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ORIGINAL PAPER

Semen characteristics of the Indian Red Jungle Fowl (*Gallus gallus murghi*)

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Abstract The Indian Red Jungle Fowl is a wild native gallus subspecies of Southern Asia. Semen has never been studied in this species. In order to better know the male reproductive capacities, experiments were conducted to study the semen characteristics, impact of ejaculate collection frequencies, and timing of collection on sperm quality parameters. Mean sperm concentration 800 million/mL, total sperm per ejaculate (0.015 billion), motility (63.5 %), live/total sperm (92.4 %), intact acrosome (75.5 %), and plasma membrane integrity (89.2 %) were recorded. Percentage of abnormal sperm (head, mid-piece, and tail) was 8.1 % and recovered mainly mid-piece abnormalities. The motile sperm percentage was positively correlated with intact acrosomes (r=0.34) and plasma membrane integrity (r=0.41). Total sperm per ejaculate (billion) was maximum at 72 h of collection followed by 24 and 48 h of collection. Daily and weekly sperm production (billion) was found maximum at 24 h of collection compared to 12, 48, and 72 h of collection. Sperm motility was higher at 24, 48, and 72 h of collection compared to 12 h of collection, but the number of live sperm were higher at 12 h of collection compared to 24, 48, and 72 h. Sperm concentration was better in the morning time, while the values for sperm viability and plasma membrane integrity were higher in the semen collected at evening time. In conclusion, the Indian Red Jungle Fowl shows a semen production quantitatively relatively low for the species as compared to domestic chicken and contrasted parameters of quality. The semen production is affected by the frequency of collection with an optimum for a daily collection preferentially held in the evening period. These results may now be used for artificial insemination and conservation program.

Keywords Semen characteristics · Sperm quality · Indian Red Jungle Fowl

Introduction

The Indian Red Jungle Fowl (*Gallus gallus murghi*) is the native gallus subspecies of Southern Asia (Genome Sequence Center 2006) with a limited distribution in Deva Vata National Park, Azad Jammu, and Kashir, Pakistan (Johnsgard 1999; Subhani et al. 2010). The Indian Red Jungle Fowl is an important member of Phasianidae family and is one ancestor of the domestic chicken (Delacour 1951; Eriksson et al. 2008). The global status of this species is least concern (IUCN 2008); while in Pakistan, it is threatened by many factors including habitat destruction, poaching, egg collection, predation, and genetic hybridization (Subhani et al. 2010).

The captive breeding/propagation of the Indian Red Jungle Fowl is a viable activity that might be helpful in the conservation of this unique bird. One key factor to develop

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conservation programs is the management of the reproduction capacities of the individuals that requires, for the males, a good knowledge of the gamete production capacities (semen characteristics, impact of collection frequency, and timing of semen collection) and fertilization ability including fertilization after artificial insemination. These studies may lead to the development of semen cryopreservation programs to manage genetic diversity with the use of artificial insemination. All these studies have to be built for the Indian Red Jungle Fowl.

Semen characteristics of many bird species such as domestic chickens (Lake 1966; Saeid and Al-Soudi 1975; Tuncer et al. 2006; Malik et al. 2013), turkeys (Burrows and Quinn 1937), and pheasants (Mantovani et al. 1993; Jalme et al. 2003) have previously been studied. They include quantitative and qualitative traits. The quantitative traits lead to define the daily, weekly, or seasonal number of sperm produced by the animals and the mean capacity of the testis production and length of maturation of sperm in the epididymis and deferent ducts. These production parameters differ between species (De-Reviers 1972; De-Reviers 1980; Brillard and De-Reviers 1981; Etchu and Egbunike 2002; Adeyemo et al. 2007). The gametes capacities are then followed by in vitro semen evaluation of so called quality traits that may (at least partly) predict fertility based on semen evaluation tests (Saacke et al. 1980; Blesbois et al. 2008). Some quality parameters for avian species such as semen volume and membrane integrity were found to be the best variables for predicting the fertilization potential in Black Castellana roosters (Santiago-Moreno et al. 2009). Most of the parameters derive from criteria applied to mammalian species such as bulls, rams, boars, and stallions (Foote 1978). But some of them would be specific to avian species such as the number of sperm measured on the vitelline membrane or the number of holes made by spermatozoa on these membranes (Rabbani et al. 2006; Stewart et al. 2004). However, these last tests need to destroy eggs produced after natural mating or artificial insemination and are not encouraged for rare breeds or subspecies such as the Indian Red Jungle Fowl. Thus, complementary quantitative and noninvasive qualitative traits are joined in order to ensure the highest possible number of sperm of the highest possible quality for insemination and conservation programs. The standard noninvasive sperm parameters include motility, sperm concentration, live or dead counts, acrosomal status, plasma membrane integrity, and morphology of spermatozoa (Malik et al. 2013).

In nature, the Indian Red Jungle Fowl lives in small mixed flocks during non-breeding season. However, during breeding season (spring and summer), one male maintains a territory with three to five hens. Hens produce four to seven eggs per clutch (Delacour 1951) while in captivity, the clutch size increases up to 10–15 eggs if eggs are removed daily from the pen (personnel observation). Reproduction phenomena in the Indian Red Jungle Fowl are complex with multiple environmental and physiological factors contributing to successful fertilization. Successful semen collection from the subspecies of jungle fowl has been little documented (Malik et al. 2013) and is lacking in the Indian Red Jungle Fowl. Therefore, in order to prepare a conservation program, this study was conducted to report for the first time successful semen collection and semen characteristics of the Indian Red Jungle Fowl. Zootechnical parameters such as the impact of collection frequency and the time of semen collection in the daily cycle were also recorded.

Materials and methods

Experimental birds

Eight mature male Indian Red Jungle Fowl birds having mean body weight of 1.7 kg (mean age 1.5 year for the semen characteristics analysis, to 2.0 years for the experiment on the impact of semen frequency) were used in this study. The animals were housed individually in pens of 106.68 × 121.92 cm. The birds were offered commercially available poultry cock breeder feed (100 g/day) and were exposed to 16 light hours a day. Fresh water was available to the birds all the daylong throughout the experimental period. The housing was done at ambient temperature at 30 °C during day and night. At night, temperature was maintained by heating lamps while in day, it was maintained by cooling the air by exhaust fans.

Semen collection and quantitative evaluation

The birds were subjected to semen collection training through abdominal massage as described by Burrows and Quinn (1935). Semen collection training was started to get the birds prepared for semen collection as well as for neat and clean ejaculate without feces. This training was continued until we were able to get sufficient semen for further processing and experimental work. The successful ejaculate was available after 4 weeks of training. Semen was collected from individual birds in a graduated plastic tube. Semen volume was measured in microlitres using micropipette. Initial sperm motility of each ejaculate was determined as described by Zemjanis (1970) by mixing 10 μL semen samples in 500 μL of phosphate buffer saline (pH 7.2, 300 mOsmol/kg). The percentage of motile spermatozoa was determined by putting a drop of semen sample on a prewarmed glass slide (37 °C) under phase contrast microscope (×400, Olympus BX20, Japan).

Sperm concentration was measured by taking 1 μL of semen and 200 μL of formal citrate solution (1 mL of 37 % formaldehyde in 99 mL of 2.9 % (*w*/*v*) sodium citrate) with

Neubauer hemocytometer (Marienfeld, Germany) under phase contrast microscope (×400, Olympus BX20, Japan).

Total sperm per ejaculate was obtained by multiplying the total volume with the concentration. Daily (total sperm production multiplied by one) and weekly sperm production (total sperm production multiplied by seven) was calculated by unitary method (De-Reviers and Williams 1981).

Experimental design

Eight male Indian Red Jungle Fowl birds were used in the study. The study was conducted in December 1, 2013 to June 22, 2014; five replicates for each male for each experiment were studied. For the experiments on semen characteristics and morphology, semen was collected once daily. For the experiment on effect of ejaculation frequencies, the cocks were subjected to four semen collection frequencies of twice a day, once a day, after alternate days, and after 3 days. Collection was done at 7:00 a. m. and 7:00 p.m. for experiment on the effect of ejaculation frequencies and for the experiment on the effect of collection time (morning vs. evening). The ejaculate volume, the concentration, motility, membrane integrity, and acrosome integrity of the sperm cells and the morphological abnormalities were examined for each experiment.

Extender preparation

The Beltsville Poultry Semen Extender (BPSE) was used as a diluent (Sexton and Giesen 1982). The extender was composed of 60 mL distilled water, potassium citrate (0.0384 g), sodium glutamate (0.5202 g), magnesium chloride (0.0204 g), fructose (0.3 g), dipotassium hydrogen phosphate (0.7620 g), potassium di-hydrogen phosphate (0.039 g), TES (0.3170 g), and sodium acetate (0.2580 g). The pH of this diluent was 7.3, having osmotic pressure of 330 mOsmol/kg. All samples were diluted 1:5 with BPSE and were processed for further investigations. All chemicals used in this study were from Sigma-Aldrich, Co., 3050 Spruce Street, St Louis, USA.

Semen quality assays

Motility

Sperm motility was assessed by placing a drop of semen sample, previously diluted to 1:5 (*v*/*v*) in the BPSE on a pre-warmed (37 °C) glass slide under a phase contrast microscope (×400) (Zemjanis 1970). Percentage of motile sperm was subjectively evaluated on a scale ranging from 0 to 100 %.

Plasma membrane integrity

Plasma membrane integrity of the Indian Red Jungle Fowl spermatozoa was assessed by using hypo-osmotic swelling test (HOS) as described by (Santiago-Moreno et al. 2009). The HOS solution was prepared by adding 1 g of sodium citrate to 100 mL of distilled water. Previously diluted 25 μL semen was mixed with 500 μL of a HOS solution (100 mosm/kg) and incubated at 37 °C for 30 min. A drop of incubated solution was placed on a pre-warmed (37 °C) slide and fixed in buffered 2 % glutaraldehyde. The spermatozoa showing swollen heads, swollen and coiled tails were classified as normal spermatozoa having intact plasma membrane. A total of 200 spermatozoa were counted at four separate fields under a phase contrast microscope (×1000 with oil immersion).

Sperm viability

Viability (% live/total sperm) of the Indian Red Jungle Fowl spermatozoa was examined by adding eosin-nigrosin to the Lake's glutamate solution. Lake's glutamate solution (Bakst and Cecil 1997) was prepared by adding sodium glutamate (0.01735 g), potassium citrate (0.00128 g), sodium acetate (0.0085 g), and magnesium chloride (0.000676 g) in 100 mL distilled water. Water soluble nigrosin (5 g) and water soluble eosin-bluish (1 g) were added into Lake's glutamate solution. Twelve drops of stain were mixed with one drop of semen. A smear was made on a glass slide, fixed and air dried. A total of 200 spermatozoa were assessed per slide under a phase contrast microscope (×1000 with oil immersion). The mixture provides a clear background in the smear to enhance the contrast of white, unstained "live" sperm or the pinkish stained "dead" sperm.

Acrosomal integrity

Acrosomal integrity of the Indian Red Jungle Fowl spermatozoa was assessed through Giemsa stain (Jianzhong and Zhang 2006). The stain was prepared by adding Giemsa (3 g) and phosphate buffer saline at pH 7.0 (2 mL) into 35 mL water. Smear was prepared by taking a drop of semen sample on a clean glass slide, dried and fixed in neutral formal-saline (5 % formaldehyde) for 30 min. Fixed slides were kept in Giemsa stain for 1.5 h. Sperm with normal acrosome appeared to be evenly stained; abnormal spermatozoa were unevenly stained while spermatozoa having ruptured acrosome were remained unstained. A total of 200 spermatozoa were observed at least in four separate fields under a phase-contrast microscope (×400; Olympus BX20, Japan) at magnitude ×1000 with oil immersion.

Table 1 Semen characteristics of the Indian Red Jungle Fowl

Bird ID	Semen quality parameters						
	Volume (μl)	Concentration (million/ml)	Total sperm per ejaculate (million)	Motility (%)	Acrosomal integrity (%)	Plasma membrane integrity (%)	Sperm viability (%)
1	11.6±2.2	730±0.3	8.0	64.0±7.5	75.9±3.7	87.7±2.6	91.6±2.5
2	17.3±4.3	280±0.2	4.0	60.0±8.9	73.6±5.5	87.8±3.0	92.4±1.8
3	23.2±9.6	680±0.2	15.0	44.0±9.8	76.1±6.8	90.2±3.1	89.6±2.2
4	14.4±3.4	1620±0.8	23.0	64.0±7.5	80.1±2.9	92.4±2.5	92.0±1.8
5	11.3±2.2	1160±0.6	13.0	72.0±15.0	79.3±11.3	92.0±3.2	94.7±1.8
6	30.2±10.4	360±0.2	11.0	52.0±12.0	65.3±6.7	86.9±5.6	89.6±1.6
7	14.5±2.4	1110±0.2	16.0	76.0±9.8	77.9±5.2	93.1±3.6	95.9±1.6
8	32.0±6.6	470±0.3	15.0	76.0±4.0	75.8±3.4	83.5±8.7	93.7±2.0
Overall	19.3±2.9	800±0.2	15.0	63.5±4.0	75.5±1.6	89.2±1.2	92.4±0.8

Sperm abnormalities

Sperm abnormalities were assessed by fixing the sperm in formal citrate solution (prepared by adding 1 mL of 37 % commercial formaldehyde in 99 mL of 2.9 % (*w*/*v*) sodium citrate). Head abnormalities (macro-heads, acephalic, round head, bent, deformed, and detached heads), mid-piece abnormalities (defective and shorter), and tail abnormalities (tail coiled below the head, tail loose, coiled, deformed, multiflagellate, and disjoined) were studied (Alkan et al. 2002).

Statistical analysis

Prior to analysis, all percentage data were normalized with an arcsine transformation. Results are reported as non-transformed means (±SEM). The differences between males or between ejaculation frequencies or between morning and evening were analyzed by analysis of variance using MSTAT-C, Version 1.42 (Michigan State University, East Lansing, MI, USA). Post hoc comparison between the means was done through Fisher's protected LSD test. Pearson correlation estimates for semen quality trait were also performed with Megastat Version 7.25 Mc-Graw-Hill New Media, New York, for excel.

Results

Semen characteristics and morphology of the Indian Red Jungle Fowl

Semen collection from eight mature Indian Red Jungle Fowl cocks was routinely done at the frequency of two ejaculates per week. Semen volume (mean 19.3 μL), concentration (800×10^6 sperm/mL), total sperm per ejaculate (mean 0.015 billion), motility (mean 63.5 %), viable (mean 92.4 %), intact acrosomes (mean 75.5 %), and plasma membrane integrity (mean 89.2 %) are shown in Table 1. Different semen quality characteristics were correlated (Table 2). Sperm motility was positively correlated with acrosomal integrity ($r=0.34$; $P<0.05$) and plasma membrane integrity ($r=0.41$; $P<0.05$). Semen volume was negatively correlated with sperm motility, concentration, acrosomal integrity, sperm viability, and plasma membrane integrity. The data on the sperm morphological abnormalities are shown in Table 3. The total amount of abnormal forms found in the semen ejaculate of the Indian Red Jungle Fowl was quite low, 8.1 % out of which the maximum abnormalities found were mid-piece abnormalities (56.17 %) followed by tail abnormalities (22.2 %), and head abnormalities (21.8 %).

Table 2 Correlation between different semen quality parameters of Indian Red Jungle Fowl spermatozoa

	Volume	Motility	Concentration	Acrosomal integrity	Plasma membrane integrity	Livability
Volume	1.000					
Motility	−0.110	1.000				
Concentration	−0.239	0.084	1.000			
Acrosomal integrity	−0.077	*0.342	0.249	1.000		
Plasma membrane integrity	−0.270	0.185	0.266	0.027	1.000	
Livability	−0.314	*0.412	0.019	−0.077	0.167	1.000

*Significant at $P\leq0.05$

Table 3 Sperm abnormalities in Indian Red Jungle Fowl semen

Abnormalities	Type	Percentage
Head	Macrocephalic	1.1±0.1
	Acephalic	0.9±0.1
	Round	1.6±0.1
	Bent	3.0±0.2
	Deformed	1.2±0.1
	Detached	2.8±0.2
	Overall	1.767±0.371
Mid-piece	Defective	5.0±0.5
	Shorter	4.1±0.5
	Overall	4.55±0.5
Tail	Loose	1.3±0.1
	Coiled	3.3±0.4
	Muliflagellate	1.0±0.1
	Deformed	1.2±0.2
	Disjoined	2.2±0.2
	Overall	1.8±0.428
Total abnormalities (%)		8.1±0.7

Impact of ejaculate frequencies on the semen characteristics of the Indian Red Jungle Fowl

The data on the impact of ejaculation frequencies on the quality of the Indian Red Jungle Fowl spermatozoa are given in Table 4. Total spermatozoa per ejaculate (million) were found maximum ($P<0.05$) when semen was collected after 72 h compared to 24 and 48 h of collection. Daily sperm production (million) and weekly sperm production (million) was found maximum ($P<0.05$) at 24 h of collection compared to 12, 48, and 72 h of collection. There was an overall increase ($P>0.05$) in sperm concentration as the interval between the collection of semen was increased from 12 h (1.39±0.1) to 24 h (3.05±0.3), 48 h (2.23±0.3), and 72 h (4.13±0.8). Sperm motility (%) was significantly improved ($P<0.05$) when time interval between the collection was increased from 12(65.0±2.8) to 24 h (85.0±2.1), while it remained similar when semen was collected at 24 h (85.0±2.1), 48 h (84.3±2.5), and 72 h (81.0±6.3) of intervals. The sperm acrosomal integrity and plasma membrane integrity of the Indian Red Jungle Fowl spermatozoa remained similar ($P<0.05$) when semen was collected at 12-, 24-, 48-, and 72-h intervals. The percentage of live sperm was recorded higher ($P<0.05$) at 12 h (89.8±0.7) of collection compared to 24 h (84.7±1.5), 48 h (85.3±1.7), and 72 h (85.0±3.0) of collection intervals.

Table 4 Effect of collection frequency on characteristics (mean±SE) of Indian Red Jungle Fowl spermatozoa

Sperm parameters	Collection frequency			
	12 h	24 h	48 h	72 h
Total sperm per ejaculate (million)	102±0.01^{c}	303±0.03^{a}	340±0.07^{a}	555±0.03^{b}
Daily sperm production (million)	204±0.03^{b}	303±0.03^{a}	170±0.04^{b}	185±0.01^{b}
Weekly sperm production (million)	1263±0.20^{b}	2122±0.21^{a}	1191±0.27^{b}	1292±0.09^{b}
Concentration (million/ml)	1390±0.1^{c}	3050±0.3^{a}	2230±0.3^{b}	4130±0.8^{a}
Motility (%)	65.0±2.8^{b}	85.0±2.1^{a}	84.3±2.5^{a}	81.0±6.3^{a}
Acrosomal integrity (%)	78.4±1.4	73.9±2.8	77.5±2.6	77.5±5.1
Plasma membrane integrity (%)	85.5±1.8	90.9±0.8	84.7±1.3	91.5±3.8
Sperm viability	89.8±0.7^{a}	84.7±1.5^{b}	85.3±1.7^{b}	85.0±3.0^{b}

The values within the column with different superscript differ significantly ($P<0.05$)

Influence of semen collection timing (morning vs. evening)

The data on the effect of semen collection time (7:00 a.m. for morning and 7:00 p. m. for evening) on spermatozoa quality parameters of the Indian red jungle fowl is given in Table 5. Semen volume, motility, and viability did not differ significantly ($P>0.05$) at either time. However, the sperm concentration was higher when semen was collected in the morning time, while sperm viability and plasma membrane integrity was significantly higher in the semen collected at evening time.

Discussion

Assessment of semen characteristics ejaculate frequency and timing of semen collection are important indicators of the

Table 5 Effect of semen collection time (morning vs. evening) on spermatozoal quality parameters of Indian Red Jungle Fowl spermatozoa

	Timing of semen collection	
	Morning	Evening
Volume (μl)	74.8±5.6	74.0±5.7
Motility (%)	71.7±3.2	65.6±3.8
Concentration (million/ml)	1730±0.20^{a}	1050±0.2^{b}
Acrosomal integrity (%)	69.4±1.9^{a}	87.7±1.1^{b}
Plasma membrane integrity (%)	78.6±3.2^{a}	92.7±0.6^{b}
Sperm viability (%)	89.8±1.2	89.8±0.8

The values (mean±SE) with different superscript differ significantly ($P<0.05$) in a row

reproductive potential required for the genetic exploitation of the breeding individuals and also for conservation of threatened species (Marzoni et al. 2000; Bah et al. 2001; Tuncer et al. 2006; Peters et al. 2008; Madeddu et al. 2009; Ajayi et al. 2011; Malik et al. 2013). Semen quality tests (sperm motility, plasma membrane integrity, livability, and acrosomal integrity) are used in routine semen evaluation for artificial insemination (Graham et al. 1990; Froman et al. 1999; Parker et al. 2000; Snook 2005; Partykaa and Lukaszewiczb 2012).

In the present study, we showed for the first time successful semen collection in the Indian Red Jungle Fowl and different characteristics of semen production. Semen collected by the massage methods showed sperm concentration, volumes, and total number of sperm per ejaculate generally lower of what is usually observed in the domestic chicken, Malaysian Red Jungle Fowl and broiler breed (Malik et al. 2013; broiler breeder (Mc-Daniel and Sexton 1977). The proportion of motile sperm was relatively low when compared to commercial parents. However, sperm viability, acrosome integrity and plasma membrane integrity was recorded equivalent to other studies on different breeds of chicken (Graham et al. 1990; Froman et al. 1999; Parker et al. 2000; Snook 2005; Partykaa and Lukaszewiczb 2012). It is interesting that sperm motility, plasma membrane integrity, viability, and acrosomal integrity were observed similar ($P>0.05$) in all eight experimental birds. It is suggested that these finding may be due to similar age, uniform food, and managemental conditions provided in captivity. The data showed positive correlation between motility, acrosome integrity, and plasma membrane integrity. Similar trends have been reported in Rhode Island Red, white breeder cocks (Nwagu et al. 1996), seven strains of chicken (Peters et al. 2008), and Spanish breeds of chicken (Prieto et al. 2011).

It is well recognized in domestic birds that quality of semen differs due to ejaculate frequency among breeds and species (Santayana 1985; Fan et al. 1988; Riaz et al. 2004; Peters et al. 2008; Ghonim et al. 2009; Zahraddeen et al. 2005). Semen quality was recorded higher with daily semen collection in Taiwan country chicken breed (Fan et al. 1988) and Domyati ducks (Ghonim et al. 2009). In the present study, it was observed that the optimum semen output can be achieved with daily semen collection frequency in wild Indian Red Jungle Fowl spermatozoa. Daily sperm production and weekly sperm production decreased but overall sperm concentration increased with the increase in collection interval. The progressive decline in the spermatozoa concentration was recorded with the increase in frequency of collection which is in agreement to the previous studies on poultry birds (Santayana 1985) and turkey (Zahraddeen et al. 2005). The acrosomal and plasma membrane integrity of Indian Red Jungle Fowl spermatozoa remained similar ($P>0.05$) in all collection frequencies according to previous studies in other poultry birds (Bilgili and Renden 1984; Bilgili et al. 1985; Donoghue et al. 1995; 1996). It is the same for the proportion of morphologically normal sperm. However, we must also note changes in motility (decrease) and livability (increase) with the highest collection frequency (12 h) that would indicate distortions in the maturation process (non-complete motility acquisition, less selection of the "good" sperm) in the epididymis and deferent ducts and suggest that sperm do not stay for enough time in these compartments with this "too high" frequency of 12 h each.

In the present study, semen volume, sperm motility, and viability remained similar for either semen collected in the morning or evening. The sperm concentration was recorded higher when semen was collected in the morning, while sperm viability and plasma membrane integrity were significantly higher with semen collected in evening. These results do not confirm previous studies, which show that timing of semen collection had no effect on semen volume and concentration (Riaz et al. 2004). It has previously been reported that artificial insemination in domestic fowl at evening time could result in more fertilization rate compared to insemination at morning time. The reason behind this is that male copulate naturally more intensively at evening time as compared to morning time and sperm competition is also increased at the evening which could result in the better ejaculate having more volume and concentration (Pizzari and Birkhead 2001; Preston et al. 2003). The other explanation for the higher concentration but the lower semen quality in early morning collections compared to evening collections could be related to environmental factors in our study. Indeed, drinking water was available to animals all day but birds do not drink and do not eat during the night. This could directly affect the volume of semen that would be lower in the early morning because of water retention resulting from lack of drinking water during the night and thus increase the sperm concentration. How this could affect semen quality is less clear and could be an indirect effect. Whatever the case, it is clear from our results that good quality semen can be collected in the evening at regular daily collection and these can be used for artificial insemination for captive conservation of this bird.

From a general point of view, if the different semen characteristics were very homogeneous in each experiment, they varied between each experiment (i.e., of a factor 5 for sperm concentration). This leads us also to suggest that the animals were not exactly in the same physiological status over the 6 months of experiment. This potential "seasonal" effect is now in study in our lab.

It is concluded that semen of the Indian Red jungle Fowl can be effectively collected with massage method, giving semen with reasonable qualitative and quantitative parameters for further fertilization. Optimum semen output can be achieved with daily semen collection frequency practiced preferentially at the evening time.

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