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## Effect of Sexing Process Using Percoll Density Gradient Centrifugation and Frozen on Motility And Damage to Spermatozoa Membrane of Filial Ongole

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### Abstract

Artificial insemination using semen Sexing aim to improve the genetic quality and to get calf with gender according to expectations. Sexing using density gradient centrifugation percoll and sedimentation egg whites have been successfully frozen. This study aims to determine the effect of sexing process using density gradient centrifugation percoll and Egg white sedimentation on motility and membrane damage spermatozoa. The material used is bull Filial Ongole semen have motility which has more than 65%. Treatment ; T0 = without sexing; T1 = Sexing. Each treatment was conducted repeat 10 times. The design used the Block Design and descriptive analytics. Media diluent used is andromed<sup>®</sup>. Percentage motility by using a Light microscope magnification 100X. while damage to the membrane using Electrone Scanning microscope (SEM) with a magnification of 10,000 X. The result is the percentage motility ; T0 = 64,25 ± 3,94%; T1 After sexing , X-sperm and Y- sperm = 53± 7,93 % dan 48,55 ± 8,28%, . Motility before freezing at T0 = 59,75 ± 4,48%. T1 before Freezing , X-sperm and Y- sperm = 47 ± 6,13% dan 42,1 ± 7,36% and Post thawing motility X-sperm and Y- sperm T0 = 44,2± 2,79%, T1 = 31,45± 7,20% and 27,45± 8,69%. On Raw semen looked membrane intact spermatozoa. Y sperm after sexing were damaged membranes of the head and in spite of the sperm head to tail, whereas the X sperm membrane damage not appear. After freezing both the control and the results of many spermatozoa sexing damaged membranes and head with tail no intact.

**Keywords:** *Artificial Insemination , Before Freezing motility , Post thawing motility , Filial Ongole Cattle , sexing sperm.*

### 1. INTRODUCTION

Artificial insemination using semen Sexing aim to improve the genetic quality and to get calf with gender according to expectations (Susilawati, 2010) and (Hafez and Hafez, 2008). Sexing using density gradient centrifugation percoll have been successfully frozen. This

study aims to determine the effect of sexing process using density gradient centrifugation percoll on motility and membrane damage spermatozoa

## 2. MATERIALS AND METHODS

The material used is bull Filial Ongole semen have motility which has more than 65%. Treatment ; T0 = without sexing; T1 = Sexing. Each treatment was conducted repeat 10 times. The design used the Block Design and descriptive analytics. Media diluent used is andromed®. Percentage motility by using a Light microscope magnification 100X. while damage to the membrane using Electrone Scanning microscope (SEM) with a magnification of 10,000 X

## 3. RESULT AND DISCUSSION

The result is the percentage motility ; T0 =  $64,25 \pm 3,94\%$ ; T1 After sexing , X-sperm and Y- sperm =  $53 \pm 7,93\%$  dan  $48,55 \pm 8,28\%$ , . Motility before freezing at T0 =  $59,75 \pm 4,48\%$ . T1 before Freezing , X-sperm and Y- sperm =  $47 \pm 6,13\%$  dan  $42,1 \pm 7,36\%$  and Post thawing motility X-sperm and Y- sperm T0 =  $44,2 \pm 2,79\%$ , T1 =  $31,45 \pm 7,20\%$  and  $27,45 \pm 8,69\%$ .

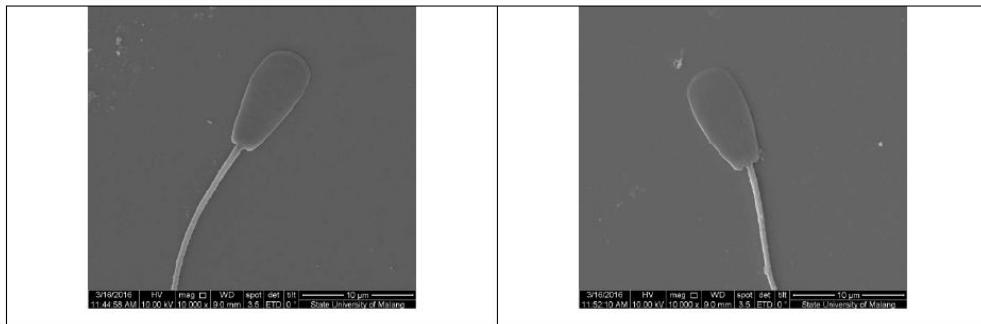
**Table 1.** Percentage of sperm motility after Sexing, Before Freezing and Post Thawing Motility.

Treatment		After Sexing	Before Freezing	Post Thawing
T0		$64,25 \pm 3,94^b$	$59,75 \pm 4,48^b$	$44,2 \pm 2,79^b$
T1	X	$53 \pm 7,93^a$	$47 \pm 6,13^a$	$31,45 \pm 7,20^a$
	Y	$48,55 \pm 8,28^a$	$42,1 \pm 7,36^a$	$27,45 \pm 8,69^a$

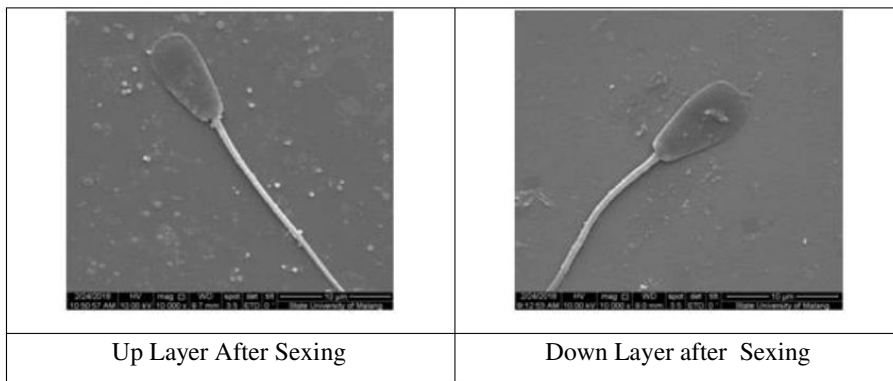
Before freezing dan post thawing showed a decline . It is seen that the control shows the percentage motility higher than the density gradient centrifugation method percoll The percentage of sperm motility results sexing bottom ( X- sperms) higher ( $P < 0,01$ ) than the top layer ( Y- sperms) . Likewise, the percentage of motility post-thawing there is a very real difference ( $P < 0,01$ ) between control and after sexing so it can be said without sexing sperm motility still shows the percentage of better than semen sexing. Reduced motility occur for a variety of treatments ranging from the process of separation, washing, refrigeration and freezing which causes sperm requires a lot of energy to maintain physiological conditions. In addition jugadisebabkan for circular movements that occur on spermatozoa due to centrifugal force so that the spermatozoa damaged. This is in accordance Susilawati (2013) that the centrifugal force generated by centrifugation cause a decrease in the quality and membrane damage. Susilawati (2014) states that centrifugation may result in damage to the membrane of spermatozoa The function is as a protective cell membrane, so that when the membrane is damaged, it can cause damage to the organelles found in cells, such as mitochondria and lysosomes Mitochondria is the venue for cell respiration to produce energy, so that in case of damage can interfere with metabolic processes that will influence the movement of spermatozoa.

**Analysis using Scanning Electrone Microscope**

The observation of fresh spermatozoa by scanning electron microscope, showed an intact spermatozoa in sperm head membranes and also on the relationship between the tail and head. the bond between the head and tail of spermatozoa spermatozoa will determine the viability and motility, because the viability and motility of spermatozoa is determined by whether or not the structure of the membrane, because the membrane function is to protect the spermatozoa.



**FIGURE 1 .** Scanning electrone microscope of raw sperm showed an intact spermatozoa in sperm head membranes and also on the relationship between the tail and head



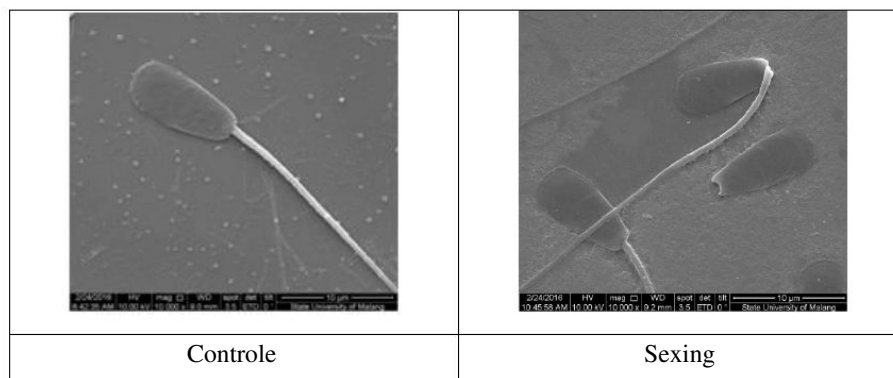
**FIGURE 2 .** Scanning electrone microscope of sperms after sexing of up layer and down layer shows the intact head separate spermatozoa part of head of spermatozoa membrane appears to be damaged or flaking, but also there the head and tail intact good has also membrane head is also in good condition.

Based on Figure 2 above shows the impact of sexing process on the top layer (Sperma Y) is the release of the head and tail as well as membrane rupture head of spermatozoa but there is also the head of spermatozoa membrane also connect the tail and head are in good condition. It is possible indeed dead spermatozoa or ugly membrane will float diata as the



events of spermatozoa washing, then the dead spermatozoa were on the top layer. On Raw semen looked membrane intact spermatozoa. Y sperm after sexing were **damaged membranes of the head and in spite of the sperm head to tail**, whereas the X sperm membrane damage not appears. After freezing both the control and the results of many spermatozoa sexing damaged membranes and head with tail no intact (Susilawati et al, 2014) decline in sperm quality after sexing highly significant

Spermatozoa in sperm freezing without sexing results show that many membranes leaking or distend (Shown in the figure 3). This shows that the impact of the freezing process is going to damage the membrane of spermatozoa, especially in the membrane spermatozoanya head.



**FIGURE 3.** Scanning electron microscope of post thawing sperms control and after sexing

The impact of the process and freezing the spermatozoa sexing is a leakage of the membrane and the release of the head of spermatozoa with tails, was evident in figure 3 Post thawing Sexing top layer of the top layer shows the damage the membrane on the tail and the head of spermatozoa, as well as the discovery of debris-debris membrane damage results. which indicates the number of spermatozoa that were damaged in the head with tails intact spermatozoa The level of damage to the top layer to the bottom layer is better in the bottom layer, ie damage not only on the intact acrosome membrane spermatozoa Wahyudi et al (2014) Spermatozoa sexing using gradient centrifugation percoll able to fertilize and produce pregnancy

#### 4. CONCLUSIONS

1. The sperm after sexing showed the decreasing of quality and the damaged of membrane
2.  $T_0 = 64,25 \pm 3,94\%$ ;  $T_1$  After sexing , X-sperm and Y- sperm =  $53 \pm 7,93\%$  dan  $48,55 \pm 8,28\%$ . . Motility before freezing at  $T_0 = 59,75 \pm 4,48\%$ .  $T_1$  before Freezing , X-sperm and Y- sperm =  $47 \pm 6,13\%$  dan  $42,1 \pm 7,36\%$  and Post thawing motility X-sperm and Y- sperm  $T_0 = 44,2 \pm 2,79\%$ ,  $T_1 = 31,45 \pm 7,20\%$  and  $27,45 \pm 8,69\%$



3. On Raw semen looked membrane intact spermatozoa. Y sperm after sexing were damaged membranes of the head and in spite of the sperm head to tail, whereas the X sperm membrane damage not appear. After freezing both the control and the results of many spermatozoa sexing damaged membranes and head with tail no intact

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