

CONCEPTION RATES AND SEX CONCOMITANT OF BALI CALVES FOLLOWING OESTRUS SYNCHRONIZATION AND ARTIFICIAL INSEMINATION OF FROZEN-SEXED SEMEN UNDER FARM CONDITIONS

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ABSTRAK

Penelitian ini bertujuan untuk menentukan angka kebuntingan dan kelahiran sapi Bali sesudah sinkronisasi estrus dan inseminasi buatan (IB) dengan semen beku sexing serta kesesuaian jenis kelamin anak. Penelitian dilakukan pada dua lokasi peternakan di Kabupaten Lombok Barat. Sinkronisasi estrus dilakukan melalui pemberian satu suntikan 5 cc Lutalyse[®] yang mengandung 25 mg PGF_{2α} (Lutalyse, Sterile Solution, Pharmacia Animal Health) secara *intra muscular*. Pengamatan dilakukan pada sapi yang memberikan respon dan diinseminasi pada saat estrus. IB juga langsung dilakukan pada sapi-sapi yang menunjukkan birahi alami. Diagnosa kebuntingan dilakukan melalui palpasi rektal pada hari ke-90. Pada Peternakan 1, angka kebuntingan sapi-sapi yang mendapat perlakuan dan yang tidak mendapat perlakuan PGF_{2α} masing-masing sebesar 29/63 (46,03%) dan 71/75 (94,66%). Pada Peternakan 2, nilai-nilai yang bersesuaian masing-masing adalah 38/89 (42,70%) dan 10/23 (43,48%). Angka kelahiran anak pada kedua farm diperoleh 100%. Angka kesesuaian jenis kelamin untuk anak betina yang lahir di Peternakan 1 adalah 94,00%, sedangkan di Peternakan 2, untuk anak sapi yang terlahir jantan adalah 93,75%. Dibawah kondisi penelitian ini, sinkronisasi estrus yang diikuti dengan IB semen beku hasil sexing tampaknya dapat diaplikasikan di lapang sehingga menghasilkan angka kebuntingan dan kelahiran anak yang pantas diterima, serta angka kesesuaian di atas 90% untuk masing-masing jenis kelamin betina maupun jantan.

Kata kunci: inseminasi buatan, sapi Bali, sinkronisasi estrus, semen beku-sexing, angka kesesuaian jenis kelamin

ABSTRACT

The objective of this study was to determine the conception rate of Bali cattle synchronized and artificially inseminated with frozen thawed sexed-semen concomitant with its calves sex. This experiment was conducted in two cattle farms in West Lombok Regency. Oestrus was synchronized with a single injection i.m. of 5 cc Lutalyse[®] containing 25 mg of PGF_{2α} (Lutalyse, Sterile Solution, Pharmacia Animal Health). The observation was conducted in the responded cattle that artificially inseminated at once oestrus. Pregnancy diagnosis was conducted by rectal palpation at about day-90. In Farm 1, the conception rate (CR) of PGF_{2α} -treated and non-treated animals were 29/63 (46.03%) and 71/75 (94.66%), respectively. However, in Farm 2 the corresponding values were 38/89 (42.70%) and 10/23 (43.48%), respectively. CR in both farms were found being 100%. Sex concomitant rate for female calves born in Farm 1 was found being 94/100 (94.00%), whilst in Farm 2, sex concomitant rate for male calves born was recorded to be 45/48 (93.75%). It was concluded that under farm conditions oestrus synchronization using PGF_{2α} in conjunction with AI of frozen-thawed Bali sexed spermatozoa might be used with acceptable conception and calving rates that above 90 % concomitant with its respective calve-sex rates.

Keywords: artificial insemination, Bali cattle, frozen sexed-semen, oestrus synchronization, sex concomitant rate

INTRODUCTION

Artificial insemination (AI) technology is the most effective tool for genetic improvement in cattle. The AI service in West Nusa Tenggara (WNT) Province began in the 1976 using frozen semen from Simmental, Limousin, Brangus, Brahman, Friesian Holstein bulls among others. In enabling sustainable production of cattle crossbreds, AI program has to be developed in order to increase the socio-economic status of village farmers as the program had a positive impact on the livestock industry in the province. Thus, in the future AI will continue to play an important role as one of the reproductive technologies for cattle farming in WNT Province.

The added value of AI technology can be increased especially when supported by the technology of sperm separation to get calves with pre-determined sex. Currently, the only known means of effectively producing separate populations of X and Y sperm in mammals is thorough the use of DNA differentiation by high-speed flowcytometry (Johnson *et al.*, 1989). Applicable to virtually all mammals, this method can produce population of X or Y sperm with greater than 90% purity and subsequent offspring whose phenotypic sex is consistent with initial purity of the stored sperm population (Rath and Johnson, 2008).

Accuracy of sperm sexing by flowcytometry is estimated to reach 90% (Seidel and Garner, 2002), whereas Johnson and Seidel (1999) using this technique obtained 85-95% of births according to sex. However, the procurement of the equipment in the university laboratories and AI Centers in Indonesia is difficult to achieve as the price is very expensive. It seems that the use conventional methods of sperm separation need to be applied under WNT Province. Some conventional methods that have been used previously to separate sperm bearing X- and Y-chromosomes included sedimentation, electrophoresis, Sephadex G-50 filtration, centrifugation, column albumin (BSA) and other methods (Garner, 2001). Hafez and Hafez (1993) stated that because of the differences in the weight, density, or size of the X and Y chromosomes as a result of differences in the size of different components of the sperm, X and Y sperm can be separated. X chromosome is larger and carries more DNA than the smaller Y-chromosomes (Gordon, 2004).

According to Hafez and Hafez (1993), only

two laboratory methods for separation of animal and human X and Y sperm appear valid, reproducible, and clinically applicable: albumin separation, which yields 75% to 80% Y sperm, and Sephadex filtration, which yields 70% to 75% X chromosome. In Indonesia using column albumin method for sperm separation, Kaiin *et al.* (2005) reported that the percentages of motility of Hungarian frozen-thawed sexed-sperm yields 44% sperm bearing X- and 43% sperm bearing Y-chromosomes.

Most of the previous works conducted to examine the effect of sex-sorted sperm on fertility of cows reared with AI were carried out either on research stations or under ranch management system. However, adequate information is lacking on performance of AI in conjunction with sexed-semen under farm conditions. Therefore, the present study was conducted with the objective to examine the conception rate of Bali cattle synchronized and artificially inseminated with frozen-thawed Bali sexed-semen and its sex concomitant under farm conditions in WNT Province.

MATERIALS AND METHODS

Semen Collection and Processing of Sexed-Sperm

Semen collection and processing of sexed-sperm were conducted at Regional AI Center, Banyuwulek, WNT Province. Semen was collected from four bulls by using artificial vagina once a week. Each bull to be ejaculated was allowed to mount a teaser bull. Ejaculated semen was observed macroscopically for volume, color, smell, consistency and pH; and microscopically for mass and progressive motility, concentration, live sperm, abnormality and intact acrosome. Semen volume was recorded directly from the graduated semen collection tube. Each ejaculate having less than 70% sperm motility was discarded.

Separation of spermatozoa was carried out by means of albumin column method. Briefly, semen concentration of 300 ($\times 10^6$)cell/mL was suspended on the top of gradient albumin column. Each semen fraction was collected and put into centrifuge tube, then washed using Brackett & Oliphant (BO) medium and centrifuged at 2500 r.p.m for 10 min. The separated sperm was then diluted in tris-buffered diluent containing 20% egg yolk and glycerol as cryoprotectant (v/v). The diluted separated-sperm was then filled into 0.25

mL straw and sealed, cooled at 5°C (equilibration) for 4 h. Finally, the processed sexed-sperm was frozen and stored in liquid nitrogen (-196°C).

Oestrus Synchronization and AI of Sexed-Sperm

This experiment were conducted from November 2011 to February 2012 at 250 cows in two cattle farms, in West Lombok Regency where all private cattle owned by farmers and taken care by management of small holders, hence the management were similar in each farm. Selection of animals was conducted by per rectum palpation of corpus luteum functional of the ovaries. Animals with palpable corpora lutea were then synchronized with a single i.m. injection of 5 cc Lutalyse® containing 25 mg of PGF_{2α} (Lutalyse, Sterile Solution, Pharmacia Animal Health). The response cattle were artificially inseminated with frozen thawed sexed-semen once oestrus. Artificial insemination with frozen thawed sexed-semen was also performed directly to those animals observed in natural oestrus.

All animals in Farm 1 and Farm 2 were inseminated with frozen-thawed X- and Y-bearing spermatozoa, respectively. Pregnancy diagnosis was conducted to each cattle by rectal palpation at about 90 days after last insemination.

Data Analysis

Data regarding to semen parameters recorded were analyzed using analysis of variance (ANOVA). The difference among means was tested by Duncan's Multiple Range Test. Conception rates and sex concomitant rate were analyzed using Chi-square and treatment differences would have been considered different at an alpha level of 0.05.

RESULTS AND DISCUSSION

Characteristics of Fresh Semen

The overall characteristics of Bali bull fresh semen are presented in Table 1. The mean volume of ejaculate observed throughout the study was 6.47 ± 2.52 mL with the color of semen varied from white to cream and the smell from normal to specific as well as consistency from moderate to viscous. The pH of fresh semen was 7 (alkaline), indicated normal pH of Bali bull semen. The mean percentages of mass motility observed in this study varied from good (++) to very good (+++), while the progressive motility was 75.00 ± 4.33%. The average sperm

Table 1. Evaluation of Macroscopic and Microscopic of Fresh Bali Bull Semen

Semen Characteristics	Mean ± SD (n = 9)
Volume (mL)	6.47 ± 2.52
Color	white to cream
Smell	normal to specific
Consistency	moderate to viscous
pH	7
Mass motility	++ to +++
Motility (%)	75.00 ± 4.33
Concentration (x10 ⁶ sperm/mL)	1.741.00 ± 421.01
Intact plasma membrane (%)	90.44 ± 3.84
Abnormality (%)	3.78 ± 0.83

++=good, +++=very good

concentration was 1.741.00 ± 421.01 (x10⁶) spermatozoa/mL. The mean percentages of intact plasma membrane and the abnormality of spermatozoa noted in this study were 90.44 ± 3.84% and 3.78 ± 0.83%, respectively.

The semen characteristics of Bali bulls observed in this study were similar to those reported by previous authors. Kaiin *et al.* (2005) reported that semen volume of Hungarian bull was 7.00 ± 2.00 mL which was slightly lower than semen volume recorded in this study, that was 6.47 ± 2.52 mL; however, higher than that of Ongole Grade Cattle (PO), that was 5.56 ± 1.43 mL reported by Rasyid *et al.* (2006). The color, consistency, pH, smell and mass motility of Bali bulls semen recorded in this study agreed with the findings of Kaiin *et al.* (2005) in Hungarian bull semen. The mean percentage progressive sperm motility observed in this study was lower (75.00 ± 4.33%) than that of Kaiin *et al.* (2005), who recorded 79.60 ± 6.00% progressive sperm motility in Hungarian bull semen. Though, it was higher compared to progressive sperm motility of PO bull observed by Rasyid *et al.* (2006), that was 71.36 ± 16.45%. The present study shows that sperm concentration of 1.741.00 ± 421.01 (x 10⁶) sperm/mL was higher than that of PO bull's semen, that was 1.651.27 ± 813.46 (x 10⁶) sperm/mL (Rasyid *et al.*, 2006) and Hungarian bull's semen, that was 1025.00 ± 225.00 (x 10⁶) sperm/mL (Kaiin *et al.*, 2005). In addition, the

intact of plasma membrane was $90.44 \pm 3.84\%$, which was higher than that observed by Kaiin *et al.* (2005), that was $48.60 \pm 9.60\%$. Similarly, Bali bull had lower sperm abnormality ($3.78 \pm 0.83\%$) than that of Hungarian bull ($11.60 \pm 5.10\%$) and PO bull's semen, that was ($7.48 \pm 5.88\%$). The difference in these characteristics of semen might be attributed to differences in the breed, age, body size, level of nutrition, frequency of semen collection, and various other factors (Kaiin *et al.*, 2005).

Characteristics of X- and Y-bearing Spermatozoa after Sexing and Freezing of Fresh Semen

Table 2 shows the characteristics of fresh semen, X and Y bearing spermatozoa after sexing and freezing of fresh semen in Bali bulls. In this study, the averages motility and intact plasma membrane (acrosome) of spermatozoa decreased significantly ($P < 0.05$) when fresh semen was sexed into X- and Y-bearing spermatozoa, and these continue decreased significantly ($P < 0.05$) in frozen thawed sexed-semen. However, no significant differences ($P > 0.05$) was observed in the mean percentages of motility, intact plasma membrane and abnormality of spermatozoa between X and Y bearing spermatozoa after sexing and freezing of fresh semen. The decreased in motility and intact plasma membrane of spermatozoa might be due to the effect of separation of spermatozoa by centrifugation that caused damage membrane cells. It was noted that the mean percentages of abnormalities of X and Y bearing spermatozoa did not change much when fresh semen was frozen.

Data shown in Table 2 clearly indicate that sexing sperm using egg albumin technique is still

able to maintain motility and viability of frozen-thawed spermatozoa. The mean percentage of motility after thawing of frozen semen was slightly above 45 percent, so that the frozen thawed Bali sexed-semen might be used for AI in cattle.

Oestrus Synchronization Response

The percentages of cattle showing oestrus in treated and non-treated $\text{PGF}_{2\alpha}$ of Bali cattle at different farms are presented in Table 3. The number of animals that showed signs of oestrus in non-treated $\text{PGF}_{2\alpha}$ was significantly higher (76.53 %) than that of treated $\text{PGF}_{2\alpha}$ (41.45%) in Farm 1. Conversely, signs of oestrus were significantly higher in treated $\text{PGF}_{2\alpha}$ animals (58.55 %) than that of non-treated $\text{PGF}_{2\alpha}$ (23.47 %) in Farm 2. However within non-treated $\text{PGF}_{2\alpha}$ animals, oestrus was observed significantly higher in Farm 1 (76.53 %) than that of Farm 2 (23.47 %).

Oestrus in all treated animals occurred within 2-3 days after injection of $\text{PGF}_{2\alpha}$. It seems that $\text{PGF}_{2\alpha}$ lutealized corpus luteum so that progesterone levels decreased. Lower levels of progesterone will in turn affect the increase of FSH levels which stimulate maturation of follicle development that finally induce onset of oestrus in cattle. Though, it was observed that characteristics of oestrus detected in both treated- and non-treated $\text{PGF}_{2\alpha}$ animals were quite similar in terms of physical changes of the reproductive tract such as vulvular oedema and mucus discharge. This is in agreement with the findings of Amjad *et al.* (2006) who observed in Sahiwal cows.

Conception Rates

Data regarding to the conception rate of Bali

Table 2. Characteristics of Fresh Semen, X and Y Bearing Spermatozoa After Sexing and Freezing Fresh Semen

Semen Characteristics	Fresh Semen Mean \pm SD	Fresh Sexed-Semen		Frozen-Thawed Sexed-Semen	
		X Sperm Mean \pm SD	Y Sperm Mean \pm SD	X Sperm Mean \pm SD	Y Sperm Mean \pm SD
Motility (%)	75.00 ± 4.33^a	71.67 ± 2.36^b	72.78 ± 2.48^b	47.78 ± 2.48^c	45.56 ± 1.57^c
Intact sperm (%)	90.44 ± 3.84^a	86.11 ± 3.14^b	82.67 ± 3.89^b	68.89 ± 4.58^c	65.56 ± 5.42^c
Abnormality (%)	3.78 ± 0.83^a	4.11 ± 0.58^a	4.07 ± 0.85^a	4.13 ± 0.65^a	4.19 ± 0.50^a

a,b,c=different superscripts in the same row indicated significantly different ($P < 0.05$). SD=standard deviation.

cattle after artificially inseminated with frozen thawed sexed-semen either in treated and non-treated PGF_{2α} are presented in Table 4.

Results of the study showed that conception rate of non-treated PGF_{2α} animals was significantly higher (94.66%) than that of treated animals (46.03%) in Farm 1. In addition within treated PGF_{2α} animals, conception rate was significantly higher in Farm 1 than that of Farm 2 (46.03% vs 42.70%). Similarly, within non-treated PGF_{2α} animals, conception rate was significantly higher in Farm 1 than that of Farm 2 (94.66% vs 43.48%).

Low conception rates in Farm 2 either in treated or non-treated PGF_{2α} animals may be caused the corpus luteum of the animals that were too young (before day 5) to respond at practical levels. Bearden *et al.* (2004) suggested that prior

to day 5, the corpus luteum may not have sufficient receptor sites to respond to normal levels of GF_{2α}. In addition, metabolic pathways may have not developed sufficiently at this point to allow complete response. The differences in the conception rates between the two locations could also be due to differences in age and physiological state of the animal at the time of treatment, season of the year, dose and chemical nature of PGF_{2α} used (Amjad *et al.*, 2006).

Moreover, low conception rates found in Farm 2, both in treated and non-treated PGF_{2α} may be due to nutritional effect because of scarcity of fodders during late dry periods and high ambient temperature at the time that treatment was performed in this location. Inadequate of feed results in low body condition scores, and may adversely affect the follicular growth and ovulation. Other factor that might cause the differences in CR of cattle is that the distance between the AI center where frozen semen was kept and the farm locations. When inseminators would perform insemination, they had to pick up the semen to AI Centre, then brought it to the farm where its location quite far from the AI Centre, particularly in the case of Farm 2. By having arrived in the farm, it was too late to inseminate the animals. Regardless of location and type of oestrus, it was observed that from total 250 cattle used in this study the overall conception rate of Bali cattle was found to be 59.20%.

Calving Rate and its Concomitant with Calves Sex

The calving rate of the artificially inseminated frozen sexed-semen and its concomitant calves sex was shown in Table 5.

Table 3. The Percentages of Animals Showing Oestrus in Treated and Non-treated PGF_{2α} Bali Cattle at Different Farms

Location	Number of Animals Showing Oestrus (%)	
	Treated PGF _{2α}	Non-treated PGF _{2α}
Farm 1	63 (41.45) ^{a,c}	75 (76.53) ^{b,c}
Farm 2	89 (58.55) ^{a,c}	23 (23.47) ^{b,d}
Total	152 (100)	98 (100)

a,b : different superscripts in the same row indicate significantly different (P<0.05).

c,d : different superscripts in the same column indicate significantly different (P<0.05).

Table 4. Conception Rate of Bali Cattle Following Artificial Insemination of Treated and Non-treated GF_{2α} at Different Farms

Location	Conception Rate (%)		
	Total	Treated PGF _{2α}	Non-treated PGF _{2α}
Farm 1	100/138 (72.46)	29/63 (46.03) ^{a,c}	71/75 (94.66) ^{b,c}
Farm 2	48/112 (42.86)	38/89 (42.70) ^{b,d}	10/23 (43.48) ^{b,d}
Total	148/250 (59.20)	67/152 (44.08)	81/98 (82.65)

Conception Rate = Number of pregnant animals/number of inseminated animals

a,b : different superscripts in the same row indicated significantly different (P<0.05).

c,d : different superscripts in the same column indicated significantly different (P<0.05).

Table 5. Calving and Sex Concomitant Rates of Artificially Inseminated Using Sexed Semen

Location	Number of Pregnant	Number of Calves Born	Sex of Calves		Sex Concomitant Rates (%)	
			Female	Male	Female	Male
Farm 1	100	100	94	6	94/100 (94.00)	6/100 (6.00)
Farm 2	48	48	3	45	3/48 (6.25)	45/48 (93.75)
Total	148	148	97	51	97/148 (65.54)	51/148 (34.46)

Calving rate = Number of Calves Born/Number of Pregnant Animals

Data in Table 5 showed that a total 148 out of 250 cows that artificially inseminated with sexed-semen was pregnant and all pregnant animals in both farms had delivered normal-appearing offspring successfully. Thus, calving rate was recorded to be 100%. This indicates that all animals which are taken care by the management system of small holders farms could maintain its gestation until delivered.

When AI performed using X-sperm, it was expected that female offspring will be born. Table 5 indicated that the number of female calves born after AI with X-sperm in Farm 1 was 94%. While the number of male calves born after AI with Y-sperm in Farm 2 was 93.75%. This study indicated that sex concomitant rate was higher for female calves than that of data reported by Said *et al.* (2005), who recorded 81% in Simmental and Friesian Holstein cattle. Data of the study was also found within the range of data of Seidel and Garner (2002), Johnson and Saidel (1999) who separating sperm using flow cytometry that was able to reach expectation of 85-95% births according to sex of the offspring.

CONCLUSION

Oestrus synchronization using GF_{2a} can be used in conjunction with AI of frozen thawed Bali sexed-spermatozoa under farm conditions with acceptable conception and sex offspring concomitant rates. However, new methods of synchronizing oestrus in Bali cattle need to be sought in order to enhance results from AI with frozen thawed sexed-semen, thereby reduce the period of time required to detect oestrus or eliminate the need of oestrus detection entirely. Further research is needed to more precisely determine the appropriate timing of fixed-time insemination following administration of oestrus

synchronization agents and their extended application to Bali cattle in the wider areas.

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