

# Genetic Improvement of Bali Cattle through Artificial Insemination with Frozen Banteng (*Bos javanicus*) Semen: Part I – Performance of Resulting Calves (Work in Progress)

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**Abstract**— The performance of Bali cattle has been declining. To preserve their genetic quality and productivity, appropriate measures must be taken, such as revitalizing Bali cattle by breeding them with Banteng semen. This study aimed to evaluate the performance of offspring resulting from artificial insemination using frozen Banteng semen in Bali cattle. Ten Bali cows underwent synchronization twice using the PGF2a protocol. The cows were then monitored for signs of estrus 24 hours after the second injection, and breeding occurred 8-12 hours after confirming heat. Artificial insemination was performed with frozen Banteng semen from Taman Safari, Bogor, Indonesia. Insemination was conducted three times during estrus until ovulation was achieved. Pregnancy was diagnosed 20 days post-insemination. The dam's weight was recorded at the time of artificial insemination, and the calf's weight was tracked at birth and then weekly for 10 weeks using a specialized livestock scale. The birth weights recorded were  $18.33 \pm 2.42$  kg for female calves and  $20.25 \pm 2.71$  kg for male calves, with no significant difference in birth weights between the sexes ( $P > 0.05$ ). This study found that the average daily weight gain was 0.29 kg for female calves and 0.37 kg for male calves, indicating no significant growth difference between the sexes and higher than average for Bali calves born with Bali bull semen. The study concluded that Bali calves born through artificial insemination with frozen Banteng semen exhibited greater birth weight and body weight growth than average Bali calves.

**Keywords**— Bali cattle, Banteng, frozen semen, artificial insemination.

## I. INTRODUCTION

The Banteng (*Bos javanicus*) holds a protected status in Indonesia, as per Ministerial Regulation P.106/MENLHK/SETJEN/KUM.1/12/2018 (Permen 2018), and is classified as endangered on the International Union for Conservation of Nature (IUCN) Red List. Additionally, the Director General of KSDAE Decision No. 180/IV-KKH/2015 designates the Banteng as a priority species in the country. The Javanese Banteng is the ancestor of Bali cattle, which are bred in several regions of Indonesia. The domestication of the Javanese Banteng began around 3500 BC and was distributed to several regions in Indonesia [1].

Bali cattle, an indigenous breed of beef cattle in Indonesia, are found throughout the nation. These cattle were domesticated from the Banteng. Adapted to tropical climates, Bali cattle thrive and yield high-quality carcasses [2;3]. They have a relatively high fertility rate [4] low calf mortality rate [5] and can sustain themselves on low-quality feed [6]. The Central Statistics Agency [7] reported in its Agricultural Census that Bali cattle were the most raised beef cattle by Agricultural Households (RTUP) in 2023. Bali cattle accounted for 38.6% of the total beef cattle raised in this area. These cattle are predominantly raised in South Sulawesi, West Nusa Tenggara (NTB), East Nusa Tenggara (NTT), and Bali. South Sulawesi hosts the largest population, comprising 18% of the total population.

The performance of Bali cattle has declined. Prasodjo et al. [8] reported that the average birth weight of male calves was  $18.9 \pm 1.4$  kg, and that of female calves was  $17.9 \pm 1.6$  kg. In 2020, Gemuh [9] reported that the birth weights of female and male Bali cattle calves were 14.50 and 16.40 kg, respectively. Said et al. [10], also reported that the average birth weight of Bali cattle calves ranged from  $15.69 \pm 1.70$  kg (Lombok) to  $13.49 \pm 1.89$  kg (Sumbawa, West Nusa Tenggara). All three data points indicated a decline in the birth weight performance of Bali cattle.

Artificial insemination is the most widely used reproductive technology in livestock, including cattle. Banteng calves produced by artificial insemination at Taman Safari Bogor can reach 25-27 kg. To maintain the genetic quality and productivity of Bali cattle, appropriate efforts must be made, including the revitalization of Bali cattle by breeding them with Banteng semen. This study aimed to evaluate the performance of offspring resulting from artificial insemination with frozen Banteng semen in Bali cattle.

## II. MATERIAL AND METHODS

All procedure of this research performs by veterinarians at the Taman Safari Indonesia (TSI) Bogor, west Java and TSI Prigen, East Java, Indonesia. The research conducted from 2023 to 2024 at TSI Prigen, East Java. Prigen Safari Park is located at an altitude of around 800 meters above sea level.

### 2.1 Animals:

Ten Bali cows were obtained from the Bali Cattle Breeding and Forage Center (BPTU-HMT) in Denpasar, Bali. The criteria for the Bali cow were age 3-5 years, a body weight of approximately 270 kg, having given birth 1-2 times, a healthy body and reproductive system, and a normal cycle with a Body Condition Score (BCS) of 3 (Scale 1-5). The cows were transferred from the BPTU-HMT to TSI Prigen in East Java and acclimatized for one month prior to the study. The cows were housed in separate enclosures measuring 2.5 by 4 meters, where they were given green fodder and concentrate at rates of 10% and 1% of their body weight, respectively. They had unlimited access to drinking water.

### 2.2 Frozen Banteng Semen

The frozen semen used belonged to TSI, Bogor, which was frozen in Andromed<sup>®</sup> extender with a sperm motility value after thawing of 30-35% [11].

### 2.3 Estrus Synchronization

The estrus of Bali cows was synchronised using the PGF<sub>2</sub> $\alpha$  protocol twice. This process involved administering a 5 ml dose of PGF<sub>2</sub> $\alpha$  (Lutalyse) on day 0, followed by a second intramuscular injection of the same dosage 11 days later [12]. The cows were then observed for signs of estrus 24 hours after the second injection, and breeding took place 8-12 hours after confirming estrus. The response to estrus and the time taken for each cow to show estrus were documented by checking for estrus signs at intervals of 24, 48, 72, and 96 hours.

### 2.4 Artificial Insemination

The insemination timing or sperm deposition was performed when the dominant follicle measures between 1.2 and 1.4 cm [11;12], and this is linked to uterine changes. Artificial insemination utilized frozen Banteng semen from TSI, with each straw containing  $25 \times 10^6$  sperm. The post-thaw motility (PTM) was 30%, equating to approximately  $7.5 \times 10^6$  motile sperm [11]. Insemination was conducted three times during estruses until ovulation was achieved [12]. Equipment designed for AI in cattle was used for the insemination process.

### 2.5 Pregnancy diagnosis

Pregnancy diagnosis was made 20 days after insemination with transrectal ultrasound to determine the presence of the embryo in the amniotic sac and re-examination with ultrasound and rectal palpation on day 60 to ensure continuation of pregnancy [14].

## 2.6 Assessing Dam and Calves' Performance

The dam's weight is recorded at the time of artificial insemination, while the calf's weight is tracked at birth and then weekly for a duration of 10 weeks using a specialized livestock scale.

## 2.7 Statistical Analysis

An Excel t-test was employed to examine dam weights, the length of gestation, calf birth weights, and their daily weight gain. The results are shown as the mean  $\pm$  standard error of the mean (SEM).

## III. RESULT AND DISCUSSION

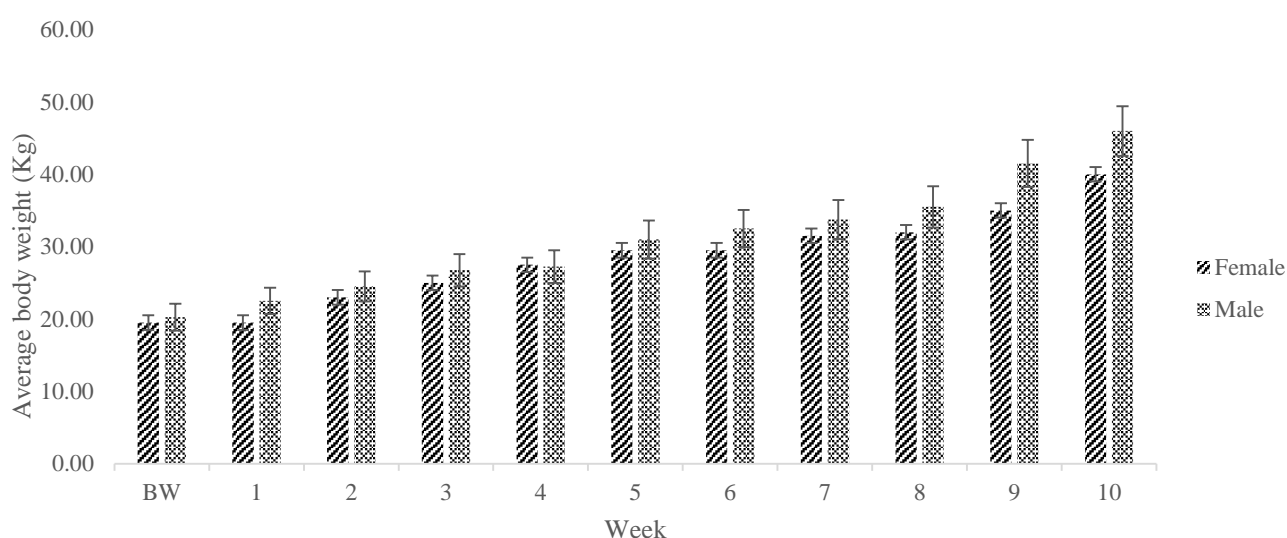
In this study, the weights of the female subjects varied between 259 and 295 kg, with no significant difference observed between those that birthed male or female calves ( $p>0.05$ ). All 10 Balinese cows that were synchronized responded to estruses. Among them, seven cows (70%) became pregnant and successfully gave birth to healthy calves, comprising four males (57%) and three females (42%). The birth weights recorded were  $18.33 \pm 2.42$  kg for female calves and  $20.25 \pm 2.71$  kg for male calves, with no significant difference in birth weights between the sexes ( $P>0.05$ ).

**TABLE 1**  
**THE PROFILE OF DAM WEIGHT AND CALVES BIRTH WEIGHT OF BALI COW INSEMINATE WITH FROZEN BANTENG SEMEN**

Variable	Calves sex		p-value
	Male	Female	
Dam weight (kg)	$272 \pm 4.01$	$278.33 \pm 4.06$	0.64
Calves birth weight (kg)	$20.25 \pm 2.71$	$18.33 \pm 2.42$	0.65

According to earlier reports, data from 2020 indicates that the birth weight of Balinese calves varied between 14.50 and 16.40 kg [9] and from  $13.49 \pm 1.89$  kg to  $15.69 \pm 1.70$  kg [10]. In this study, Balinese calves inseminated with frozen Banteng semen had birth weights ranging from  $18.33 \pm 2.42$  to  $20.25 \pm 2.71$  kg, showing an increase in birth weight. This is also higher than the  $17.9 \pm 1.6$  kg to  $18.9 \pm 1.4$  kg reported by Prasoj et al. [8] The findings of this research provide optimism for restoring Balinese cattle to their former performance levels.

The average weekly weight gain showed no difference between male and female calves ( $P>0.05$ ). The average weight gain between males and females was 2.05–2.58 kg (Figure 1; Table 2). Weight gain varies. From the first to the eighth week, the weight gain ranged from 0 to 3.50 kg. From the ninth and tenth weeks, the height gain ranged from 3 to 6 kg (Table 2).



**FIGURE 1: Weight gain in Balinese calves from crossbreeding with Banteng**

According to Chadijah [15], the daily weight increase for female Bali cattle calves is between 0.15 and 0.21 kg, while other reported that male calves gain between 0.245 and 0.256 kg per day [16]. Both studies concluded that male calves experience greater weight gain compared to female calves. Gemuh [9] reported growth of Bali calves per day between 0.29 to 0.30 kg. However, this research found that the average daily weight gain was 0.29 kg for female calves and 0.37 kg for male calves, indicating no significant growth difference between the sexes (Table 2).

**TABLE 2**  
**COMPARISON OF WEIGHT GAIN IN MALE AND FEMALE CALVES OF BALI CATTLE BRED USING BANTENG**  
**FROZEN SEMEN**

Calves sex	Delta Average gain (kg/week)										Average	p-value
	1	2	3	4	5	6	7	8	9	10	±SE	
Female	0	4	2	3	2	0	2	0.5	3	5	2.05±1.26	0.48
Male	2.3	2	2.3	1	3.8	2	1.3	1.8	6	5	2.58±1.29	

According to previous studies, the gestation period for female calve ranges from 258 to 307 days, while for males it is between 257 and 306 days [17]. Other research indicates a duration of 255 to 295 days for females and 256 to 295 days for males' calves, with an average gestation period of 283.9 to 284.9 days [8]. The gestation period for Bali cattle inseminated with frozen Banteng semen ranged from 293.67±2.01 to 295.75±1.12 days (Table 1), with no observed difference between male and female calves.

**TABLE 3**  
**GESTATION PERIOD IN BALINESE CATTLE AFTER INSEMINATION WITH FROZEN BANTENG SEMEN**

Gestation length	Calves sex		p-value
	Male	Female	
Minimum (Days)	294	289	
Maximum (Days)	297	296	
Average ±SEM	295.75±1.12	293.67±2.01	0.47

This study's data is limited by the small number of female participants, and the assessment of body weight gain was conducted only up to the 10th week. Additional research is necessary to extend the evaluation until weaning and even until the calves reach puberty, so that improvements in performance can be more clearly observed compared to Bali cattle inseminated with Bali bulls. Ongoing studies are exploring the enhancement of Bali cattle using frozen Banteng semen. Despite the limited number of female cattle, results from these insemination efforts suggest possible improvements in both birth weight and overall body weight gain.

#### IV. CONCLUSION

The study concluded that Bali calves born through artificial insemination with frozen Banteng semen exhibited greater birth weight and body weight growth compared to the average Bali calves.

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#### CONFLICT OF INTEREST

The author declares that there is no conflict of interest with the results of this study.

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