The Thai Journal of Veterinary Medicine

Volume 42
Issue 4 *December, 2012*

Article 5

12-1-2012

Effect of Semen Collection Techniques on Semen Quality and Sperm Motility Parameters in Siamese Fighting Cock (Gallus gallus)

Nathavut Kanatiyanont

Kornchai Kornkaewrat

Piyawan Suthanmapinunt

Anuchai Pinyopummin

Original Article

Effect of Semen Collection Techniques on Semen Quality and Sperm Motility Parameters in Siamese Fighting Cock (*Gallus gallus*)

Nathavut Kanatiyanont[1,2] Kornchai Kornkaewrat[3]
Piyawan Suthanmapinunt[4] Anuchai Pinyopummin[5]*

Abstract

Abdominal massage (AM) is a common method for semen collection in avian species. There are few data concerning electro-ejaculation (EE) in this species. This experiment was carried out to study the effect of semen collection techniques (AM and EE) on semen quality and sperm motility parameters in three Siamese fighting cocks (*Gallus gallus*). EE was done under general anesthesia by isoflurane vaporizer. In experiment 1, either low voltage (5-10 volts) or high voltage (5-30 volts) protocols for EE stimulation was applied. Ejaculations were obtained from all attempts (n= 4) in both protocols. High voltage protocol gave greater percentage of sperm motility (p= 0.01), but caused bleeding in 2/4 attempts. In experiment 2, comparison between AM and low voltage EE techniques (n= 15), the semen quality and sperm motility parameters were not significantly different (p> 0.05). After incubation for 6 hours at 37^0C, all sperm motility parameters, except linearity, were also similar (p> 0.05). All cocks safely recovered from EE. Therefore, in addition to AM, low voltage EE technique could be the alternative method for semen collection, especially for the untamed and aggressive male birds. The ability to collect semen and the evaluation of semen are important factors for the management of breeding program or the application of reproductive technologies in avian species.

Keywords: abdominal massage, electro-ejaculation, semen quality, Siamese fighting cock

[1]*Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.*
[2] *Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand.*
[3] *Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.*
[4] *Department of Farm Resources and Production Medicine, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.*
[5] *Department of Large Animal and Wildlife Clinical Science, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.*
***Corresponding author: E-mail:** fvetacp@ku.ac.th*

บทคัดย่อ

ผลกระทบของวิธีการรีดเก็บน้ำเชื้อต่อคุณภาพของน้ำเชื้อ และค่าพารามิเตอร์การเคลื่อนที่ของตัวอสุจิในไก่ชนพันธุ์ไทย

*ณฐวุฒิ คณาติยานนท์ [1,2] กรไชย กรแก้วรัตน์ [3] ปิยวรรณ สุธรรมาภินันท์ [4] อนุชัย ภิญโญภูมิมินทร์ [5]**

การรีดเก็บน้ำเชื้อในสัตว์ปีกโดยทั่วไปใช้วิธีการนวดกระตุ้นท้อง การใช้เครื่องกระตุ้นการหลั่งน้ำเชื้อด้วยไฟฟ้ามีข้อมูลอยู่น้อย การศึกษานี้ได้ศึกษาถึงผลของวิธีการรีดน้ำเชื้อทั้งสองวิธีต่อคุณภาพน้ำเชื้อ และค่าพารามิเตอร์การเคลื่อนที่ของตัวอสุจิในไก่ชนพันธุ์ไทย จำนวน 3 ตัว ซึ่งการใช้เครื่องกระตุ้นการหลั่งน้ำเชื้อด้วยไฟฟ้า ทำหลังจากการทำให้สลบด้วยไอโซฟลูเรน การทดลองที่ 1 เปรียบเทียบระหว่างความต่างศักย์ต่ำ (5-10 โวลต์) และความต่างศักย์สูง (5-30 โวลต์) ที่ใช้ในการกระตุ้นด้วยไฟฟ้า พบว่าทั้งสองแบบสามารถทำให้เกิดการหลั่งน้ำเชื้อได้ทุกครั้ง (แบบละ 4 ครั้ง) การใช้ความต่างศักย์สูงได้น้ำเชื้อที่มีเปอร์เซ็นต์อสุจิที่เคลื่อนที่มากกว่าน้ำเชื้อที่กระตุ้นด้วยความต่างศักย์ต่ำ (p= 0.01) แต่ทำให้เกิดเลือดออกบริเวณที่สัมผัสแท่งกระตุ้น (2/4 ครั้ง) การทดลองที่ 2 เปรียบเทียบวิธีการรีดน้ำเชื้อระหว่างการนวดกระตุ้นท้องและการใช้เครื่องกระตุ้นการหลั่งน้ำเชื้อด้วยไฟฟ้า พบว่าคุณภาพน้ำเชื้อและค่าพารามิเตอร์การเคลื่อนที่ของตัวอสุจิจากสองวิธีการรีดไม่มีความแตกต่างกันทางสถิติ (p> 0.05) และหลังการเก็บรักษาที่ 37^{0}ซ. เป็นเวลา 6 ชั่วโมง ค่าพารามิเตอร์การเคลื่อนที่ของตัวอสุจิ ยังคงไม่มีความแตกต่างกัน (p> 0.05) ยกเว้นค่า linearity หลังการใช้เครื่องกระตุ้นการหลั่งน้ำเชื้อด้วยไฟฟ้า ไก่ทุกตัวฟื้นและเป็นปกติ ดังนั้นการรีดน้ำเชื้อด้วยเครื่องกระตุ้นไฟฟ้าโดยใช้ความต่างศักย์ต่ำจึงเป็นอีกทางเลือกหนึ่ง ในการเก็บน้ำเชื้อจากนกที่ไม่เชื่องและก้าวร้าว ทั้งนี้ความสามารถในการรีดเก็บและประเมินคุณภาพน้ำเชื้อเป็นปัจจัยสำคัญที่ช่วยในการจัดการผสมพันธุ์ และสำหรับใช้ในเทคโนโลยีการสืบพันธุ์อื่นๆต่อไป

คำสำคัญ: การนวดกระตุ้นท้อง การรีดน้ำเชื้อด้วยเครื่องกระตุ้นการหลั่งน้ำเชื้อด้วยไฟฟ้า คุณภาพน้ำเชื้อ ไก่ชนพันธุ์ไทย

[1]*ศูนย์เทคโนโลยีชีวภาพเกษตร มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ. นครปฐม 73140*

[2]*ศูนย์ความเป็นเลิศด้านเทคโนโลยีชีวภาพเกษตร สำนักพัฒนาบัณฑิตศึกษาและวิจัยด้านวิทยาศาสตร์และเทคโนโลยี สำนักงานคณะกรรมการการอุดมศึกษา กรุงเทพฯ 10900*

[3]*ภาควิชาเวชศาสตร์คลินิกสัตว์เลี้ยง มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ. นครปฐม 73140*

[4]*ภาควิชาเวชศาสตร์และทรัพยากรการผลิตสัตว์ มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ. นครปฐม 73140*

[5]*ภาควิชาเวชศาสตร์คลินิกสัตว์ใหญ่และสัตว์ป่า มหาวิทยาลัยเกษตรศาสตร์ วิทยาเขตกำแพงแสน จ. นครปฐม 73140*

***ผู้รับผิดชอบบทความ E-mail:** fvetacp@ku.ac.th*

Introduction

Avian semen collection technique by abdominal massage (AM) was initially employed in domestic chickens (Burrow and Quinn, 1935), and extensively applied for wild species, e.g. crane (Gee, 1983), parrot (Brock, 1991; Stelzer et al., 2005), parakeet (Anderson et al., 2002), pheasants (Jalme et al., 2003), vulture (Umapathy et al., 2005; Madeddu et al., 2009), penguin (Waldoch et al., 2007), bird of prey (Blanco et al., 2009) and rhea (Goes et al., 2010), with some limits to success (Brock, 1991; Jalme et al., 2003; Stelzer et al., 2005; Umapathy et al., 2005; Madeddu et al., 2009). Another technique for avian semen collection is the electro-ejaculation (EE) technique, which requires anesthesia. However, this technique has less reports in avian, e.g. drake (Watanabe, 1957; Setioko and Hetzel, 1984); parrot (Harrison and Wasmund, 1983); waterfowl (Samour, 1985) and hornbill (Ng et al., 1998), and is rarely used for routine semen collection. To our knowledge, only one study of the use of EE in chicken was reported (Kono and Hiura, 1983). However, recent availability of a safe inhalant anesthesia protocol for avian (Gunkel and Lafortune, 2005), together with the non necessity for semen collection training, especially in untamed and aggressive male birds, could make EE become a method of choice. This technique will allow those birds to be evaluated for their semen quality or fertility before entering reproductive management program.

In mammals, semen collection method may affect semen quality, e.g. volume, sperm motility or longevity. There were comparative studies between artificial vagina (AV) versus EE in cattle (Leon et al.,

1991), ram (Marco-Jimenez et al., 2005) and llama (Giuliano et al., 2008) or manual massage versus EE in dog (Christensen et al., 2011). Moreover, studies in primate and human using penile vibratory stimulation (PVS) and EE showed different quality of ejaculated semen (Yeoman et al., 1998; Brackett and Lynne, 2000; Schneiders et al., 2004). The varied compositions of seminal plasma in semen which is secreted from accessory sex glands, caused by collecting methods, may contribute to semen quality differences (Yeoman et al., 1998; Christensen et al., 2011).

For avian semen, unlike mammalian semen, the seminal fluid may come from paracloacal vascular body, lymphatic folds, dorsal proctodeal glands, ejaculatory groove region or tissues in the vicinity of the papilla of the ductus deferens, depending upon the species (Fujihara, 1992). In chicken, seminal fluid or transparent fluid (TF) mainly comes from lymphatic folds, resulting in variable sperm concentration by the ratio of TF addition to semen at ejaculation (Fujihara, 1992). TF has a favorable effect on sperm motility (Ashizawa and Wishart, 1987; Fujihara, 1992), no detrimental effect on sperm during in vitro storage (Wambeke and Fujihara, 1993), and contribution to protecting sperm from peroxidation (Surai et al., 1998).

The objective of the present study was to investigate the efficacy of electro-ejaculation technique for semen collection and its semen quality, in comparison to those of abdominal massage technique in Siamese fighting cock (*Gallus gallus*), a Thai native chicken breed. This result could be of benefit to the breed itself, and could be used as a model for implementation to other avian species.

Materials and Methods

Animal: Three Siamese fighting cocks, 2-3 years of age, were reared in individual cages (60x60x60 cm^3) and fed on food and water *ad libitum*. The experiment was conducted between September 2011 and May 2012. All cocks had no experience of semen collection.

Semen collection methods: Semen samples were collected from each cock every 2-4 weeks by either AM or EE techniques, alternatively. The animals were fasted for 6 hours before semen collection.

Abdominal massage (AM): Each cock was trained for AM technique (Burrow and Quinn, 1935) for 2 weeks. Thereafter, semen, avoidance of feces and urine, was collected after AM from cloaca by microtubes and used for the study.

Electro-ejaculation (EE): Cock was generally anesthetized by isoflurane vaporizer (Gunkel and Lafortune, 2005; Escobar et al., 2011) and placed in dorsal recumbent position. Feathers at cloacal area were cut, and the cloaca was washed with isotonic normal saline. The bipolar electric probe with three longitudinal electrodes was made from 1 ml. syringe (Fig 1). The probe was connected to an electro-stimulator (Model 304, P.T. Electronics, USA). For electrical stimulation, the electrode was positioned and contacted at the dorsal body wall. Electrical

Figure 1 The cloacal probe was modified from 1 ml. syringe

stimulation was applied as either low or high voltage series. Semen was collected and pooled in microtubes at the end of each series stimulation. Antibiotic paste was topically applied to the cloaca. The entire procedure took approximately 40 min/cock.

Semen evaluation: Semen was measured for volume using a micropipette. Some samples were also measured for pH using pH indicator stripes (Universalindikator, Merck Germany). Then, it was diluted to 1:50 with 37^0C warmed modified Tyrode's medium (TALP) (Sontakke et al., 2004). Sperm concentration, motility and velocity parameters were evaluated by Computer Assisted Semen Analysis (CASA; HTM-IVOS, Hamilton Thorne Bioscience, USA). For dead sperm count and sperm morphology, a smear was prepared from a mixture of diluted semen and 2% eosin-nigrosin. The percentage was based on 200 sperm count. Sperm morphology was analyzed according to the classification of Stelzer et al. (2005).

Sperm motility and velocity parameters evaluated by CASA were: total motile sperm; percentage of progressive motile sperm; average path velocity (VAP; µm/sec); progressive velocity (VSL; µm/sec); curvilinear velocity (VCL; µm/sec); straight line velocity (STR; µm/sec); amplitude of lateral head displacement (ALH; µm); beat cross frequency (BCF; Hz) and linearity of track (LIN; %). The instrument setting was adapted from Sontakke et al. (2004).

Experiment 1 Comparison of low and high voltages protocols for EE stimulation

To determine the efficacy of EE for semen collection, the two voltage series of stimulation, low and high voltage, were employed after general anesthesia. The ejaculated semen from both protocols was also evaluated for their quality.

Low voltage stimulation protocol: The protocol was modified from the method described by Ng et al. (1998) and the method used in mammal (Wildt et al., 1986). Briefly, three voltages series between 5-10 volts were applied. The first stimulation series was 5-6-7 volts, the second series was 6-7-8 volts and the last series was 8-9-10 volts. Stimuli were done 10 times for each voltage. Each stimulus took 3 sec with a gap of 3 sec between the stimuli. The animals rested for 5 min between each stimulation series.

High voltage stimulation protocol: The protocol was based on the method used in duck and goose (Samour et al., 1985). Two to three stimulation series were utilized. Each stimulation series began at 5 volts and gradually increased in 5-volt stages up to 30 volts. Stimuli were done 7 times for each voltage. Each stimulus took 3 sec and with a 3-sec interval between

them. Resting period was 5 min between each stimulation series.

Experiment 2 Comparison of AM and low voltage EE techniques

To compare the quality of semen obtained by AM and low voltage EE, each cock semen was collected by AM and EE, alternatively. Semen was evaluated as described above. Sperm motility and velocity parameters were measured freshly (0 hour) and after incubation at 37^0C for 6 hours.

Statistic analysis: Two-way analysis of variance (ANOVA) and paired *t*-test were used to analyze the semen quality and sperm velocity parameter. (Saunders and Trapp, 1994; Pallant, 2005).

Results

Comparison of low and high voltage protocols for EE stimulation: Under general anesthesia, all cocks responded to the electrical stimulation by protrusion of the phallus and leg spasm in both low and high voltage protocols. Semen could be obtained in all attempts. Ejaculation occurred mostly in the second series of stimulation at every voltage stimulus. Comparison of semen quality obtained from low and high voltage protocols is shown in Table 1. Both protocols yielded acceptable semen quality. There was a significant difference ($p< 0.05$) in the percentage of motile sperm. The other parameters, meanwhile, did not differ ($p> 0.05$) between protocols. However, in high voltages protocol two of four attempts resulted in slightly bleeding at the electric probe contacted area. The blood contaminated semen fractions were discarded and not included in the results. All cocks recovered safely after anesthesia. The injuries occurring in high voltage protocol were healed without any complication.

Table 1 Comparison of efficacy of semen collection and semen quality obtained between low and high voltage protocols for EE stimulation.

Parameters	Low voltages (Mean ±SD)	High voltages (Mean±SD)	*p*-value
Number of ejaculates/attempt	4/4	4/4*	
Semen volume (µl)	300.2±253.2	133.7±83.2	0.10
Sperm concentration ($x10^9$/ml)	13.2±9.6	15.1±6.0	0.72
Total sperm/ ejaculate ($x10^9$)	5.8±8.0	1.8±0.9	0.39
Motile sperm (%)	80.5±5.0 [a]	91.0±3.3 [b]	0.01
Normal sperm (%)	80.2±8.4	91.3±3.3	0.09
Dead sperm (%)	6.3±2.2	7.0±1.4	0.58

* Some semen fraction from two ejaculates were discarded due to blood contamination.
Values in the same row with different superscripts differed significantly ($p< 0.05$).

Comparison of AM and low voltage EE techniques: On Day 4 of AM training, some cocks responded with erectile abdominal feathers and phallus everting when squeezing at paracloacal area. All cocks started ejaculating on Day 8 and were used in the study after Day 14 of training. For EE, only low voltage protocol was used in all cocks.

Semen quality from both methods was compared (Table 2). The semen obtained by AM had a greater sperm concentration than EE ($p< 0.05$). However, the total sperm per ejaculate and other parameters were insignificantly different ($p> 0.05$). The semen pH were 7.6±0.7 (n= 4) in AM and 7.8±0.9 (n= 8) in EE, with insignificant difference between the two methods ($p> 0.05$).

Sperm motility and velocity parameters evaluated freshly after collection (0 hour) were insignificantly different ($p> 0.05$) between the two methods (Table 3). After incubation at 37^0C for 6 hours, all parameters except LIN were also insignificantly different ($p> 0.05$). The results indicated that sperm obtained from both AM and EE techniques could maintain their motility similarly.

Discussion

The present study indicated that semen collection by EE technique can be accomplished in Siamese fighting cocks with high success rate and safety. Semen obtained by EE has similar quality to those obtained by AM. The advantage of EE is non necessity for semen collection training as a requirement for AM, but with the condition of available anesthesia protocol and equipment.

As previously reviewed by Gee et al. (2004) that small electrode or high current can cause injury during electro-ejaculation, high voltage EE protocol employed in this study resulted in bleeding in some attempts. Therefore, low voltage EE protocol is safer and can give similar results.

Table 2 Comparison of semen quality obtained between AM and low voltage EE techniques

Parameters	AM (Mean±SD)	EE (Mean±SD)	*p*-value
Number of ejaculates	15	15	
Semen volume (µl)	194.2±159.8	163.8±163.8	0.61
Sperm concentration	29.9±16.5 [a]	17.4±13.0 [b]	0.02
Total sperm/ ejaculate ($x10^9$)	6.4±6.9	2.8±4.3	0.10
Motile sperm (%)	86.9±20.6	85.1±8.7	0.76
Normal sperm (%)	88.2±7.2	87.6±7.8	0.83
Dead sperm (%)*	4.8±2.3	5.9±2.1	0.22

* Data of 12 ejaculates for each technique
Values in the same row with different superscripts differed significantly ($p< 0.05$).

Table 3 Comparison of sperm motility parameters between AM and low voltage EE techniques

Sperm motility parameters	AM (Mean±SD)	EE (Mean±SD)	*p*-value
Number of ejaculates	15	15	
At 0 hour			
Total motility (%)	86.9±20.6	85.1±8.7	0.76
Progressive motility (%)	47.0±15.5	42.8±12.8	0.42
VAP (µm/sec)	114.0±19.6	101.2±22.0	0.10
VSL (µm/sec)	87.0±20.9	75.8±23.4	0.17
VCL (µm/sec)	166.3±25.9	153.2±25.3	0.17
STR (µm/sec)	73.2±7.1	70.9±8.2	0.42
ALH (µm)	5.9±0.8	5.5±0.8	0.31
BCF (Hz)	24.2±3.0	25.7±4.7	0.31
LIN (%)	50.9±6.7	47.6±8.7	0.25
At 6 hours after incubation at 37^0C			
Total motility (%)	84.4±19.7	72.6±16.5	0.08
Progressive motility (%)	56.7±14.2	47.0±13.8	0.06
VAP (µm/sec)	114.1±31.1	98.1±27.8	0.15
VSL (µm/sec)	97.0±26.9	81.3±25.5	0.11
VCL (µm/sec)	158.4±36.5	145.8±29.7	0.30
STR (µm/sec)	81.6±5.2	78.6±5.6	0.14
ALH (µm)	5.5±0.9	5.4±0.9	0.83
BCF (Hz)	26.7±3.6	27.4±4.0	0.59
LIN (%)	58.8±7.0 [a]	52.8±8.5 [b]	0.04

Values in the same row with different superscripts differed significantly ($p< 0.05$).

Semen quality of Siamese fighting cocks obtained by both AM and EE techniques in this study was comparable to that of previous studies in Thai native cocks using AM technique: semen volume [0.13-0.30 ml (this study) vs. 0.27-0.41 ml (Chotesangasa, 2001; Sunathai et al., 2004)], total sperm/ejaculate [1.8-6.4x10^9 (this study) vs. 2.2-3.2x10^9 (Chotesangasa, 2001) and sperm total motility [80.5-91.0 % (this study) vs. 93.8-96.6 % (Vongphalab and Phasuk, 2007). Other characteristics were within the ranges reported for cocks of this species (Garner and Hafez, 2000).

The methods of semen collection affected semen quality in several mammalian species (Leon et al., 1991; Yeoman et al., 1998; Brackett and Lynne, 2000; Schneiders et al., 2004; Marco-Jimenez et al., 2005; Giuliano et al., 2008; Christensen et al., 2011). The variation in seminal fluid composition may contribute to different results (Yeoman et al., 1998; Christensen et al., 2011). The present study did not find any significant differences ($p> 0.05$) in semen characteristics between AM and EE. This may due to the different source of seminal fluid. In avian species, the fluid is secreted from the cloacal area that is being squeezed during the collection in both AM and EE techniques. Therefore, the similar semen composition could be obtained. A study in drake that has intromitten phallus revealed the different semen quality among the use of artificial vagina, electro ejaculation and manual massage (Setioko and Hetzel, 1984). The dissimilar structure as non-intromitten phallus of domestic chicken might be responsible for the similar results between the two methods in the present study.

In conclusion, in addition to AM, another common semen collection technique in avian is the low voltage EE technique, which may be the alternative technique, especially for untamed and aggressive male birds. As demonstrated in the Siamese fighting cocks, the technique is safe and semen quality can be evaluated. The ability to collect and evaluation of semen from any avian species are important factors for the management of breeding program or application of reproductive technologies.

Acknowledgements

This research is supported by the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education. (AG-BIO/PERDO-CHE).

References

Anderson, S.J., Bird, D.M. and Hagen, M.D. 2002. Semen characteristics of the Quaker parakeet (*Myiopsitta monachus*). Zoo Biol. 21: 507-513.

Ashizawa, K. and Wishart, G.J. 1987. Resolution of the sperm motility-stimulating principle of fowl seminal plasma into Ca^{2+} and an unidentified low molecular weight factor. J Reprod Fertil. 81: 495-499.

Brackett, N.L., Lynne, C.M. 2000. The method of assisted ejaculation affects the outcome of semen quality studies in men with spinal cord injury: A review. Neuro Rehabilitation. 15: 89-100.

Blanco, J.M., Wildt, D.E., Höfle, U., Voelker, W. and Donoghue, A.M. 2009. Implementing artificial insemination as an effective tool for *ex situ* conservation of endangered avian species. Theriogenology. 71: 200-213.

Brock, M.K. 1991. Semen collection and artificial insemination in the Hispaniolan parrot (*Amazona ventralis*). J Zoo Wildl Med. 22(1): 107-114.

Burrows, W.H. and Quinn, J.P. 1935. A method of obtaining spermatozoa from the domestic fowl. Poult Sci. 14: 251-254.

Chotesangasa, R. 2001. Effects of mating ratio, cock number in the flock and breeder age on fertility in Thai native chicken flock. Kasetsart J Nat Sci 35: 122-131.

Christensen, B.W., Asa, C.S., Wang, C., Vansandt, L.,

Bauman, K., Callahan, M., Jens, J.K. and Ellinwood, N.M. 2011. Effect of semen collection method on sperm motility of gray wolves (*Canis lupus*) and domestic dogs (*C. l. familiaris*). Theriogenology. 76: 975-980.

Escobar, A., Thiesen, R., Vitaliano, S.N., Belmonte, E.A., Werther, K. and Valadao, C.A.A. 2011. Cardiorespiratory effects of isofurane anesthesian in crested Caracarus (*Caracarus plancus*). J Zoo Wildl Med. 42(1): 12-17.

Fujihara, N. 1992. Accessory reproductive fluids and organs in male domestic birds. Worlds Poult Sci J. 48: 39-56.

Garner, D.L. and Hafez, E.S.E. 2000. Spermatozoa and seminal plasma. In: Reproduction in Farm Animals. 7th ed. E.S.E. Hafez and B. Hafez (eds). Lippincott Williams and Wilkins, USA. 96-109.

Gee, G.F. 1983. Crane reproductive physiology and conservation. Zoo Biol. 2: 199-213.

Gee, G.F., Bertschinger, H., Donoghue, A.M., Blanco, J. and Soley, J. 2004. Reproduction in nondomestic birds: Physiology, semen collection, artificial insemination and cryopreservation. Avian Poult Biol Rev. 15(2): 47-101.

Giuliano, S., Director, A., Gambarotta, M., Trasorras, V. and Miragaya, M. 2008. Collection method, season and individual variation on seminal characteristics in the llama (*Lama glama*). Anim Reprod Sci. 104: 359-369.

Goes, P.A.A., Cavalcante, A.K. da S., Nichi, M., Perez, E.G. de A., Barnabe, R.C. and Barnabe, V.H. 2010. Reproductive characteristics of captive greater Rhea (*Rhea americana*) males reared in the state of São Paulo, Brazil. Braz J Poult Sci. 12(1): 57-62.

Gunkel, C. and Lafortune, M. 2005. Current techniques in avian anesthesia. Sem Avian Exot Pet Med. 14: 263-276.

Harrison, G.J. and Wasmund, D. 1983. Preliminary studies of electroejaculation to facilitate manual semen collection in psittacines. Proceedings of the Annual Meeting of the Association of Avian Veterinarians, Krakow. Poland. 207-213.

Jalme, M.S., Lecoq, R., Seigneurin, F., Blesbois, E. and Plouzeau, E. 2003. Cryopreservation of semen from endangered pheasants: the step towards a cryobank for endangered avian species. Theriogenology. 59: 875-888.

Kono, K. and Hiura, Y. 1983. Semen collection by rectal electro-ejaculation in the domestic fowl. Jpn Poult Sci. 20: 267-270.

Leon, H., Porras, A.A., Galina, C.S. and Navarro-Fietro, R. 1991. Effect of the collection method on semen characteristics of Zebu and European type cattle in the tropics**.** Theriogenology. 36: 349-355.

Madeddu, M., Berlinguer, F., Ledda, M., Leoni, G.G., Satta, V., Succu, S., Rotta, A., Pasciu, V., Zinellu, A., Muzzeddu, M., Carru, C. and Naitana, S. 2009. Ejaculate collection efficiency and post-thaw semen quality in wild-caught Griffon vultures from the Sardinian population. Reprod Biol Endocrinol. 19: 7-18.

Marco-Jime´nez, F., Puchades, S., Gadea, J., Vicente, J.S. and Viudes-de-Castro, M.P. 2005. Effect of semen collection method on pre- and post-thaw Guirra ram spermatozoa. Theriogenology 64: 1756-1765.

Martı´nez-Pastor, F., Martı´nez, F. , A´lvarez, M., Maroto-Morales, A., Garcı´a-Alvarez, O., Soler, A.J., Garde, J.J., de Paz, P. and Anel, L. 2009. Cryopreservation of Iberian red deer (*Cervus elaphus hispanicus*) spermatozoa obtained by electroejaculation. Theriogenology 71: 628-638.

Ng, S.C., Mathews, J., Wong, H.M., Bongso, A. and Seager, S.W.J. 1998. Characterisatoin of sperm from the Oriental pied hornbill *Anthracoceros albirostris convexus* collection by electroejaculation. In: The Asian hornbills: ecology and conservation, Proceedings of 2nd International Asian Hornbill Workshop, *Thai Studies in Biodiversity* No. 2. P. Poonswad (ed.). Bangkok: 85-92.

Pallant, J.F. 2005. SPPS survival manual: a step by step guide to data analysis using SPPS. Ligare, Sydney. 318 pp.

Samour, J.H., Spratt, D.M.J., Hutton, R.E. and Jones, D.M. 1985. Studies on semen collection in waterfowl by electrical stimulation. Br Vet J. 141: 265–268.

Saunders, B.D. and Trapp, R.G. 1994. Basic and clinical Biostatistics, 2nd ed. Appleton & Lange, USA. 344 pp.

Schneiders, A., Sonksen, J. and Hodges, J.K. 2004. Penile vibratory stimulation in the Marmoset monkey: A practical alternative to electro-ejaculation, yielding ejaculates of enhanced quality. J Med Primatol. 33: 98-104.

Setioko, A.R. and Hetzel, D.J.S. 1984. The effect of collection method and housing system on semen production and fertility of Alabio drakes. Br Poult Sci. 25: 167-172.

Sontakke, S.D., Umapathy, G., Sivaram, V., Kholkute, S.D. and Shivaji, S. 2004. Semen characteristics, cryopreservation, and successful artificial insemination in the blue rock pigeon (*Columba livia*). Theriogenology. 62: 139-153.

Stelzer, G., Crosta, L., Burkle, M. and Krautwald-Junghanns, M.E. 2005. Attempted semen collection using the massage technique and semen analysis in various psittacine species. J Avian Med Surg. 19: 7-13.

Sunathai, S., Cheunchomjitr, T., Phothikanit, K. and Vongphalab, T. 2004. Frozen semen production of Thai indigenous cocks. KKU. Vet J. 40: 26-33.

Surai, P.F., Blesbois, E., Grasseau, I., Chalah, T., Brillard, J.P., Wishart, G.J., Cerolini, S. and Sparks, N.H.C. 1998. Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. Comp Biochem Physiol B Biochem Mol Biol. 120: 527-533.

Umapathy, G., Sontakke, S., Reddy, A., Ahmed, S. and Shivaji, S. 2005. Semen characteristics of the captive Indian white-backed vulture (*Gyps bengalensis*). Biol Reprod. 73: 1039-1045.

Vongphalab, T. and Phasuk, Y. 2007. Development of semen cryopreservation and artificial insemination techniques in Thai native chicken.

The Thailand Research Fund Report. 129 p.

Waldoch, J., Root, T., Ramer, J. and Proundfoot, J. 2007. Semen collection and characterization in Rockhopper penguins (*Eudyptes Chrysocome chrysocome*). J Zoo Wildl Med. 38(1): 13-17.

Wambeke, F.V. and Fujihara, N. 1993. The effect of adding transparent fluid to fowl semen on fertility and hatchability after 24 h of storage. Asian-Aust J Anim Sci. 6(3): 447-450.

Watanabe, M. 1957. An improved technique of the artificial insemination in ducks. J Fac Fish Anim Husb, Hiroshima Univ. 1: 363–367.

Wildt, D.E., Howard, J.G., Hall, L.L. and Bush, M. 1986. Reproductive physiology of the clouded leopard: I. Electroejaculates contain high proportions of pleiomorphic spermatozoa throughout the year. Biol Reprod. 34: 937-947.

Yeoman, R.R., Sonksen, J., Gibson, S.V., Rizk, B.M. and Abee, C.R. 1998. Penile vibratory stimulation yields increased spermatozoa and accessory gland production compared with rectal electroejaculation in a neurologically intact primate (*Saimiri boliviensis*). Hum Reprod. 13: 2527-2531.