The comparison of artificial insemination success between unsexed and sexed sperm in Ongole Crossbred cattle

T Susilawati, A Mahfud, N Isnaini, A P A Yeki, A N Huda, A T Satria and Kuswati
Animal Science Faculty, Brawijaya University, Jl. Veteran, Malang, Indonesia

Corresponding author: trinil_susilawati@yahoo.com

Abstract. The aim of this study was to compared the success of artificial insemination (AI) between unsexed compared to sexed sperm in Ongole Crossbred cattle. Both types of sperm were frozen semen and produced by Artificial Insemination Center Singosari Malang Indonesia. In this study, Percoll Density Gradient Centrifugation was applied as protocol to select Y sperm (sexing). In total, 154 heads of female cattle divided equally into two groups namely unsexed and sexed sperm. All females were received double dosage of frozen semen during AI time. The parameters observed were percentage of Non Return Rate (NRR), Conception Rate (CR) and Pregnancy Rate (PR). The results showed that unsexed and sexed group values at NRR-1 were 90.91 and 77.92%, NRR-2 were 88.31 and 76.62%, CR were 79.22 and 57.14% and PR were 84.42 and 75.33%, respectively. According to the result, it is concluded that AI using unsexed sperm shows better result compared to the sexed.

1. Introduction
Sex determination at the early stage able to reduce management cost thorough selective management in beef cattle. In this regard, sexing sperm could be used as an effort to separate sperm. Percoll Density Gradient Centrifugation (PDGC) is a technique which can be use to separate sperm X and Y based on the sperm weight [1]. At this point, PDGC is able to separate Y sperm with ratio 83.1% and decrease motility around 10%. The principle of this method was based on head size of sperm X and Y [1]. Previous study shows that sperm motility of unsexed and sexed before freezing was 64.25 ± 3.94 and 53.70 ± 7.93% respectively [2]. In term of reproduction parameter evaluation, shows that AI conception rate using unsexed and sexed frozen was 35.48 and 43.47% [3]. Based on the aforementioned data, this study aimed to compare the success of AI using unsexed and sexed sperm in Ongole Crossbred cattle on the reproductive performance that are NRR, CR and PR.

2. Material and methods

2.1. Material
In total 154 heads of female (cow) Ongole Crossbreed were divided equally into 2 groups namely unsexed and sexed sperm group AI. The female criteria was body condition score (BCS) > 4, body weight 200 to 400 kg, have given birth normally, and has good estrous quality. The frozen semen was
produced by the Center for Artificial Insemination Singosari Malang. In this study, sperm sexing was done by using PDGC method.

2.2. Methods
The AI technique was deep insemination using double dosage. Insemination time was done 8 hours after heat signs. After that, inseminated cows were injected with bio ATP and given an additional of concentrate (1 kg for 3 days). After 19-21 days post AI, NRR was observed by visually evaluation of estrous sign for 3 cycles. Then it followed with rectal palpation after 3 months to detect the pregnancy.

3. Results and discussion
The results showed frozen semen had Post Thawing Motility (PTM) in unsexed sperm was 36.00 ± 1.00%, while sexed was 31.40 ± 1.84% (P<0.05) compared to frozen sexing semen PTM. Previous study [1] has PTM at 36.50 ± 13.13%. Sexing semen using PDGC method damaging membrane structure, therefore spermatozoa would decreased its motility and viability up to 50% [1, 4]. Long-term storage has an effect to motility, mitochondrial function and plasma membrane integrity of the sperm. The loss of energy will interfere capacitation and acrosome reactions process, due to inactivation of ATP-ase and acrosine, as well as other acrosomal enzymes which disturbing spermatozoa membrane. This would lead to the loss of part or all of the acrosome [4]. Plasma membranes are very important and fundamental in fertilization process [5], since its regulate many functions of spermatozoa such as transport of sodium, potassium [6] and calcium [7] on exchange which critically regulates mitochondrial motility and function. Plasma membranes have receptors for binding and fusion between spermatozoa and the pellucida zone and receptors that regulate spermatozoa capacitation [8]. An intact plasma membrane is also needed for fusion with the outer acrosome membrane and induction of the acrosome reaction [9].

The reproductive performance of AI using unsexed and sexed sperm in Ongole Crossbred was presented in Table 1.

| Parameter (%) | Unsexed | Sexed |
|---------------|---------|-------|
| NRR-1         | 90.91   | 77.92 |
| NRR-2         | 88.31   | 76.62 |
| CR            | 79.22   | 57.14 |
| PR            | 84.42   | 75.33 |

The results showed decrease of NRR-1 to NRR-2. The reduction in cow on AI using unsexed sperm on NRR-1 to NRR2 was 90.91 to 88.31%, whereas in cows on AI using frozen sexed sperm was from 77.92 to 76.62%. A decrease in the NRR-1 value to NRR-2 is probably caused by a lack of observation during estrus or silent heat which was occurs at NRR-1. One of the causes of repeated breeder after AI is embryonic loss before implantation or ovary hypofunction [10]. This also in the NRR-2 value has decreased compared to the value of CR that is in cattle in AI using unsexed frozen semen from 88.31 to 79.22%, while for sexed from 76.62 to 57% [11]. Cattle crossbreeding between PO and Simental or Limousin were easy to face stress in hot climate and also due to high levels of lignin in feed. As the result, it will cause silent heat and anovular estrus [10].

Conception Rate of unsexed sperm was better compared with sexed sperm (79.331 vs. 57.143%). Its shows that AI with sexed sperm shows lower result. This can be explained due to in sexed sperm, the ability of sperm to fertilize the oocyte were disturbed. It could be by the acrosome damage during cryopreservation [12]. In this regards, cryopreservation was associated with changes in acrosome reactions resulted in damage of plasma membrane [13].

The pregnancy percentage in AI using unsexed and sexed sperm was 84.42 and 75.33 %. Both groups showed high pregnancy rate. Presumably this due to the application of AI with double dosage.
and deep insemination technique. In addition, the injection of bio ATP and concentrate after AI might lead to better cow physiology, therefore no embryonic death occurs due to insufficient progesterone to maintain pregnancy. If AI pregnancy rates with conventional semen are consistently 60% or better, then only sexed semen in herd should be considered. It should be used only in healthy cycling females in good body condition. AI companies suggest using sexed semen only in heifers; however, research indicates that cycling mature beef cows are also good candidates [14]. According to previous study [15] AI with deep insemination technique would produce higher pregnancy rate compared to the conventional method. Frozen semen with motility of 20–40% does not show differences in the pregnancy rate of motility > 40%. For that, in the condition of semen with motility < 40%, deep insemination technique is recommended when performing AI.

4. Conclusion
The AI using unsexed sperm shows better reproductive performance compared to the sexed in this study.

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