatozoa domination chromosome Y*

Genetic and DNA material contained in spermatozoa X can cause differences in density in both results of the analysis of variance showed that percoll had a very significant effect [P <0.01] on the dominance of local goat spermatozoa. This means that different compositions will produce different spermatozoa dominance. The Results show that [P1] and [P4] very significantly, [P1] and (P3) show very significant [P <0.01] medium and P1 and P2, Like P2 and P3, show a significant [P <0.05]. This means that the higher the percoll composition used, the higher the Y sperm dominance obtained.

**Table 5**. The average dominance of spermatozoa Y after being the separation

| Percoll Composition | The dominance of spermatozoa Y [%] |
|---|---|
| $P_1$ [10 : 90] | $62.7 \pm 22.8^{a}$ |
| $P_2$ [20 : 80] | $78.8 \pm 16.0^{b}$ |
| $P_3$ [30 : 70] | $90.3 \pm 5.8^{c}$ |
| $P_4$ [40 : 60] | $87.0 \pm 5.2^{bc}$ |

Composition [$P_1$] and [$P_2$] spermatozoa Y can also penetrate percoll even though the proportion is lower than that; the dominance of spermatozoa X can also penetrate the composition [$P_1$] and [$P_2$] more than the proportion $P_4$ and $P_3$. $P_1$ and $P_2$ of spermatozoa X which have a larger head size can still

penetrate Percoll layer with the proportion of 37.3% and 21.2%, this is considered and the filter formed by percoll is still less dense due to its low composition. Composition [$P_3$] and [$P_4$], spermatozoa X which have a larger head size are only able to penetrate percoll layers with a low proportion of 9.7% and 13%.

The highest average percentage of Y spermatozoa domination [$P_3$] was 90.3 ± 5.8%. While the lowest average percentage of Y spermatozoa [$P_1$] was 62.7 ± 22.8%. At [$P_1$], [$P_2$] and [$P_3$] the percentage of Y chromosome-dominated spermatozoa increases and then (P4) decreases. After incubation for 10 minutes, Y spermatozoa will try to penetrate the separation medium or percoll. The higher the composition or dosage of percoll, the more tightly the filter is formed so that only spermatozoa have a small size can penetrate the medium. Spermatozoa have smaller and lighter head sizes, so their movements are faster and more concentrated [9]. Spermatozoa X which have a larger head size. In the study, the dominance of spermatozoa with high proportions was able to penetrate the composition [$P_3$] and [$P_4$] while X spermatozoa that penetrated the Percoll layer had a low proportion.

Spermatozoa head length for livestock is 9 microns, 1-2 microns thick, 13 microns long neck and 44-50 microns long tail [5]. [6] Average size of 8.21 ± 0.72 microns, width 4.09 ± 0.42 microns and an area of 33.5 ± 5.92 microns. Spermatozoa head area is less than 36.5 microns while the spermatozoa Y is more than 36.5 microns [15]. In this study, the spermatozoa Y area was less than 34.22 microns and X spermatozoa were more than 34.22 microns. The results of the study showed that the average length of spermatozoa heads without local treatment was 8.61 ± 0.61 microns, width 3.96 ± 0.49 microns and an area of 34.22 ± 5.57 microns. The size of spermatozoa after separation on [$P_1$] length 8.20 ± 0.59 microns, width 3.91 ± 0.43 microns and area 32.11 ± 4.28 microns. The length size for [$P_2$] is 8.07 ± 0.65 microns, width is 3.89 ± 0.47 microns and the area is 31.46 ± 5.17 microns. The length of spermatozoa [$P_3$] was 7.96 ± 0.54 microns, 3.53 ± 0.42 microns wide and 28.14 ± 4.13 microns. While the length of sperm for ($P_4$) is 7.98 ± 0.73 microns, width 3.64 ± 0.53 microns and an area of 29.25 ± 5.69 microns.

Spermatozoa head area is less than 36.5 microns while the spermatozoa Y is more than 36.5 microns. In this study, the spermatozoa Y area was less than 34.22 microns and X spermatozoa were more than 34.22 microns [15]. Difference size of the local goat spermatozoa in the study can be caused by various types of animals. The dominance of Y spermatozoa from the results of separation in the study was calculated based on the spermatozoa head area. An assumption that the spermatozoa area is less than 34.22 microns is spermatozoa Y and more than 34.22 is spermatozoa X. The effects of different Percoll compositions on spermatozoa can be seen [Table 4].

## 4. Conclusions

Different percoll compositions affect motility, mortality, and dominance of Y spermatozoa but have no effect on Y sperm abnormalities. The addition of percoll doses can increase the dominance of spermatozoa Y resulting from a high proportion of $P_3$ and $P_4$ by 90.3 ± 5.8 and 87 ± 5.2%. Higher percoll doses can reduce motility and increase the mortality of separating spermatozoa, but are still quite good for the implementation of Artificial Insemination.

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