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Research article

Semen characterization of Dang Surat Thai native chicken

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Abstract

Native Dang Surat chicken play an important role in poultry breeding of southern Thailand but their production performance falls short of commercial counterparts. Nevertheless, native birds have superior disease resistance while meat texture and taste of eggs are popular among Thais. Husbandry of native breeds are confined to smallholders who can only fulfil local niche outlets. To satisfy the wide-spread demand throughout Thailand and further afield, local-breed sires are crossed with high productivity hens. To maintain a constant supply of F1 hybrids, a colony of Dang Surat cocks are maintained by the Department of Livestock who select by phenotypes, not semen quality that determines male productivity. Accordingly, semen volume, pH, color and consistency, sperm motility, concentration, viability were measured. Morphology was also assessed by using eosin-nigrosin staining. Semen was slightly alkaline (pH, 8.0±0.1), mean volume=265±23 µL, had high sperm concentrations, 2740±76 x106/ml, of which 98.9±0.1% were viable. Abnormal sperm heads, tails and mid pieces, cytoplasmic droplets and detached heads comprised 1.5±0.3, 3.7±0.4, 0.3±0.1 and 0.8±0.1%, respectively. The findings showed normal seminal fluids and sperm parameters similar to commercial poultry semen in general. Our data will help develop efficient cockerel selection, natural flock breeding plan, and assisted reproduction by cryopreservation and artificial insemination to better meet market demands.

Keywords: Artificial insemination, Cryopreservation, Dang Surat cock, Poultry breeding, Rooster, Semen characteristics

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INTRODUCTION

Native poultry is important to socio-economic aspects of smallholder communities for their superior characteristics of adaptability to the local environment, tolerance to common diseases, and their preference for taste of their meat and eggs. However, productivity of native breeds compares poorly with commercial breeds (Tongsiri et al., 2019). Currently, the practice is to improve meat quality and increase egg productivity is to cross native cocks with different exotic breeds thereby producing new commercial lines.

Almost two decades ago, the Dang Surat chicken, a native of Thailand, was identified and established by the Thai Department of Livestock Development as a native poultry breed chicken of Southern Thailand. They have been maintaining parent stock that is phenotypically selected each year at Surathani Breeding Center. This local breed has provided roosters for our well-known "Ligor chicken", a commercial cross-breed developed by Walailak University (Angkanawisut, 2021). However, there is a dearth of information on male reproductive performance of Dang Surat parent stock.

Despite the ability to produce a viable and complex trait of progenies in both genders (Wolc et al., 2019), the reproductive potential of male breeders has not been explored for its potential compared to the hens. Semen is composed of sperm and seminal plasma secreted by the epididymis and vas deferens. Sperm quality is a predictive factor for male fertility. This is the primary determinant of high fertilization capacity and subsequent hatchability of eggs in the fowl (Froman et al., 1999; Peters et al., 2008; Sun et al., 2019). Hence, rooster reproductive performance based on semen characteristics should be a suitable selection criterion for efficient poultry production. This information can be used as a management tool for sire selection. It will allow poultry breeders the opportunity to select only good fertilizing potential males for flock natural mating or artificial insemination.

Significant variations between breeds in semen volume, pH, sperm motility, viability, and abnormal sperm percentage have been reported (Goneppanavar and Prabhu, 2013; Tesfay et al., 2020) but there is no such information on the Dang Surat. Therefore, the present study aimed to characterise semen and sperm in order to determine the performance of Dang Surat roosters. This data will help formulate the most effective and efficient policy of maintaining flock of these birds.

MATERIALS AND METHODS

Animal

Twenty-four healthy 20-month-old Dang Surat cocks, with an average weight of 3.0 ± 0.39 kg (range 2.2-3.6 kg), were purchased from the Surat Thani breeding center, Department of Livestock Development. The roosters were housed in a conventional open-sided house with a concrete floor under their natural environmental and climatic conditions prevailing in southern Thailand. They were weighed, identified using leg bands, and reared in individual cages measuring 50 cm × 30 cm × 40 cm (length × width × height) with free access to feed and water. The cocks were fed with the commercial meat-type chicken

feed that contained 12% crude protein, 120 grams per head per day, and water was provided *ad libitum*. They were left to acclimatize to the new environment for one month before undergoing training in semen collection for two weeks. The experiment was conducted from January to March where the photoperiod was approximately 14 hours of day light per day.

Semen collection

Individual semen collections were done in the morning, between 08:00 a.m. to 9:00 a.m. twice a week on Tuesday and Friday. The dorso-abdominal massage technique (Burrows and Quinn, 1937) was used to collect the semen. Semen from each cock was placed directly in a 1.5 ml microtube (Eppendorf, Humburg, Germany) at room temperature. The ID number of chickens, and the time/date of each collection were recorded. The samples were then transferred to the laboratory for more evaluation within 30 minutes.

Semen evaluation

Volume, pH, and color/consistency of every undiluted semen sample were measured. The ejaculate volume was determined by aspirating the semen into a calibrated positive displacement pipette. The pH was measured by Hydrion™ pH indicator test papers (Micro Essential Lab, USA). The osmolarity was measured in 10 µL aliquots using a vapor pressure micro-osmometer (Wescor 5500, Wescor Inc., UT, USA)

Semen color/consistency was visually estimated under day-light scored as 1 = clear (watery), 2 = cloudy, 3 = milky, and 4 = creamy (high consistency) as described previously (Evans and Maxwell, 1987) (Figure 1A). Semen contaminated with feces, uric, or blood was discarded (Figure 1B), and only clean samples were used for semen analysis.

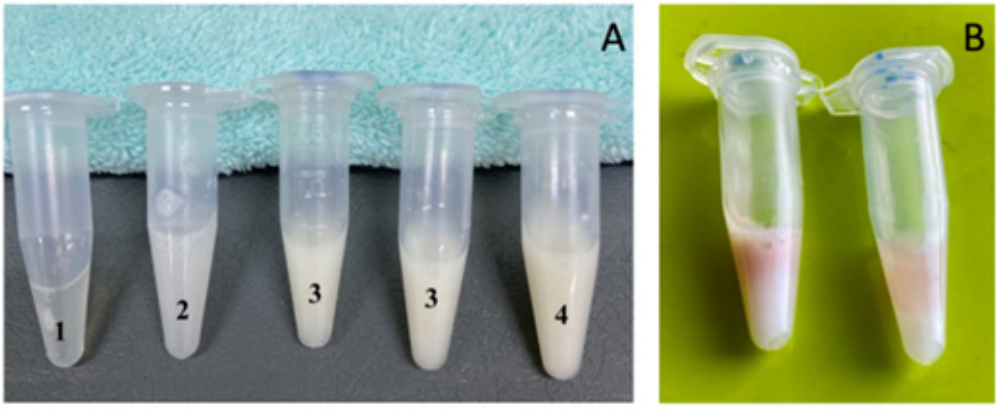

Figure 1 (A) Gross evaluation of color and consistency of semen; 1= Clear (watery); 2 = Cloudy; 3 = Milky; 4 = Creamy. (B) Samples of semen contaminated with blood or feces.

Mass motility

A drop of fresh semen on a clean slide and the wave motion was observed by light microscopy (100x magnification), without coverslip. The mass motility was estimated by the vigor of the wave motion on a 5-point scale (0 = no movement, 1 = very poor, weak movement, 2 = poor but some movement, 3 = fair, slow movement, 4 = good, vigorous movement, 5 = very good, wave-like movement) as described previously (Getachew, 2016).

Spermatozoan motility

A drop of semen was placed on a glass slide, covered by a cover slip and motility was observed by bright-field light microscopy (400x magnification). The proportion of motile sperm was estimated as a percentage sperm present.

Progressive motility

Sperm moving in a single direction were expressed as a proportion of total cells present.

Sperm concentration

Each sample was diluted 1:400 in 0.9% saline. A drop of 10 µl of the diluted semen was pipetted into each hemocytometer chamber and cells allowed to settle. The sperm concentration was assessed by the average number of sperm counted in both chambers under microscopy at 400x magnification (Bakst and Cecil, 1997).

Viability and Morphology of spermatozoa

Spermatozoa viability was expressed as percentages of live sperm compared to total cells. Dead sperm stained pink with eosin-nigrosin while viable cells appeared white by bright field microscopy. Morphologically normal or abnormal sperm with any pink discoloration of the head region was considered dead or non-viable sperm. Nigrosin provides a background to enhance visual differentiation between the non-viable and viable sperm (Bakst and Dymond, 2013). A minimum of 200 sperms were counted on each slide and the percentage of viability and abnormality were reported.

Ethics Statement

The study was performed under the local ethical guidelines and met the requirement of the Institutional Animal Care and Use Committee. (Animal Ethics Committee of Walialak University Approval No. WU-ACUC)-64036).

Statistical analyses

Sample size calculation using a 95% confidence interval and 20% margin of error required 20 roosters for semen quality analysis. Means ± standard error (SEM) of each semen parameter was reported as a descriptive analysis. To compare the means of concentration and identify color of chicken semen, the generalized linear model (GLM) was used. For Post Hoc multiple comparisons, Tukey's method was applied. The level of significance for all statistical analyses were set at 0.05.

RESULTS

The mean volume of semen was 265 ± 23 µl (SEM) while the sperm concentration was 2740±76 (x10^6) sperm/ml. Twelve semen collections were collected from each of 24 Dang Surat roosters and the semen characteristics were evaluated. The results were summarized in Table 1.

Table 1 Semen quality of Dang Surat roosters (n=24) 12 replications

Parameters	Mean	SEM	Range
Semen volume (µL)	265.0	23.0	500-95
Semen pH	8.0	0.1	8.4-7.6
Mass motility (0-5)	3.6	0.2	4.7-2.4
Individual motility (%)	79.9	1.7	93.8-61.1
Progressive motility (%)	42.1	1.5	57.3-24.8
Sperm concentration (10^6/ml)	2739	76	3654-2097

The appearance of fresh semen from our collection was mostly milky in color (74.6%). The rest were cloudy (20.0%), and creamy (5.4%). The highest concentrated semen of 4073 ± 316 (x10^6) /ml (mean ± SEM) could be indicated by its creamy-colored viscous appearance (Table 2). The milky appearance had a mean concentration of 2857 ± 62 (x10^6) /ml. The cloudy appearance had the lowest concentration (2040 ± 147 (x10^6) /ml). There were robust differences among three group which suggests our visual distinctions is a reflection of sperm concentration.

Table 2 Percentage and average sperm concentration (10^6/ml) in different appearance of semen

Color/Consistency	%	Mean	SEM	Range
Creamy	5.4	4073[a]	316	5625-2500
Milky	74.6	2857[b]	62	5300-438
Cloudy	20	2037[c]	147	4750-125

[a,b,c] Means with different superscript are significantly different (p-value < 0.001).

The average pH of semen was 8.0 ± 0.1 and the pooled semen osmolality was 338 mOsmol/kg. The viability of sperm assessed by eosin-nigrosin staining was 98.9 ± 0.1% (mean±SEM) of which abnormal spermatozoa was 6.3 ± 0.5%. The percentage of the Sperm with head defects was 1.5 ± 0.3%, tail and midpiece was 3.7 ± 0.4%, cytoplasmic droplets and detached head were 0.3 ± 0.1% and 0.8 ± 0.1% respectively (Table 3).

Table 3 Sperm viability and morphologically abnormality of Dang Surat roosters

Parameters	Mean	SEM
Sperm viability (%)	98.9	0.1
Sperm abnormality (%)	6.3	1.5
Head defects (%)	1.5	0.3
Tail and mid-piece defects (%)	3.7	0.4
Cytoplasmic droplets (%)	0.3	0.1
Detached heads (%)	0.8	0.1

The heads had abnormal morphologies such as macrocephalic and microcephalic, round heads, bent heads, spiraled heads, looped heads, knotted heads, etc. were recorded. Tail and midpiece abnormality involved defects such as angled neck/tail, coiled tail, bent tail, knotted tail, and broken tail. The cytoplasmic droplet can be both proximal and distal cytoplasmic droplets. Examples of sperm defects observed are shown in Figure 2.

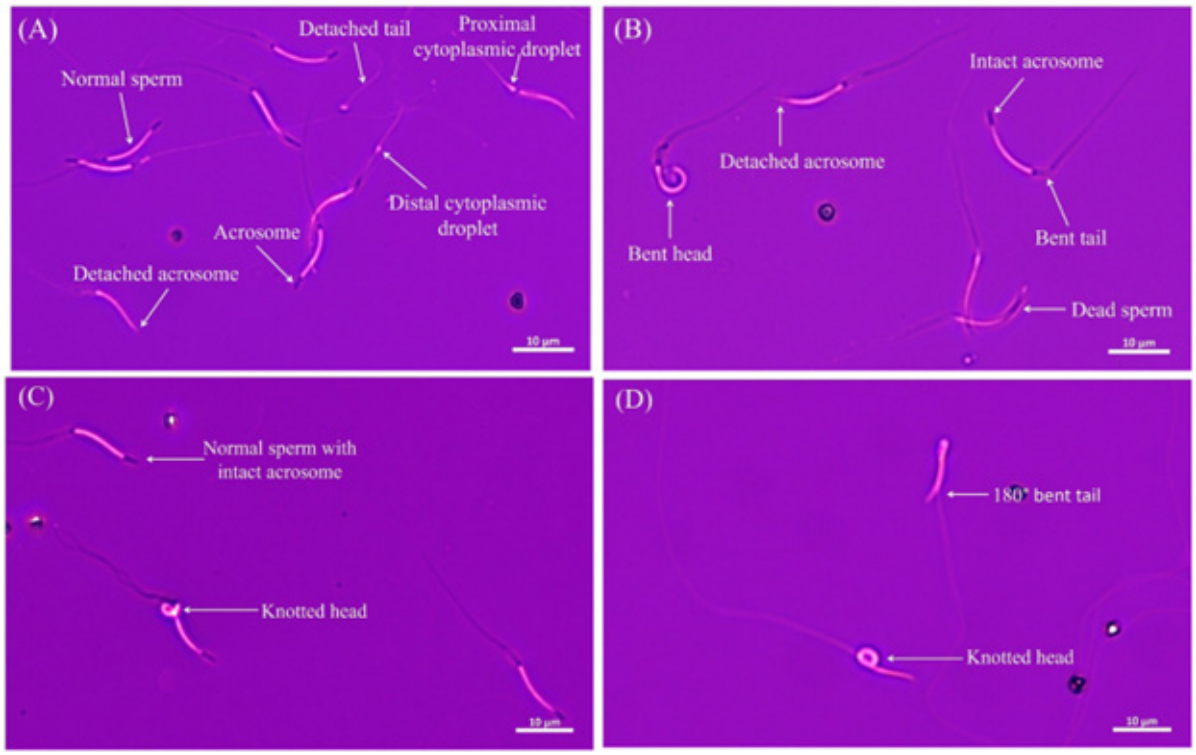

Figure 2 Dang Surat spermatozoa stained with eosin-nigrosin reveals normal and abnormal sperm cell morphology and acrosome integrity. (A) normal sperm with acrosome; proximal and distal droplet; (B) dead sperm, bend head, bent tail, detached acrosome; (C) knotted head and normal spermatozoa; (D) 180° bent tail, knotted head. Magnification = 40x, Scale bar = 10 µm.

DISCUSSION

Sperm quality assessment

Semen quality is a measure of male fertility, or its ability to fertilize. The protocol involves both sperm quantity and quality. Several parameters are commonly used to define semen quality, e.g., semen volume, pH, sperm concentration, percentage of motility and progressive motility, percentage of live/dead and normal/abnormal sperm morphology, and other parameters. In both humans and domestic animal species, the determination of reference values for high-quality semen is important for insemination programs (van der Horst, 2021). The semen quality assays can be used to predict fertilizing ability in order to select the 'best breeders', and cull the low-performing males, hence, improving the reproductive efficiency of the flock.

Quality of semen may vary with breed and strain, age, body weight of cocks, semen collection technique, and diluents used in the semen analysis (Peters et al., 2008; Tuncer et al., 2008).

Poultry semen has a low volume at high concentration and contains about 4-6 billion spermatozoa/ml. In the Thai native chicken, Pradu-hangdum, Khunkaew et al. (2021) reported the sperm concentration of 5.24±0.58 billion sperm/ml. The mean semen volume of the 20-month-old Dang Surat rooster was 265±23 µl (Table 1). This accords with other Thai native roosters: 215±80 µl (range 80-500 µl) for Pradu-hangdum (Khunkaew et al., 2021); 282 ± 89 µl for one-year-old Thai red junglefowl (Polsang et al., 2022), 255 ± 90 µl 7-8-month old and 208 ± 60 µl for 10-11-month old Thai native roosters (Inyawilert et al., 2019). Semen volumes may be age dependent: Thai native rooster, 8-13 months of age were not significant but tended to be lower in younger roosters (Chotesangasa, 2001). Sonseeda et al. (2013) reported no difference in ejaculatory volume of semen between 10-22 months and 18-30 months of age. Our results showed that those retired sires (20-month-old) from Surat Thani breeding center still produced normal amount of semen. Stress caused by environmental factors such as rain, external parasite, other aggressive roosters, etc. could predispose low volume and poor semen quality. In roosters, semen is composed of sperm and seminal plasma from the epididymis, vas deferens and testes. Semen pH varies slightly between different breeds and bird species. This variation in ejaculated semen pH may be due to many factors. Semen pH of chicken semen be slightly alkaline (7.5 ± 0.1 to 8.3±0.6) (Isidahomen, 2016) or optimally between 7.0 and 7.4 (Getachew, 2016): sperm motility was generally high in slightly alkaline semen. Our semen mean pH was rather more alkaline (8.0 ± 0.1) than others. The pH of semen may decrease with time between collection and measurement. Semen samples that contain many dead sperm may also cause the increase pH. Clearly, semen pH is variable and therefore an unreliable indicator of semen quality.

Semen evaluation can be done at the time of collection. Good quality semen should be viscous and creamy-white and no contamination of fecal material, urate or blood that will lead to lowered fertility (Bakst and Dymond, 2013). Contamination with blood may result from excessive force during the collection process or injury.

Semen color may depend on the avian species and generally indicates ejaculate density. A creamy color in this study suggested a high sperm concentration. The semen of the domestic fowl secreted by various reproductive glands, varies from a dense opaque suspension (creamy) composed of high sperm density to watery fluid with lower number of sperm (Peters et al., 2008). However, the evaluation can be subjective. Our data showed that the samples of fresh semen with the highest sperm concentration were cream-colored with thick consistency while samples with the lowest sperm concentration were watery, clear, and colorless.

The motility, viability and fertilizing ability of avian sperm depends on in vitro handling and storage conditions. Factors such as storage temperature, pH of extenders, osmolarity, sperm dilution rate, and seminal plasma, can affect *in vitro* sperm motility, viability and fertilizing ability (Kumar Sarkar, 2020).

Sperm motility and the fertilizing ability of undiluted rooster semen stored *in vitro* usually decreases within 1 hour of collection (Carter et al., 1957). Sperm motility is actually an expression of many aspects of sperm

physiology, such as glycolysis and oxidative phosphorylation. Hyperactivity is crucial for fertilization; however, sperm need to hyperactivate at the final stage of capacitation before they attach and bind to the oocyte.

The diluter can dramatically change sperm swimming behavior and lead to mis- interpreting sperm fertility (van der Horst, 2021). Semen diluents must be isotonic, as the osmotic pressure created by the solution may be detrimental to the sperm cell. An osmotic pressure of 375 mOsm/kg is considered optimal for short-term semen storage (Getachew, 2016). Normal saline has been used as a routine diluter for avian semen (Parker and McDaniel, 2004; Klimowicz et al., 2005; Paranzini et al., 2018) and was the diluent in our investigation. Extender composition also influence motility while high dilutions can stimulate sperm hyperactivation followed by exhaustion (Dumpala et al., 2006).

Our overall sperm motility rates were generally good with saline; however, mean progressive motility was considerably lower perhaps reflecting hyperactivity-exhaustion. Our results also showed gradual decrease in progressive motility with delayed examination. Hence, the media and testing delays influenced our semen evaluation.

Sperm can maintain its fertilizing ability in extenders with osmolarities from 250 to 460 mOsmol/kg, but are ideally 325 to 350 mOsmol/ kg (Sexton, 1977). The osmolality of seminal plasma of domestic fowl was 310-338 mOsmol/kg (Surai and Wishart, 1996). The osmolality of Thai native chicken semen was 305 to 338 mOsmol/kg (Thananurak et al., 2020), while our average value was similar (338 mOsmol/kg).

Sperm motility failed after 48 hours storage in saline at low temperature (Lake and Mc, 1959), perhaps reflecting energy exhaustion. Sperm motility from both undiluted and diluted chicken semen was lowest after storage at 41°C compared to storage at 25, 15 or 5°C (Dumpala et al., 2006). For our experiments, semen was collected at ambient temperature, about 30°C, then transported to the laboratory (ambient temperature 26°C) for semen evaluation. Other factors influencing semen quality are breed and strain, age, body weight, and collection methods (Islam et al., 2002; Tuncer et al., 2006; Peters et al., 2008; Tarif et al., 2013).

Sperm morphometric characteristics

Sperm morphometry (size and shape) and function are important determinants of male reproductive success. In sperm evolution, roosters are polygamous, the sperm usually exhibit high quality with high motility, low abnormality and less immature sperm (abnormal size and shape of head) due to the high sperm competition (Santiago-Moreno et al., 2016). However, recent studies suggest that sperm morphometry can be phenotypically plastic, i.e., adjusted to varying population structures. Thus, roosters in a highly competitive environment may produce spermatozoa with larger midpieces whereas without social competition, longer sperm tails may offer increased reproductive success (Immler et al., 2010). Our samples contained few abnormal spermatozoa (6.3 ± 0.5%) and amongst these, tail and midpiece (3.7 ± 0.4%) and head region (1.5 ± 0.3%) defects predominated.

Environmental pollutants also affect spermatogenesis and modify sperm head dimensions. The semen extender and sample preparation, fixation and staining also influence sperm morphometric measurements (Siudzinska and Lukaszewicz, 2008; Urióstegui-Acosta et al., 2014; Yániz et al., 2015).

In our study, eosin-negrosin staining helped to distinguish morphology and acrosomal integrity. It proved simple, inexpensive and quick and is recommended for routine screening of avian semen.

CONCLUSIONS

Semen screening for key parameters that influence fertilisation is important for selecting the most prolific rooster breeding stock. Semen quality may be judged by its color, ejaculatory volume, sperm motility and percent deformity that are heritable traits that can form the basis of genetic selection in flocks. This study was the first to characterize relevant semen parameters of Dang Surat cocks and the data will underpin selection methods that improve and manage sires for genetic improvement. Our data provide basic information for developing cryopreservation and for artificial insemination.

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AUTHOR CONTRIBUTIONS

Kittima Lewchalermvong: First draft, reading the final manuscript.
Pawinee Kulnanan: Semen evaluation and analysis.
Kanpapat Boonchay: Project administration. semen evaluation,
Sunsaneeya Thaikoed: Semen collection and evaluation.
Yotsapat Phetcharat: Semen collection and poultry husbandry.
Viboon Yiengvisavakul: Literature search, manuscript updates.
Thanis Damrongwatanapokin: Statistic analysis, read and approve the manuscript for publication.
Carmencita Lavilla: Experimental design, review and editing, manuscript updates and final version.
Jureerat Sumretprasong: Funding acquisition, supervision, data analysis, writing the final manuscript.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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