Conception Rates of Holstein-Friesian Cows Inseminated Artificially with Reducing Frozen Semen Doses

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ABSTRACT

Conception rates of Holstein Friesian (HF) acceptors were evaluated in artificial insemination (AI) mating using three frozen semen doses of the straws per volume of 0.25 cc. Frozen semen consisted of a standard dose by 25 x 10⁶ sperms (L.1) and two reduced doses by 20 x 10⁶ sperms (L.2) and 15x10⁶ sperms (L.3). This field study was conducted in dairy cattle area of Lembang NBMCU, from August 2011 to March 2012. Frozen semens were obtained from two HF national progeny tested young bulls. Conception was detected by rectal palpation, around 60-90 days after AI mating. Conception rates (CR %) were calculated as a ratio between pregnant acceptors to the total AI mated cows. Some general linear model analyzes were conducted by considering fixed variables of semen dose (3), bull (2), inseminator (6) and parity (5). The results showed that three frozen semen doses did not significantly affect (P>0.05) on CR (%). By decreasing semen dose to L.3 gave CR (%) similar to L2 even to L1. It was concluded that frozen semen doses of HF bulls at the levels of 20 million to 15 million sperms per straw per volume of 0.25 cc resulted CR (%) similar to the standard one.

Key words: insemination, semen dose, conception rates

INTRODUCTION

Artificial insemination (AI) has become a way of mating offering many advantages, such as to accelerate genetic improvement of livestock and to serve estrous females in a large number and a wide scale, so AI mating provides promising economic benefits. A bull through a wide use of his frozen semen can serve several hundreds or even thousands of estrous cows in one year. This mating therefore is powerful to exploit maximum advantages of top bulls in dairy cattle industry (Bucher et al., 2009). Around 60% of breeding cattle population have been globally using AI mating in the effort to distribute widely superiority of the best bulls. Distribution of top bulls by AI mating in the dairy cattle industry can considerably be higher (Bhakat et al., 2011).
In frozen semen industry, the number of spermatozoa per straw are crucial that can result in a high fertility of cows as AI acceptors (DeJarnette et al., 2010). The success of AI mating using frozen semen at lower doses, from which the ability to give conception rates of acceptors similar to that of the standard dose, could maximize the use of superior bulls through the production of their straws (Andrabi et al., 2006). Artificial insemination using frozen semen instead of fresh semen, however, required number of sperm per dose of insemination for achieving a good fertility of AI acceptors (Saha et al., 2010). A field study in dairy cattle for the purpose of investigating the effectiveness of AI frozen semen mating at a low dose (15 million sperms) and a very low dose (2 million sperms) on straws per volume 0.25 cc was conducted by Anderson et al. (2004). The result showed that the previous dose of 15 million sperms was capable of achieving optimal conception rates of the acceptors, whilst the latter one of 2 million sperms failed to maintain and caused a decreasing conception rates of the acceptors.

Further developments of using frozen semen in the AI mating have been conducted, particularly in dairy cattle and beef cattle in regards to accelerate the increased specific sexed population. One of purposes for combining the use of frozen sperm with sexing technology in AI mating is for getting a faster genetic progress of breeding stocks. To determine the minimum level of sex-sorted frozen sperm dose in achieving optimal conception rates in dairy cattle and beef cattle, many studies have been done such as by Seidel & Schenk (2008), Schenk et al. (2009), Frijters et al. (2009), DeJarnette et al. (2010), Larson et al. (2010) and Sá Filho et al. (2010). DeJarnette et al. (2010), as an example, compared conception rates of Holstein cows and heifers in AI mating by 2.1x10^6 or 3.5x10^6 sex-sorted sperms. Conception rates of both of the sexed frozen semen doses were comparable. Further, some field results are seemingly prospects for improved efficiency of sex-sorting semen procedures with lower frozen semen doses for commercial application in the dairy cattle industry.

HF dairy cattle in Indonesia for their mating system are almost entirely using AI mating with frozen semen produced by National Artificial Insemination Agencies. To maintain the genetic quality of HF dairy cattle, identification of superior HF domestic bulls has been done through the National Progeny Test. Frozen semen of HF bulls produced by Lembang Artificial Insemination Agency (Lembang AIA), as one of the two National Artificial Insemination Centers, up to now has still been implementing a standard concentration of straw per volume 0.25 cc containing around 25 million sperms. Frozen semen from the National Progeny Tested bulls are targeted to be produced more efficiently. This might be realized, among others, through the production of more frozen semen straws per collection per bull. This can be realized if the number of frozen semen per straw can be produced at lower levels of concentration over the current standard, but they are still capable of giving conception rates of AI acceptors as well as to the standard one. Another important consideration is that if optimum doses of sex-sorted frozen sperms per straw for AI mating can be determined at field in dairy cattle, so these sexed-sorted frozen semen for AI mating can be expected for getting a wider application in beef cattle and buffalo as well.

This study, therefore was conducted to obtain information on the effectiveness of using different frozen semen concentrations at the volume 0.25 cc produced by Lembang AIA in artificial insemination mating, providing two lowered frozen sperm doses (20 x 10^6 sperms and 15 x 10^6 sperms) compared to the standard one (25 x 10^6 sperms) on conception rates of HF acceptors at small dairy farmers in Lembang, West Java.

### MATERIALS AND METHODS

#### Location and Period

This field research for studying three different doses of frozen semen by AI mating was conducted from August 2011 to March 2012. Research location was done in small dairy farmers, mostly raising Holstein Friesian (HF) dairy cattle under semi intensive management. These small farmers in raising HF dairy cattle were distributed in a number of locations under Lembang North Bandung Milk Collecting Unit (Lembang NBMCU), Lembang Subdistrict, Lembang, West Java.

#### Frozen Semen

Three different doses of frozen semen were produced by Lembang AIA by following various standard processes of collection, evaluation, dilution, processing and packaging as usually done (Lembang AIA, 2011). An artificial vagina was used to shelter fresh semen. Macroscopic examination was done for volume, color and consistency; microscopic was analyzed for mass and individual movements by microscope; and examination of concentration was conducted by spectrophotometer. Semen dilution was performed with an equilibration time of 5 h. Calculation of concentration of sperm per collection of fresh semen per bull used this formula: Number of straw= (semen volume x motility x sperm concentration) / Straw concentration

This study of three different doses of frozen semen used straws with volume 0.25 cc, consisting of Level I for a standard dose containing 25 million sperms, Level II as the first reduced sperm dose containing 20 million sperms and Level III as the second reduced sperm dose containing 15 million sperms.

#### Bull

Two HF bulls as participants of National Progeny Test were used as producers of frozen semen, namely Farrell (No. Code: 30 686) and Forsa (No. Code: 30 698). Both males were descendants of ancestors with high genetic potential of milk production as well as good growth, reproduction and libido.
Acceptor

AI acceptors were HF dairy cows and heifers, with a minimal number of acceptors subjected for each combination based on classes of sperm concentration (3) and males (2) were approximately 50 animals. So that a total number of AI acceptors for all of dose and male combinations were targeted at a minimum number by around 300 heads. These cows were identified for having good body condition, normal reproductive organ and healthy, as well as clear signs of estrus at the time of estrus coming. Cows were also not in disorder reproduction and repeat breeder. These HF cows considered as candidates of AI acceptors were within 1-8 parities (556 heads). Most of them were in 1-4 parities for 517 heads (93%), while the rest were in 5-8 parities for 39 heads (7%).

Inseminator

Artificial insemination services were conducted by six inseminators having AI operating area surrounding HF dairy cattle subdistrict of Lembang NBMCU. Each inseminator was targeted of performing six combinations of treatments (frozen semen dose= 3 levels and HF bull= 2 heads) in a proportion manner. Inseminators were instructed to record code and date of each straw used. Based on the ability of conducting AI mating on HF dairy cows studied, these six inseminators were considered as inseminator with good skill as indicated by the number of HF acceptors successfully mated by AI mating using the three frozen semen doses.

Pregnancy Detection

Pregnancy was detected by rectal palpation between 60-90 d after AI mating to check the occurrence of corpus luteum (CL) at the left or the right of ovaries of individual acceptor cow. An acceptor cow was considered coming into pregnant if at any time after 21 d of AI mating it was not returned to estrus as well as corpus luteum (CL) was able to be palpated on the ovary after 60-90 days of the AI mating. Instead, a cow was considered unpregnant if signs of estrus returning about 21 d after AI mating.

Variables of Observation

Variables of observation were expressed as conception rates (CR %) as calculated by a ratio between the number of AI acceptors positively identified pregnant over the total number of AI acceptors in a determining population. As an example, to calculate conception rates of the AI mating acceptors mated by the standard frozen semen dose (L1) from Farrell bull was obtained by dividing the number of pregnant acceptor to the total number of AI acceptors mated by the standard dose (instead of L2 and L3 doses) produced by Farrell bull (instead of Forsa bull).

Sperm Quality

Sperm quality was examined from the motility of sperms after thawing stated as Post Thawing Motility (PTM). These three frozen sperm doses were collected for testing samplings from sperm containers at Lembang AIA, Lembang NBMCU and from thermos of inseminators in the field. Straws collected from thermos of inseminators were thawed at a right time when AI mating soon being done to estrous acceptors.

Analysis of Data

Conception rates of HF cows as AI acceptors were analyzed by General Linear Model (GLM) for classes with unbalance data. Some GLM were developed by considering one or more factors of frozen semen dose (3), bull (2), inseminator (6) and parity (5) as fixed variables. Initial GLM analysis was performed for all HF cows fulfilling the criteria as AI acceptor in this study. Results showed that all factors did not give significant effects (P>0.05) on conception rates. However, the inseminators had a very significant effect (P<0.01) on conception rates.

To determine further inseminator effect, T-test was done for conception rates among inseminators in this study. Based on this initial result, it was known that all of inseminators had good ability in performing AI mating. One inseminator, however, was identified having a very good skill indicated by achieving a high numbers of AI acceptors as well as in resulting high pregnancy rates than others. Conception rates of AI mating acceptors from all inseminators were therefore included in the analysis. While the influence of parity were considered into 5 parities (1, 2, 3, 4 and ≥ 5). Parity (5) was considered as a fixed variable together with other two factors of bull (2) and frozen semen dose (3).

Possible interaction between two factors were considered, so interaction between dose x bull was put into the GLM analysis. T-test was conducted to determine significant differences between the average values of conception rates (%).

RESULTS

Acceptors by Frozen Semen Dose

Holstein Fresian (HF) female cows as acceptors of AI mating of three frozen semen doses of 25 x 10⁶, 20 x 10⁶ and 15 x 10⁶ sperms and two HF bulls of Farrel and Forsa reached a total number of 680 heads. However a number of HF acceptors that fulfilled the criteria to be analyzed for conception rates of the AI acceptors using three frozen semen doses in this study were only 556 heads. The remaining 124 HF cows (18.2%) were eliminated as the acceptors. Elimination was considered for some reasons, namely due to these cows of losing code of the frozen semen dose used, returning estrus around 21 d after AI mating and being dead or sold before pregnancy examination. Another reason was due to reproductive problems such as endometritis, repeat breeder and persistent corpus luteum.
Acceptors by Bull

HF acceptors inseminated by frozen semen from Farrel bull amounted for 279 heads (50.2%), while those from Forsa bull amounted for 277 heads (49.8%). A minimum number of AI acceptors for each combination of bull and frozen semen dose was targeted at least 50 heads. From this study, the total number of acceptors inseminated by frozen semen at 25 x 10⁶, 20 x 10⁶, and 15 x 10⁶ sperms for Farrel bull were 105 heads (18.9%), 87 heads (15.6%), and 87 heads (15.6%), respectively; while those for Forsa bull were 83 heads (14.9%), 108 heads (19.4%), and 86 heads (15.5%), respectively. Further, the total number of AI acceptors for both of these two bulls at respective frozen semen doses were successively 188 heads (33.8%), 195 heads (35.1%), and 173 heads (31.1%). This showed that the number of AI acceptors for each of dose and bull combination fulfilled the target. Similarly, the number of HF cows as AI acceptors were also proportional for three tested frozen semen doses.

Acceptors by Parity

The parity or number of a cow giving birth is a condition to indicate a biological maturity of animals. As described earlier in this study, AI mating was targeted to all acceptors within 1-≥5 parities. The number of the HF cows and heifers as AI mating acceptor were the number of pregnant acceptors to the number of all acceptors being pregnant, when an individual cow gets pregnancy examination. Conception rates are directly related to the production of which determining productivity of each cow (Saha et al., 2014). Conception rate (CR %) was formulated as a result of the ratio between the number of pregnant acceptors to the number of all inseminated acceptors for any certain population.

Results from variance analysis of the GLM were consistent for the classified groups that only inseminator gave significant effect (P<0.05) on conception rates (%) of AI mating acceptors. By contrast, frozen semen dose, bull as well as interaction of dose with bull and of dose with inseminator were not statistically significant (P>0.05) on conception rates of the AI acceptor (Adj. R² models= 10.32%). As was previously described, differences in conception rates among inseminators occurred as one inseminator (Inseminator 1) had very good skill in AI mating and in gaining conception rates than the other inseminators.

Acceptors by Inseminator

The number of AI mating acceptors and their conception rates conducted by six inseminators for the three frozen semen doses are presented in Table 1. Performance of inseminator 1 was the best among the other five inseminators for intern of getting higher AI mating acceptors and conception rates. The total number of AI acceptors inseminated by this inseminator for those three frozen semen doses were 196 heads (35.3%) and the average of conception rates was around 81.6%-89.6%. Whilst the other five inseminators achieved the number of AI acceptors were between 53-126 heads (9.5%-22.7%) with the conception rates between 43.9%-79.7%.

Variance Analysis of Conception Rates

Conception rates (CR %) were used as variables to assess the success of AI mating of HF acceptors by using three frozen semen doses in this study. Conception rates become a proper indicator to determine the success rates of AI mating, compared for example to the numbers of not returning pregnant or non return rate (NR %) (Perry et al., 2011). This is because conception rates can be seen in a more definite success of AI acceptors being pregnant, when an individual cow gets pregnancy examination. Conception rates are directly related to the production of which determining productive life of each cow (Saha et al., 2014). Conception rate (CR %) was formulated as a result of the ratio between the number of pregnant acceptors to the number of all inseminated acceptors for any certain population.

Role of Inseminator in Artificial Insemination Mating

Inseminator as an executor of AI mating is one of the key factors in determining the success of pregnancy

| Inseminator | Dose of frozen semen per volume 0.25 cc | Acceptor (head) | Acceptors by Bull |
|-------------|--------------------------------------|-----------------|------------------|
| Ins. 1      | 25 x 10⁶                             | 84              | 81.6 ± 4.0       |
| Ins. 2      | 20 x 10⁶                             | 20              | 78.1 ± 4.1       |
| Ins. 3      | 15 x 10⁶                             | 49              | 45.0 ± 5.3       |
| Ins. 4      |                                    | 33              | 75.2 ± 4.5       |
| Ins. 5      |                                    | 24              | 43.5 ± 5.1       |
| Ins. 6      |                                    | 20              | 64.9 ± 5.2       |

Note: ns showed average values at the same row were not statistically significant (P>0.05)
rates of AI acceptors. The number of HF acceptors in-
seminated among individual inseminator varied. Initial
analysis of General Linear Model (GLM) considering
the fixed factors of doses of frozen semen (3), bull (2),
inseminator (6) and parity (5) proved that inseminators
gave a very significant effect (P<0.01) on the pregnancy
rates of AI acceptors. (Total R-Sq= 6.58%). Inseminator
1 was identified for having a very good AI mating skill
compared to those other five inseminators as indicated
either by high number of AI mating acceptors or by high
pregnancy rate (Table 1). However, the number of AI
acceptors and conception rates achieved by this insemi-
nator were proportional for each of those three frozen
semen doses.

Previous examination on the effects of insemina-
tor for AI success as reported by Sarder (2006) showed
working experiences of an inseminator gave a signif-
ificant impact on conception rates of AI acceptors.
Inseminator categorized as a professional inseminator
with good ability and experiences had a better AI suc-
cess rates than the other less skill and experiences in-
seminators. Inseminator was identified as one factor that
could give effect to on conception rates of the AI accep-
tors beside the other factors such as farm management,
lambing period, lambing interval and peak milk pro-
duction (Buckley et al., 2004). Inseminator with a good
experience and skill leads to a good guarantee for a
successful AI mating beside of quality of frozen semen,
right time of insemination and health status of acceptors
(Drost, 2007). Variation on the performances among in-
seminators to make acceptors becoming pregnant were
directly influenced by their skill and experiences. The
more skill and the longer experiences of inseminator
increased fertility rates of acceptors being successfully
pregnant (Herawati et al., 2012).

Quality Test of Frozen Semen

Proper assessment of post thawing sperm quality
is an important aspect to achieve a high success rate of
AI mating, since it can provide insights upon fertilizing
capacity of cryopreserved spermatozoa (Januškauskas &
Žilinskas, 2002). Many processes of frozen semen since
dilution, chilling, freezing, storage, transportation and
thawing for AI mating could affect the viability and
fertilization capacity of spermatozoa (Saha et al., 2014;
Muoño et al., 2015). According to Arifiantini et al. (2005)
the success of using frozen semen to AI mating could
be seen from the values of recovery rate (RR), as the
expression of the number of spermatozoa successfully
recovered from the freezing process and the number of
survival spermatozoa in vitro after thawing that was
called longevity.

To check the feasibility of frozen semen qual-
ity after thawing was conducted by collecting sampling
tests of straws of the three different frozen semen doses
stored at Lembang AIA, AI containers at Lembang
NBMCU and AI thermos of inseminator in the field.
Quality test of frozen semen was evaluated through
the thawing process by following a standard procedure
(Lembang AIA, 2011). Shrivatava (2013) suggested that
frozen-thawed semen may be safely used for artificial
insemination up to 15 min by keeping frozen-thawed se-
men straw either at 4 °C or at warm water at 30 °C after
thawing of frozen semen at 37 °C for 30 s.

Based on the provisions of National Standardi-
zation Agency of Indonesia (BSN, 2008) of the quality
requirements in producing frozen semen among others
are to be unpermitted of containing microorganisms in
causing infectious diseases, spermatozoa motility at
least 40 % and degree of movement of individual sper-
matozoa at least 2 at the time of frozen semen thawed.
Results from this study generally showed that after
thawing frozen semen in container storage at Lembang
AIA had an excellent quality of sperms and very feasible
for AI mating. Results of thawing frozen semen from
Forsa bull and Farrell bull at each sperm dose (25 x 10⁶,
20 x 10⁶, and 15 x 10⁶ sperms) showed the movement of
individual spermatozoa at the scores between 2-3 and
PTM between 40-45. Similarly, the results of thawing
frozen semen from a container at Lembang NBMCU for
each treatment and bull showed individual sperm motil-
ity at the scores between 2-3 and PTM between 40-45.

Sampling test moreover were extended at field con-
ditions providing in the villages of Cibodas (Forsa bull),
Pojok (Farrel and Forsa bulls) and Pasar Kemis (Forsa
bull). The test results also gave sperm quality after
thawing similar to the previous tests at Lembang AIA
and Lembang NBMCU. Individual spermatozoa move-
ment was on the score of 2-3 and PTM between 40-45.
All of these results showed an indication of good han-
dling processes were conducted for three different
frozen semen doses of straws along phases of distributions
and storages. One crucial factor in affecting the storage
of frozen semen straws is the availability of sufficient
liquid N₂ in a container. A study in central dairy cows
in Pakistan proved without the addition of liquid N₂ at
2 liter capacity in stored frozen semen containers resulted
deterioration of sperm motility after 10-d storage times
(Das et al., 2002). Thus the availability of liquid N₂ had to
be guaranted properly along the process of distributions
and storages of three frozen semen doses in this study.

Microscopic examination of cattle in Poland found
spermatozoa with normal morphology by 50% from
where those progressive movements by 80% did not
lead to morphological changes (Barszcz et al., 2012).
According to López-Gatius (2012) at field conditions,
post thawing of frozen semen gave good results for AI
mating in exotic beef cattle by the achievement of aver-
age motility by 38.0±2.74%, proportion of life semen by
45.2±9.86% and abnormal semen by 6.0±2.83%.

Conception Rates of Artificial Insemination Mating
Acceptors

Study of the effectiveness of the AI mating based on
the treatments on reducing doses of frozen semen per
0.25 cc volume for the AI mating acceptors is presented
in Table 2. All of three straws containing frozen sperm
doses of 25 x 10⁶, 20 x 10⁶, and 15 x 10⁶ sperms did not
statistically give significant effects on conception rates
of HF cows as the AI mating acceptors. By decreasing
frozen semen concentration up to 20 x 10⁶ sperms per
0.25 mL straws resulted conception rates of the accep-
tors as well as to the standard frozen semen. A further declining of sperm concentration to 15 x 10⁶ sperms per straw was still capable of maintaining conception rate similar to the previous sperm concentration, even it was still able to maintain CR similar to the standard dose of 25 million sperms.

Further investigation on conception rates of HF acceptors by considering individual bull proved also that by decreasing concentration of the standard frozen semen dose to two reduced doses of 20 x 10⁶ sperms and 15 x 10⁶ sperms did not give significant effects on conception rates of these AI acceptors. Conception rates of the AI acceptors as the joining of these two bulls also gave consistent results, from which by reducing frozen semen doses up to the two lower levels provided similar conception rates to the standard one. Conception rates of the AI acceptors for semen doses of 25 x 10⁶, 20 x 10⁶, and 15 x 10⁶ sperms were 64.7±4.5%, 65.2±4.6%, and 66.3±4.3%, respectively.

In relation to these results, Saha et al. (2014) reported that the genetic composition of bulls as frozen semen producers gave no significant effect on conception rates, namely in the range of 50.0%-77.8%. Based on these results, it could be stated that AI mating by reducing doses of frozen semen up to a lowered level containing 15 million sperm per 0.25 cc could maintain conception rates of the AI acceptors as well as by using frozen semen at the standard dose. This was applicable for all ages of the AI mating acceptors.

CONCLUSION

Conception rates of HF acceptors by frozen-semen AI mating using the two reducing sperm doses (20 x 10⁶ and 15 x 10⁶ sperms) gave similar success rates to the standard one (25 x 10⁶ sperms) per 0.25 cc.

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Table 2. Conception rates (%) of artificial insemination mating acceptors by semen dose

| Bull   | Dose of frozen semen per volume 0.25 cc | 25 x 10⁶ Dose | 20 x 10⁶ Dose | 15 x 10⁶ Dose |
|--------|---------------------------------------|---------------|---------------|---------------|
| Farrel | Acceptor (head)                       | 60.0 ± 5.4⁰   | 62.1 ± 4.8⁰   | 71.3 ± 5.2⁰   |
| Forsa  | Acceptor (head)                       | 69.4 ± 5.8⁰   | 68.3 ± 4.6⁰   | 61.2 ± 6.0⁰   |
| Total  | 188                                   | 64.7 ± 4.5⁰   | 65.2 ± 3.4⁰   | 66.3 ± 4.3⁰   |

Note: ns showed average values at the same row were not statistically significant (P>0.05).
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