Male calf proportion of artificial insemination results by using sexed sperm with double dose on Ongole crosbred cows

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Abstract. The technology of artificial insemination is very popular to increase the population of cattle in Indonesia. This technology could be more valuable with sexing sperms in order to get the offspring with expected sex. The aim of the research is to know the proportion of male calves that was born from crossbred Ongolecows which were inseminated using double doses semen sexed for Y sperm. The materials were 43 calves from Artificial Insemination Center Singsosari. Twenty four calves was born from cows inseminated with non-sexed semen and nineteen calves with sexed semen. The percentage of sperm carrying X and Y chromosomes was performed by measuring the size of the sperm’s head by using a microscope. The result showed that the proportion of Y sperm in sexed semen was 80.79 %, while the X sperms was 19.21%. Furthermore, the percentages of the male calves that born from double dose sexed Y sperm was 78.95 %, while from the non sexing semen was 21.05%. It can be concluded that the artificial insemination using double dose sexed semen can result the male calves proportionate with the Y sperm proportion in the sexed semen.

1. Introduction
Artificial Insemination (AI) as a technology to improve the genetic quality of livestock can be increased in value by using semen sexing [1]. The use of sexing or the separation of spermatozoa containing X and Y chromosome is an option to support the purposes of AI to produce calves with the expected sex. One of the frequently used sexing methods is Percoll Density Gradient Centrifugation (SGDP). Percoll is a medium consisting of colloidal particles coated with polyvinylpyrrolidone (PVP) which can increase the motility of spermatozoa and can separate the cells.

Sexing with Percoll density gradient centrifugation method is capable of separating the sperms with more than 80% accuracy based on the identification of spermatozoa head measurements [1]. Sexing treatment can reduce the spermatozoa motility due to centrifugation treatment, the percentage of Y sperm motility decreased by 48.55% after sexing [2]. The value of conception rate (CR) on sexed sperm yielded lower number of of pregnant cows (55.10%) compared to non-sexed (65.31%) [3]. Therefore, AI by using sexed sperm needs to be improved by using double doses semen in order to increase the chance of successful pregnancy and the chance of success in getting calves with the expected sex.

The purpose of this research is to know the proportion of male calf from AI result by using sperm sexed for Y chromosome with double dose on crossbred Ongole cows.
2. Material and methods

2.1. Material
The material used in this study was 43 calves which was born from 24 females that had been inseminated by using non-sexing frozen semen and 19 females with double dose sexed Y sperm. The Y sexed sperm from Limousin bulls in this study was produced in Artificial Insemination Center (BBIB) Singosari. They were used to inseminate the cows. The observed parameters were the proportion of X sperm and Y sperm and the percentage of male calf from AI results.

2.2. Methods
The proportion identification of X sperm and Y sperm in non sexing and sexing Y sperm that used for AI was calculated based on the measuring of sperm head. The calculation was carried out using a 400x magnification microscope equipped with an objective micrometer. A total of 1000 spermatozoa from fresh semen was measured for spermatozoa head length and width using a micrometer then the average size of the spermatozoa was obtained. Furthermore 500 spermatozoa of non sexing was measured for the size of head of the spermatozoa. The size of sexing Y sperm that is larger or equal to the average of non sexing sperm is spermatozoa X while the head of the spermatozoa below the average is Y spermatozoa. The X sperm was bigger than Y sperm in length multiply wide [1].

The data proportion of sex calf was performed with direct field observation of sex calf which was born from crossbred Ongole that had been inseminated using non sexing semen for 24 females and double dose sexing Y sperm for 19 females.

3. Results and discussion

3.1. The percentage of sperm - X and sperm - Y in frozen sexing semen and non sexing
The proportion of sperm - X and sperm - Y in semen can be changed in percentage using the sexing method. Spermatozoa X contains more chromatin in the head resulting in a larger spermatozoa X head size [4]. The percentage of Y and X spermatozoa in non-sexing and sexing semen can be seen in Table 1.

| No sexing | Sexing |
|-----------|--------|
| Sperm X   | Sperm Y | Total (%) |
| Mean (%)  | Mean (%)|           |
| 47.23     | 52.77  | 100        |
| 19.21     | 80.79  | 100        |

Table 1 shows that in non-sexing semen the average percentage of sperm-X was 47.23% while for Y-sperm was 52.77%. This is in accordance with the results of a study conducted by [1] with the percentage of X and Y spermatozoa before sperm-X sexing was 52.10% and sperm-Y spermatozoa were 47.9%. Sexing method change the natural proportion of spermatozoa X and Y to the desired proportion [5]. After sexing using centrifugation of gradients density percoll (SGDP) method the percentage of Y-sperm increased to 80.79% while the sperm-X percentage dropped to 19.21%. According to Darminto et al. (2009) the sexing method can increase the ratio of spermatozoa carriers of X chromosomes (females) and Y (males) whose initial 50:50 can be changed to approximately 70:30. To prove whether the proportion of the number of Y spermatozoa in the female can produce calves with male sex, further field observations was performed on sex calf observation of AI sexing results.

3.2. The Percentage of accuracy of calf sex using sexing semen and Non Sexing
The accuracy of sex calf which was born from female that has been inseminated with sexing semen is an indicator to know the accuration of sexing semen method. The percentage of male calf was born can be seen in Table 2.
Table 2. The proportion of male calf in non sexing and double dose sexing Y sperm

|                     | Non Sexing | Double Dose Sexing Y sperm |
|---------------------|------------|---------------------------|
|                     | Proportion of Spermatozoa Mean (%) | The Proportion of Spermatozoa Percentage (%) | Proportion of Spermatozoa Mean (%) | The Proportion of Spermatozoa Percentage (%) |
| Sperm X/ Female     | 47.23      | 45.83                      | 19.21                              | 21.05                                      |
| Sperm Y/ Male       | 52.77      | 54.17                      | 80.79                              | 78.95                                      |
| Total (%)           | 100        | 100                        | 100                                | 100                                        |

Table 2 shows the results for non-sexing frozen semen that having sperm-X proportion of 47.23% resulting in female calves totaling 11 or having a percentage of 45.83%. Whereas for sperm-Y in non-sexing frozen semen, the percentage of 52.77% produces male calf as many as 13 or has a percentage of 54.17%. Thus the accuracy of calf sex produced by non-sexing frozen semen is appropriate for the proportion of spermatozoa. In addition the results of AI with double dose sexing Y sperm frozen had a Y-sperm proportion of 19.21% resulting in calf with female sex as much as 4 tails or had a percentage of 21.05%. Whereas for sperm - Y with a percentage of 80.79% it produces male calf with a total of 16 tails or 78.95%. Thus the accuracy of calf sex produced by double dose Y sexing sperm frozen semen on the proportion of spermatozoa is appropriate. It was proven that sexing can separate Y and X spermatozoa in semen so that the percentage proportion between Y and X spermatozoa that was originally almost the same can change to around 80%: 20% [6]. With the increasing percentage of Y spermatozoa in sexing semen, the percentage of male calf births will also increase.

The difference in the high percentage of female calf births compared to male calves is likely due to the differences in survival sperm which was resulted from the effect of the freezing process on sperm - X and sperm - Y and ends in the fertilization process. Sperm Y has the ability to move progressively faster than sperm X so that when it is inseminated on the right time on estrous could results male calf born [1]. On the other hand, the sperm - X has more energy content so it can survive longer than sperm – Y [6]. Based on [4] mentioned that Y sperm that had motility was faster than X sperm, while X sperm was slower but it more survive the large number of Y sperm so opportunity for doing the fertility was bigger and resulted male calf.

4. Conclusion
The artificial insemination using sexing Y sperm with centrifugation of gradient density percoll method can produce high proportion of male calf in 78.95% which appropriate with proportion of Y sperm in which higher that X sperm.

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