THE SUCCESS RATE OF ARTIFICIAL INSEMINATION USING LIQUID SEMEN WITH DIFFERENT DILUENTS IN BALI CATTLE

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ABSTRACT

Artificial insemination using liquid semen is beneficial for areas where liquid nitrogen is not available. The purpose of this study was to determine the success rate of artificial insemination using liquid semen with different diluents based on non-return rate (NRR) and conception rate (CR). Research location is at BPTU-HPT Denpasar Breeding Center Pulukan. The material used in this study is Bali cattle which have a Body Condition score (BCS) of 2.5-3 (scale 1-5), physically fit, not pregnant, no reproductive disorders. The method used in this study was experimental field 3 treatments and 20 replications. The number of cows used was 60, each of which was treated 20 cows: PO = AI using andromed diluent, P1 = AI using Tris aminomethane diluent + 20% egg yolk, P2 = AI using CEP-3 + 20% egg yolk diluent. Before conducting AI cows in estrus synchronization using PGF2 α (Enzaprost -T) then in AI using liquid semen that is stored 1-3 days with an average motility of each treatment P0, P1, P2: 57 $\pm$ 6,19; 60 $\pm$ 5,65; 57 $\pm$ 5,63. AI uses a double dose at the second and eighth hour after estrus. The results of each treatment P0, P1 and P2 are NRR₁: 80%, 60%, 50%; CR = 45%, 40%, 35%. The conclusion of this study is that diluted andromed (P0) has a higher success rate when compared to P1 and P2.

KEY WORDS

Artificial insemination, liquid semen, non-return rate, conception rate.

Artificial Insemination using frozen semen has been used in Indonesia. AI with frozen semen requires liquid nitrogen for storage, not all regions in Indonesia have an adequate supply of liquid nitrogen, especially outside of Java. One alternative that can be used is to use liquid semen. Liquid semen is semen that is mixed with certain diluents and stored at 3-5°C (Susilawati and Yekti, 2018). Vishwanath and Shannon (2000) adds that the liquid semen storage of at least 2-4 days. The application of AI with liquid semen has been implemented in New Zealand because it has several advantages when compared to frozen semen (Yang, et al., 2018).

Diluent requirements that can be used for liquid semen are not toxic to spermatozoa, as an energy source, isotonic, as a buffer, inhibit bacteria, be able to protect spermatozoa and as cryoprotectants. In addition, liquid semen diluents must also be easily obtained and have a relatively cheap price (Susilawati and Yekti, 2018).

One of the most used diluent andromed. Andromed has the advantage in terms of fertility, is not easily contaminated with bacteria, easy to use (Susilawati, 2011). However, andromed is an imported product that takes a long time to order and has a high price. For this reason, several diluents have been developed that can maintain the survival of spermatozoa and increase the volume of semen. Verberckmoes et al (2005) developed a Cauda Epididymis Plasma (CEP) diluent that can maintain the life of spermatozoa for 6 days.

Ducha et al (2012) said that CEP-2 + 20% egg yolk stored at 4-5°C can maintain spermatozoa membrane and motility for 8 days. Susilawati et al (2018) states that tris aminomethane + egg yolk and CEP + egg yolk can maintain the survival of spermatozoa better than using skim milk. For this reason, Tris + 20% EY and CEP-3 + 20% EY can be used as alternatives for liquid semen diluents, because they are easy to use and the ingredients are easily available.

Tris + 20% EY has a composition that is tris aminomethane, citric acid, lactose, fructose, streptomycin, penicillin, aquadest (Susilawati, 2011). Tris aminomethane and citric acid are used as buffer, lactose and fructose are useful as an energy source, streptomycin and penicillin function as anti-microbial, while the addition of egg yolk is used as cryoprotectant to keep spermatozoa during cold storage.

Cauda Epididymis Plasma (CEP-2) is a diluent that has a seminal plasma-like composition found in the epididymis. These conditions can maintain the quality of spermatozoa, membrane integrity and motility (Indriani et al., 2013; Purwoistri et al., 2013). CEP-3 + 20 EY is a modification of CEP-2 by replacing the expensive Bovine Serum Albumin (BSA) function with 0.4% egg white. The use of 0.4% egg whites can be used as a substitute for BSA in CEP-3 and can last up to 6 days in cold storage temperatures of 3-5 ° C (Sholikah, et al., 2016). Therefore, this study aims to determine the success rate of AI using liquid semen with different diluents in Bali cattle.

MATERIALS AND METHODS

This research was conducted on July 8 until November 7, 2019. The research location was at BPTU-HPT Denpasar Breeding Center Pulkan. The material used in this study were 60 Balinese cows that had a Body Condition score (BCS) of 2.5-3 (scale 1-5), physically fit, not pregnant, no reproductive disorders. cattle pen with semi intensive system. The feed given is king grass, indigofera and concentrate. Semen material used for liquid semen comes from a 4-year-old male weighing $\pm$ 450 kg. Accommodated using an artificial vagina, liquid semen is stored in a ministraw containing 0.25 ml (50 million sperm), liquid semen used for AI has motility above 40% (Table 2). AI and rectal palpation for pregnancy diagnosis are performed by officers who have attended training and are certified.

Table 1 – Composition of the Tris aminomethane + 20% EY and CEP-3 + 20% EY diluents

Composition	Tris aminomethane + 20% EY	CEP-3 + 20% EY diluent
NaCl (g/100ml)	-	0,09 g
KCl (g/100ml)	-	0,05 g
CaCl ₂ (H ₂ O) ₂ (g/100ml)	-	0,04 g
MgCl ₂ (H ₂ O) ₂ (g/100ml)	-	0,08 g
NaHCO ₃ (g/100ml)	-	0,10 g
NaH ₂ PO ₄ (g/100ml)	-	0,11 g
KH ₂ PO ₄ (g/100ml)	-	0,27 g
Fructose (g/100ml)	0,500 g	0,27 g
Lactose (g/100ml)	1,500 g	-
Tris (g/100ml)	1,363 g	1,61 g
Citric acid (g/100ml)	0,762 g	0,82 g
Penicilin (g/100ml)	0,100 g	0,009 g
Streptomycin (g/100ml)	0,100 g	0,01
Egg yolk (g/100ml)	20%	20%
Egg white (g/100ml)	-	0,4%

Andromed contains aquabidest, fructose, glyserol, citric acid, buffer, phosfolipid, spectinomycine, lincomycine, tylocin, gentamycine, but the exact composition of the andromed diluents is not available for publication. The making of andromed diluents is as follows, by mixing andromed with aquabides in a ratio of 1: 4, then homogeneous and ready to use (Susilawati, 2011).

Semen that has been accommodated is tested for quality macroscopically and microscopically. then the dilution process is carried out through 3 stages: the first stage is a

mixture of semen with diluent (ratio 1: 1 at 37°C) then cooled in the refrigerator. the second stage at 25 °C (50% of the remaining diluent to be added) and the third stage at 20 °C after that it waits until the temperature is 3-5 °C then motility after dilution is tested.

The method used in this study was experimental, with 3 treatments and 20 replications. Cattle used were 60 cows, each of which treated 20 cows, PO = AI with andromed diluent, P1 = AI with Tris aminomethane diluent + 20% egg yolk, P2 = AI with CEP diluent -3 + 20% egg yolk.

Bali cattle synchronized estrus with 2-time injection techniques. The hormone PGF2 α (Enzaprost-T) used for 1 injection is 5 ml. Detection of estrus is done through visual observations through observations on the vulva that is swollen, red (color), warm, mucous discharge, and mounting other cattle. Artificial insemination is carried out with a double dose of AI at the second and eighth hour after estrus.

The observed variables are non-return rate (NRR-1) on the 21st day after AI and conception rate (CR).

1. Non Return Rate (NRR). Non return rate is a percentage of the number of AI acceptors that are not estrus after the AI. Animals that did not estrus again after AI assumed that the cattle were pregnant (Jainudeen and Hafez. 2008).

$$\frac{\text{the number of cattle that are not show estrus again}}{\text{the number of all cattle in AI}} \times 100\%$$

2. Conception Rate (CR). Conception rate is the percentage of cows that are pregnant at the first AI (Susilawati, 2011). Detection of pregnant cows using the rectal palpation method.

$$\frac{\text{the number of cattle pregnant at the first AI}}{\text{the number of all cattle in AI}} \times 100\%$$

The data obtained were tabulated using Microsoft Excel by finding the average value, then analyzed descriptively (Harsojuwono, et al., 2011). For supporting variables namely the quantity of cervical mucus, vulva color, vaginal temperature were analyzed by the Kruskal wallis test and if there were differences, Mann Whitney continued testing.

RESULTS AND DISCUSSION

Semen quality before AI in this research had a motility percentage of P0 57 $\pm$ 6,19; P1 60 $\pm$ 5,65; P2 57 $\pm$ 5,63 (Table 2). The motility value of semen before AI is in accordance with Standart Nasional Indonesia (SNI) (minimum 40%).

Table 2 – Semen motility before AI

Treatments	Motility (%)
P0	57 $\pm$ 6,19
P1	60 $\pm$ 5,65
P2	57 $\pm$ 5,63

Table 3 – Success Rate AI

Treatments	Number of AI acceptors	NRR		CR (%)	
		Number of cattle	%	Number of cattle	%
P0	20	16	80	9	45
P1	20	12	60	8	40
P2	20	10	50	7	35

Note: P0 - Andromed diluents; P1 - Tris aminomethane diluent +20% EY; P2 - CEP-3 diluent + 20%EY.

Non return rate (NRR) is one method for estimating pregnancy in cattle. Varotto, et al (2016) states that NRR is an indicator of animal fertility and can be used to evaluate reproductive performance quickly without waiting for birth. NRR observations can be seen in

Table 3. Based on the Table, descriptively it is known that the highest NRR value is at P0: 80% with the number of cows assumed to be pregnant as many as 16, while the lowest value at P2 50% assuming 10 pregnant animals.

NRR value P2 and P1 in this study was lower than in previous studies in cattle P0, which is 81.48% (CEP-3 + 20 EY) and 81.48% (Tris aminomethane + 20 EY). The low value of NRR in this study believed to be caused by various factors, one of which is a factor of estrus synchronization. Chao et al. (2010) explained that the synchronization of estrus can affect the intensity of estrus. Kurykin et al. (2003) also argue, there is a tendency for signs of weak estrus in cattle which are synchronized using two PGF2 α injections. In addition, environmental factors such as the presence of a lactating can also be suspected to influence the success of the pregnancy. Islam (2011) states that the process of lactating can cause hormonal responses that can inhibit the emergence of estrus Aji, et al. (2017) states that lactating cattle will have an impact on the process of implantation in the uterus. lactating cattle have a higher likelihood of premature embryo death (Jousan et al., 2005). In addition, environmental factors such as the presence of lactating cattle can also be suspected to influence the success of the pregnancy. Islam (2011) states that the process of breastfeeding can cause hormonal responses that can inhibit the emergence of estrus. Aji et al. (2017) states that lactating cattle will have an impact on the process of implantation in the uterus. Lactating cattle have a higher likelihood of premature embryo death (Jousan et al., 2005).

Conception rate (CR) in this study was determined by using rectal palpation to detect pregnancy, so that it can be known more accurately. The highest CR value was in the P0: 45% treatment and the lowest was in the P2: 35% treatment. CR values in P1 and P2 that are lower than P0, may be caused by the quality of the diluent. In P1 (Tris aminomethane) and P2 (CEP-3) they contain egg yolk which is a product of livestock production. The product, consists of several components which cannot be standardized in quality and are varied, so that some components can have beneficial effects but some other components can also have adverse effects on spermatozoa function (Ratnawati, et al., 2017). whereas in P0 (andromed) is a thinner media without egg yolk and does not contain ingredients from animals so there is no risk of microbiological contamination. One of the composition of andromed which acts as cryoprotectant is glycerol. Glycerol is a substance that can diffuse into spermatozoa cells and can be metabolized into an energy source and form fructose (Susilawati, 2011). Papa et al. (2015) states that the use of diluent containing 7% glycerol in liquid semen can increase pregnancy percentage greater when compared with diluent without glycerol.

The CR value of treatment P1 40% and P2 35% in this study was lower than in previous studies in Ongole cattle, namely Tris aminomethane + 20% EY 83.3%; 59.26% (Costa et al., 2016; Saifudin et al., 2018) and CEP-3 + 20% EY 51.85% (Salim et al., 2018). The low CR value can be caused by a variety of factors, one of which is the quality of the semen used at the time of the AI, however, the motility of the semen used in this study is in accordance with SNI which is above 40% (Table 2.) so that the factors that are likely to influence the low CR value is an AI acceptor factor. Poor estrus quality can be suspected to be a factor causing low CR values. Roelofs et al (2010) also stated that there was a tendency to decrease the accuracy of estrus detection after the use of the prostaglandin hormone. The low estrous response might be influenced by individual factors in responding to the given PGF2 α hormone. The immature corpus luteum does not have PGF2 α receptors so that the induction of luteolysis with PGF2 α fails (Balumbi, et al., 2019). The estrus quality of each treatment can be seen in Table 4.

Table 4 – Scores of cervical mucus quantity, vulva color, vaginal temperature and vulvar swelling

Treatments	Scores of cervical mucus quantity *	Vulva color **	Vaginal temperature (°C)	vulva swelling% (n)
P0	2,30 $\pm$ 0,64 ^b	2,10 $\pm$ 0,51 ^a	38,96 $\pm$ 0,40 ^a	100 (20 ekor)
P1	1,80 $\pm$ 0,56 ^a	2,05 $\pm$ 0,45 ^a	38,72 $\pm$ 0,62 ^a	100 (20 ekor)
P2	1,82 $\pm$ 0,54 ^a	2,12 $\pm$ 0,40 ^a	38,70 $\pm$ 0,46 ^a	100 (20 ekor)

Note: * score of cervical mucus quantity: 1 = low/absent; 2 = moderate; 3 = abundant; ** vulva color score: 1 = pale pink; 2 = pink; 3 = red.

Based on the kruskal wallis test it is known that the quantity score of cervical mucus has a significant difference ($P < 0.05$). The score of cervical mucus quantity in treatments P1 and P2 was significantly different ($P < 0.05$) with P0. However, P1 treatment was not significantly different from P2. The highest average score of cervical mucus quantity was at P0 2.30 ± 0.64 while the lowest was at P1 1.80 ± 0.56 . Vulva color, vaginal temperature there was no significant difference ($p > 0.05$) in the two treatments. vulvar swelling occurs in all AI acceptors used in each treatment. a high Scores of cervical mucus quantity value at P0 can also be indicated that the response of estrus quality to the AI acceptor in the treatment is higher when compared to P1 and P2. Cervical mucus can function as a facilitator for spermatozoa to reach the ovum so that fertilization occurs (Berardi et al., 2015). Verma et al (2014) states that when cervical mucus has an influence on the value of conception in cattle. The appearance of cervical mucus can be used as an indicator of reproductive health related to estrous behavior and can be used as a sign to determine the exact time of artificial insemination, and the appearance of cervical mucus will increase the conception rate (Ondho et al., 2019). The quantity of cervical mucus is influenced by hormones estradiol produced by follicles. When estrous hormone estradiol is at its peak, it will trigger an increase in LH, resulting in ovulation and stimulation of cervical mucus discharge (Jainudeen and Hafez, 2008). In addition, other factors such as the condition of livestock, individuals, activities and interactions in livestock also affect the intensity of estrus (Kune and Najamudin, 2002).

CONCLUSION

The success rate of AI liquid semen using andromed (P0) (80% NRR, 45% CR) diluent is higher than Tris aminomethane (P1) (60% NRR, 40% CR) and CEP-3 (P2) (50%) diluents NRR, 35% CR).

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