

陸、附件-發表資料

## **Semen Collection in Formosan Wild Boar (*Sus scrofa taiwanus*) using Low Voltage Electroejaculation Method**

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### **abstract**

Semen was collected using a low voltage Electroejaculation (EEJ) method **with five** captive Formosan wild boars in Taiwan. Sixteen ejaculates were obtained during this study. The average of sperm concentration was  $265 \pm 323$  ( $\times 10^6$ ) per ml with  $58.93 \pm 22.78$  % of motile sperm. The erection and ejaculation zones were found around 10 to 40 times of **three** voltage stimuli when the electric probe was **inserted** 15-18 cm deep into anus. During the whole procedure, the blood pressure was maintained at below 200 mmHg and no significant difference before and after EEJ **was observed**. Low voltage EEJ was found to be non-hazardous to the anesthetized wild boars, and no abnormalities were detected in cardiac and respiratory frequencies or body temperature during the procedure.

### **Introduction**

Formosan wild boar (*Sus scrofa taiwanus*) is one of the 16 subspecies of Eurasian wild boar. This boar subspecies is native only to Taiwan. Taipei Zoo organized a conservation and breeding program to protect this species. Since farmers allow their domestic pigs to roam free and interbreed with Formosan wild boars, it has become increasingly difficult to spot purebred Formosan wild boars in Taiwan in recent years. Therefore before this special breeding program started, each individual animal's "genetic resource" was first confirmed via gene sequencing and analysis by National Taiwan University. The cryopreservation of their genetic resources was one part of the program. Reproductive cells would be collected to store. However, male wild boars were difficult and dangerous to train to collect semen using artificial vagina or gloved

hand semen collection. EEJ would be particularly useful for collecting semen of this species. Because the electric effects would cause high blood pressure and impact on cardiac output, semen collection by EEJ method was thought to be harmful for animals. This study tried to use lower voltage electricity to reduce this concern.

### **Animals and methods**

Five gene-sequencing confirmed purebred Formosan wild boars, captive in Taipei Zoo, were used in the study in 2019. The body weight of these boars was ranging from 70 to 121 Kg and the ages were 5-12 years old. General anesthesia was performed using blow dart with muscle injection of Zoletil 3-4.5 mg/kg and maintain with 2-3% isoflurane. Body temperature, cardiac and respiratory frequencies were monitored continuously to make sure the anesthesia condition. Especially, blood pressure was checked using human sphygmomanometer during operation and before EEJ. If the blood pressure higher than 200 mmHg, the EEJ procedure would suspend to take a rest waiting the blood pressure to decrease or stop the whole process.

Preputial cavity was totally disinfected by flushing large amount of 0.7% provide-iodine solution. Normal saline was then flushed into preputial cavity before EEJ. Rectal feces were removed simply by hand or warm water flush. Ultra sound image was performed using rectal probe to identify the location of prostate gland. The length between anus to prostate gland was measured.

A rectal probe which had a diameter of 1.8 cm and length of 30cm, with three linear electrodes of 0.4 cm wide and 5 cm long was used. The electric probe covered with K-Y jelly was inserted into the rectum adjacent to the prostate gland. The probe delivers an AC voltage with 2-3 volts, wave frequency of 60 Hz and a current of 500 mA. Electrical stimuli were applied in steadily increasing steps, using a sine-wave electronic electroejaculator. The probe was activated for 3 seconds each stimuli from 2 volts for 20 stimuli then change to 3 volts for another 40-50 times. The voltage during the stimulation procedure is no more than 4 volts. After stimulation, preputial cavity was squeezed to let semen flow into 35 °C warm glasses container. Two to three series were performed, with 5 min rest interval. The color of the semen varied from slightly opaque to milky white. Then the semen were assessed for volume, sperm concentration, percentage of motile, forward progressive motility, viability, acrosomal integrity and structural morphology

### **Results and conclusion**

Sixteen ejaculates were obtained during this study. The average length of anus to prostate gland, measured by rectal ultra sound machine, was  $10.26 \pm 1.40$  cm. The erection and ejaculation zones were found around 10 to 40 times of 3 voltage stimuli

when the electric probe was put 15-18cm deep into anus. The semen was easy to get gelatinous using higher than 3 volts.

Low voltage EEJ method was found to be non-hazardous to the anesthetized wild boars, and no abnormalities were detected in cardiac and respiratory frequencies or body temperature during the procedure. During the whole procedure, the blood pressure was maintained less than 200 mmHg and no significant difference before and after EEJ. Therefore the anesthesia did not cause any side effects.

**Table 1.** Physical characteristics of male Formosan wild boar reproductive system

	mean±SD	range
Length of right testis (cm)	12.77 ± 1.17	10.6 - 14.0
Length of left testis (cm)	13.40 ± 0.98	11.8 - 14.6
Length of anus to prostate gland (cm)	10.26 ± 1.40	8 - 12

**Table 2.** Comparison of physiological values of wild boars before and after EEJ

	Before EEJ	After EEJ
Body temperature (°C)	36.83 ± 0.40	36.55 ± 0.38
Respiratory rate (/min)	20.67 ± 4.99	18.00 ± 4.00
Heart rate (/min)	89.83 ± 18.60	91.67 ± 15.03
Systolic blood pressure (mmHg)	131.1 ± 14.4	143.5 ± 24.5

**Table 3.** Characteristics of Formosan wild boar semen collected by EEJ

	mean±SD	range
Opalescent color semen	6/ 16	
Milky white Color semen	4/ 16	
Opaque with gel form semen	6/ 16	
Volume (mL)	9.05 ± 6.13	3.3 – 22.7
Sperm concentration(X10 <sup>6</sup> /mL)	265 ± 323	14.4- 1127
Motile(%)	58.93 ± 22.87	13.3 - 80
movement( 0-5 )	2.96 ± 0.43	2 – 3
progressive forward (%)	36.76 ± 14.81	8.9-48.8
Viability(%)	72.72 ± 14.80	38 - 90
Acrosome integrity(%)	84.30 ± 14.16	49 – 98
Morphology abnormal(%)	11.41 ± 12.51	1- 42

## Reference

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Fig. 1. Purebred Formosan Wild Boar



Fig. 4. milky white semen(left), slightly opaque with gel (right)



Fig. 5. Sperm structure under PAP stain



Fig. 2. EEJ operation under general anesthesia



Fig. 3. ultra sound image of prostate gland

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