



Semen collection, evaluation and fertile period in Khaki Campbell and White Pekin breeds of ducks

S C GIRI¹, K V H SASTRY², S K SAHOO³ and P KUMAR⁴

Central Avian Research Institute, Bhubaneswar, Odisha 751 003 India

Received: 8 February 2016; Accepted: 7 March 2016

ABSTRACT

Collection and evaluation of semen are the first critical steps for examination of male fertility, gamete preservation and development of artificial insemination technology in ducks. Induced teaser method and manual massage method of semen collection were evaluated for the ease and reaction time in White Pekin and Khaki Campbell breeds of ducks. Semen characteristics and length of fertile period were examined in both the breeds. Method of semen collection in both the breeds of ducks did not affect semen volume and quality parameters. However, reaction time and hypo-osmotic swollen spermatozoa percent differed significantly with the method of semen collection. The length of fertile period in White Pekin is longer than Khaki Campbell ducks during artificial insemination while it was reverse during natural mating. Our findings indicated that manual message method of semen collection in ducks may be practised for a successful artificial insemination programme as the drakes can be habituated for the semen collection by massage method and donate semen quickly without much difficulty and alteration in semen quality.

Key words: Artificial insemination, Ducks, Fertile period, Semen collection

The genetic potential of ducks in terms of meat and eggs needs to be improved for commercial viability. Development of successful artificial insemination (AI) technology in ducks was at slow pace because of their unique reproductive features. Semen quality is an important index in predicting the fertility rates and the reproductive value of males. The seminal parameters that are often used for determining male's fertilizing potential include sperm concentration, viability and motility (McDaniel *et al.* 1998). Selection of drakes on the basis of semen characteristics helps in attaining maximum desirable fertility concomitantly, improving the reproductive performance of breeding stock. Development of suitable technique of semen collection is essential for achieving higher volume of semen with minimum stress to birds. Semen collection in Osaka drakes (native variety in Japan known as small Pekin duck) was done by artificial vagina method (Kasai and Izumo 2001). Teaser female technique (Chelmonska *et al.* 1988) was used for semen collection in Muscovy drakes. However, these methods have inherent limitation for application in large scale AI programs required in commercial duck breeding. Besides, a suitable method of semen collection is not available for the commercially important breeds of ducks. Therefore, a suitable semen collection method was developed and evaluated in commercially important breeds

of ducks like White Pekin (meat type) and Khaki Campbell (egg type).

MATERIALS AND METHODS

Selection and management of experimental birds: Adult drakes (40), 40-week-old, comprising 2 breeds (20 birds from each i.e. Khaki Campbell and White Pekin) were reared in individual cages (2.5 ft × 2.5ft × 2ft) fitted with small feeding and watering trays. An additional small trough was provided at one corner inside the cage filled with water allowing the bird to dip its head as and when required. All the birds were maintained under uniform husbandry conditions in accordance with institutional animal ethics guidelines. Feed (18% CP and 2650 kcal ME/kg) and drinking water was offered *ad libitum* to the drakes. Within 1 week, the birds were found accustomed to the cage environment after which half of the drakes from each variety were subjected to training for semen collection through manual massage method and the remaining half by induced teaser female duck. The practice of training continued for 2 weeks (daily during morning time: 8–9 AM) before feeding. At the end of the training period, 6 rakes from each group were selected considering their response to semen ejaculation under 2 collection methods and maintained throughout the experiment period.

Collection of semen

First method (induced by teaser duck): Induced semen collections were done at every 3 days interval. One female

Present address: ¹Senior Scientist (scgiri12@rediffmail.com), ^{2,3}Principal Scientist (kochiganti@gmail.com, sahoosk4@rediffmail.com), ⁴Technical Officer (pk9935.bbsr@gmail.com).

duck was used as dummy for each collection which was introduced to the male's cage at the beginning. The birds were kept under observation till development of courtship between them. At the peak of male excitation as manifested by mounting and pumping at the female's head continuously, the drake was quickly taken out of the cage. The male was kept in a sitting posture by holding both the legs firmly and the cloaca was pressed gently so that the copulatory organ everted with an easy thrust. The evaginated phallus was guided into a wide mouthed test tube for collection of semen from its tip.

Second method (manual message): The drake was restrained firmly by holding its both the thighs in a horizontal position at a height convenient to the collector so that the neck of the bird is rested on the shoulder of attendant. Initially, the bird was gently massaged on the back starting from neck to the tip of the tail. After few strokes of gentle massage, an intense massage was applied towards the tail end till the bird exhibited alertness for donating semen. During this time, there was evagination of phallus through cloacae with a thrust and ejaculation of semen took place simultaneously. Semen thus expressed was collected into a small wide mouth glass vial (15 ml capacity). Throughout the study, semen was collected by the same trained person.

Evaluation of semen: Semen collected from individual drake in each method was examined for volume, pH, mass motility (on a scale of 0 to 5 recorded on the basis of swirls, waves and eddies; Chemineau *et al.* 1991), sperm concentration by haemocytometer, live sperm percentage through vital staining. Reaction time (time elapsed between exposure to teaser stimulus and ejaculation) were recorded for each bird every time. Methylene blue reduction test (MBRT) and hypo-osmotic swelling test (HOST; osmolarity, 150 mOsm/l) were performed to evaluate metabolic activity and membrane integrity of spermatozoa which are considered as the indirect fertility test. The proportion of sperm cells exhibiting hypo-osmotic swollen positive response was expressed as percent (Correa and Zavos 1995).

Artificial insemination: The semen collected by different methods were pooled separately and diluted (1:1) in normal saline. Females (ducks) were inseminated at 09.00–10.00 h with approximately 100 million spermatozoa at a depth of 2 cm in the vagina with a tuberculin syringe which was repeated every third day. Eggs were collected daily, starting on the second day after the first insemination, fumigated and stored. At the end of every week, all eggs were set in

the incubator. Candling of the eggs was done on 15th day of incubation to examine the embryonic growth (fertility).

Fertile period: artificial insemination vs. natural mating

Adult female Khaki Campbell ducks (20) of the same age were divided equally into 2 groups and were reared in individual cages under uniform management conditions. The birds of the first group were inseminated with approximately 100 million spermatozoa collected from males of the same breed through manual massage method. In the second group, one adult drake per each duck was paired and allowed to mate freely. The birds were kept under continuous observation to detect the successful mating. The drakes were removed after the successful mating. The eggs laid were collected for 12 days. A similar protocol was followed for White Pekin birds to examine the fertile period during natural mating and artificial insemination. On every fourth day, the eggs collected were transferred to the incubator. Candling of the eggs was done on 15th day of incubation to examine the embryo growth (fertility).

Statistical analysis: Data were analysed by ANOVA and Duncan's multiple range tests using the software package SPSS-16.

RESULTS AND DISCUSSION

Attempts were made to evaluate and develop a suitable method of semen collection in different breeds of ducks (both egg and meat type). The number of successful attempts of semen collection in the teaser female method were significantly ($P < 0.01$) lower than the manual massage method in both the breeds of ducks (Table 1). It was observed that introduction of teaser female duck into male's cage sometimes goes without courtship resulting in no collection of semen.

The reaction time (time elapsed between initiation of stimulus and semen donation) is significantly ($P < 0.05$) longer for first method (teaser female) than the second method (manual massage) of semen collection in both the breeds of ducks. However, the reaction time is comparatively lesser in White Pekin than Khaki Campbell ducks (Tables 2, 3).

Some of the seminal parameters, viz. semen volume, pH, mass activity, percent live sperm and methylene-blue reduction time were not affected with the method of semen collection in both the breeds. However, the hypo-osmotic swelling test, an indicator of membrane integrity varied significantly with the method of collection and the breed. In Khaki Campbell, semen collected by first method

Table 1. Success rate for 2 methods of semen collection in 2 breeds of ducks

Breed	Method of semen collection	Total no of attempts of semen collection	Number of successful attempts of semen collection	Percentage of successful attempts of semen collection
Khaki Campbell	First method	60	27	45.00
	Second method	60	43	71.66
White Pekin	First method	60	35	58.33

revealed significantly ($P < 0.05$) higher number of swollen sperm than the second method (Table 2). The reverse was true in case of White Pekin (Table 3).

The percent fertility and hatchability of the duck eggs

Table 2. Physical and biochemical characteristics of semen collected by 2 methods in Khaki Campbell ducks

Parameter	No of collections (n)	Khaki Campbell	
		First method	Second method
Reaction time (min)	20	4.86 ± 0.17 ^b	3.16 ± 0.13 ^a
Volume (ml)	20	0.38 ± 0.03	0.42 ± 0.05
pH	20	7.52 ± 0.12	7.58 ± 0.08
Mass activity (0-5 scale)	20	4.78 ± 0.09	4.58 ± 0.06
Sperm conc. ($\times 10^9$ /ml)	20	2.55 ± 0.10	2.44 ± 0.10
Live sperm (%)	20	92.45 ± 0.76	93.36 ± 1.68
MBRT (min)	20	5.28 ± 0.33	5.92 ± 0.46
HOST (%)	20	83.65 ± 3.45 ^b	79.86 ± 2.88 ^a

The mean values bearing different superscripts in a row differ significantly ($P < 0.05$).

Table 3. Physical and biochemical characteristics of semen collected by 2 methods in White Pekin ducks

Parameter	No of collections (n)	White Pekin	
		First method	Second method
Reaction time (min)	20	3.12 ± 0.15 ^b	2.54 ± 0.18 ^a
Volume (ml)	20	0.68 ± 0.08	0.63 ± 0.33
pH	20	7.64 ± 0.06	7.53 ± 0.05
Mass activity (0-5 scale)	20	4.55 ± 0.06	4.64 ± 0.10
Sperm conc. ($\times 10^9$ /ml)	20	2.23 ± 0.11	2.11 ± 0.09
Live sperm (%)	20	94.66 ± 2.06	96.50 ± 0.88
MBRT (min)	20	6.65 ± 0.22	6.90 ± 0.55
HOST (%)	20	76.54 ± 2.26 ^a	81.33 ± 3.18 ^b

The mean values bearing different superscripts in a row differ significantly ($P < 0.05$).

from the 2 duck breeds which were inseminated with the semen collected by 2 different methods are presented in Table 4. The results revealed no significant difference in fertility and hatchability between 2 methods of semen collection in both the breeds (Table 4).

The length of fertile period after artificial insemination and natural mating was different in both the breeds. The length of fertile period is relatively longer in Khaki Campbell ducks than White Pekin counterparts. The White

Pekin ducks may be inseminated on every alternate day while the Khaki Campbell needs it on every fourth day to obtain higher number of fertile eggs (Table 5).

Successful semen collection method in chickens and turkeys through dorso-abdominal massage was described by Burrows and Quinn (1937) which is now a preferred method of semen collection in other birds (Blanco *et al.* 2000, Lukaszewicz 2001). Various methods were attempted for collection of semen from Muscovy drakes. The massage method (Kamar 1962) and electro ejaculation (Tan 1980) were used on the Muscovy drakes with limited success. Nishiyama *et al.* (1967) collected semen from 4 Pekin drakes with an artificial vagina similar to that used for farm animals. Semen collection was also reported in Alabio drakes (Setioko and Hetzel 1994) and in Osaka drakes (Kasai and Izumo 2001) by artificial vagina (AV) method. However, a reliable method for semen collection and insemination suitable for commercial duck production is not available. In the present investigation, a simple massage method for the collection of semen suitable for Khaki Campbell and White Pekin drakes is developed. The physical and biochemical characteristics of the ejaculates collected from the Khaki Campbell and White Pekin drakes were also evaluated.

Teaser female method which was developed for Muscovy drakes (Chelmonska *et al.* 1988) was also examined for its suitability in commercial duck breeds. The adopted methods were compared in terms of semen yields and semen characteristics in both the duck breeds. However, there was a significant difference in the number of successful attempts of semen collection between the 2 methods (Table 1). It was observed that some of the drakes which were in courtship with teaser duck were frightened while being taken out of the cage, leading to no semen collection. Sometimes, a few drakes ejaculated while being taken out of the cage leading to unsuccessful semen collection. The reason of better success rate of semen collection through manual message method might be due to the careful handling, taming and systematic preparation of the drakes by the same trained person every time. Furthermore, the drakes accustomed to donate semen quickly as the number of attempts increased with the time. The experience in the present experiment revealed that 2 weeks of training period is necessary for the drakes to be accustomed to the semen collection procedure.

Kasai and Izumo (2001) reported that method of semen collection affected semen volume, pH of ejaculate, concentration, motility and viability of sperm in Osaka drakes. However, it was noticed that the method of semen collection in Khaki Campbell and White Pekin ducks did not affect semen quality traits except for reaction time and hypo-osmotic swelling (Tables 2, 3). The percent fertility and hatchability of duck eggs collected after artificial insemination (semen collected by 2 different methods) in 2 breeds of ducks revealed no significant difference (Table 4).

The primary goal of a successful AI program is to ensure that breeding females always lay fertilized eggs. The

Table 4. Fertility and hatchability rate of duck eggs, inseminated with semen collected by 2 methods

Breed	Method of semen collection	Percent fertility	Hatchability	
			Total egg set (TES)	Fertile egg set (FES)
Khaki Campbell	First method	77.28±2.65	52.19±4.38	67.53±3.55
	Second method	79.87 ± 2.37	53.95 ± 5.95	66.54±5.09
White Pekin	First method	70.11 ± 2.86	63.88±3.21	91.11±4.33
	Second method	71.82 ± 2.40	66.73 ±2.79	92.91±4.89

advanced genetic selection has enabled hens to lay continuously over prolonged periods. However, the ability of storing sperm in uterus either in natural mating or by artificial insemination leading to higher fertile period needs to be studied. The dose and the interval of insemination are the critical components in the development of AI programs for any domestic avian species. The length of the fertile period forms the basis for determining the dose and the interval of insemination in the development of AI programs in domestic avian species. It was attempted to measure length of fertile period in both the breeds of ducks after single insemination or mating. The length of fertile period is relatively longer in Khaki Campbell ducks than White Pekin counterparts. The White Pekin ducks may be inseminated on every alternate day while Khaki Campbell needs it on every fourth day to obtain higher number of fertile eggs (Table 5).

Table 5. Fertile period in ducks under natural mating and artificial insemination

Day	Fertility percentage			
	AI		Natural mating	
	Khaki Campbell	White Pekin	Khaki Campbell	White Pekin
1 st	29.16	25.00	98.83	78.57
2 nd	66.66	72.72	90.58	68.75
3 rd	80.00	50.00	82.50	66.66
4 th	80.00	27.27	61.03	36.36
5 th	25.00	20.00	42.50	21.42
6 th	18.18	11.11	44.73	21.42
7 th	00.00	10.00	42.46	13.33
8 th	00.00	09.09	36.98	15.38
9 th	00.00	00.00	30.55	07.14
10 th	00.00	00.00	22.05	00.00
11 th	00.00	00.00	9.37	00.00
12 th	00.00	00.00	00.00	00.00

The findings of the present investigation indicated that, manual message method of semen collection in ducks may be practised for a successful artificial insemination

programme as the drakes gradually get habituated to the collection method and donate semen quickly without much difficulty and alteration in semen quality.

REFERENCES

- Blanco J M, Gee G, Wildt D E and Donoghue A M. 2000. Species variation in osmotic, cryoprotectant, and cooling rate tolerance in poultry, eagle, and peregrine falcon spermatozoa. *Biology of Reproduction* **63**: 1164–71.
- Burrows W H and Quinn J P. 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science* **26**: 19–24.
- Chelmonska B, Dymkowska B and Jerysz A. 1988. Instruction of semen collection from Muscovy drake and duck artificial insemination. *Agricultural University, Wroclaw*. 1–38 (in Polish).
- Chemineau P, Cagnie Y, Gue'rin Y, Orgeur P and Vallet J C. 1991. *Training manual on artificial insemination in sheep and goat*. FAO, Rome.
- Coerrea J R and Zavos P M. 1995. Frozen thawed bovine spermatozoa diluted by slow or rapid dilution method; Measurements on occurrence of osmotic shock and sperm viability. *Theriogenology* **44**: 963–71.
- Kamar G A R. 1962. Semen characteristics of various breeds of drakes in the subtropics. *Journal of Reproduction Fertility* **3**: 405–09.
- Kasai K and Izumo A. 2001. Efficiency of artificial vagina method in semen collection from Osaka drakes. *Journal of Applied Poultry Research* **10**: 206–10.
- Lukaszewicz E. 2001. Effects of semen filtration and dilution rate on morphology and fertility of frozen gander spermatozoa. *Theriogenology* **55**: 1819–29.
- McDaniel C D, Hannah J L, Parker H M, Smith T W, Schultz C D and Zumwalt C D. 1998. Use of sperm analyser for evaluating broiler breeder males. *Poultry Science* **77**: 888–93.
- Nisshiyama H, Nakashima N and Fukihara N. 1967. Studies on the accessory reproductive organs in the drake and addition to semen of the fluid from the ejaculatory groove region. *Poultry Science* **55**: 234–42.
- Setioko A R and Hetzel D J. 1984. The effect of collection method and housing system on semen production and fertility of Alabio drakes. *British Poultry Science* **25**: 167–72.
- Tan N S. 1980. The training of drakes for semen collection. *Annals of Zootechnology* **93**–102.