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Research Article

Effect of collection method on semen quality of ostrich (*Struthio camelus*)

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Abstract

The success of an artificial insemination program in ostriches is highly dependent on the yield of viable semen. We, therefore, tested the effect of collection method (Massage, dummy or teaser) on semen quality of male ostriches. Male and female ostriches selected for training were from a breeding flock consisting of 90 adult ostriches (3 to 7 years old). Nine males were used in training for massage, dummy or teaser distributed equally. Semen characteristics were evaluated fortnightly for three consecutive months from all males involved in all semen collection methods. Semen quality traits involved in this study were ejaculate volume, sperm concentration, total number of spermatozoa, mass motility, individual motility and percentages of dead spermatozoa, abnormal spermatozoa and abnormal acrosome. Results revealed that using teaser female method, male ostriches recorded the highest values ($P \leq 0.05$) of ejaculated volume, spermatozoa concentration, total number of spermatozoa, mass motility, and individual motility and the lowest ($P \leq 0.05$) percentage of dead spermatozoa, abnormal spermatozoa and abnormal acrosome during the whole period of experiment while massage method recorded the lowest values for these traits. In conclusion, collecting semen during mating (teaser or dummy) appears to yield better quality of semen than manual massage.

Keywords: Collection method; semen quality; ostrich

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Introduction

Now-a-days commercial ostrich (*Struthio camelus*) farming is mainly relying upon a natural mating system. Ostriches are usually kept in pairs, trios or colonies with a low male to female ratio (Malecki et al., 2008). The practice is economically unsuitable because there is a little room for the selection of viable traits. Second superior males can only mate with a few females in one season, and the high feed cost owing to all the surplus males that need to be maintained. In addition, inadequate egg production, high rate of embryo mortality, poor chick survival, suboptimal growth rates and poor response to selective breeding are some of the important problems faced by ostrich farming (Cloete et al., 1998). The development of an artificial insemination (AI) technique in the industry could potentially overcome these problems. Specific traits could be selected with genetic improvement if high quality, fertile ejaculates could be acquired from males and inseminated in a stress free manner into receptive females. However, the success of such a program relies largely on the ability to collect semen, as well as on the availability of large numbers of sperm for AI purposes.

The manual massage method to collect semen from ostriches has been used for some time. However, the method is problematical because of stress to the birds and the handlers during restraining and the manual

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extrusion of the phallus, and the fact that it cannot guarantee quality ejaculates routinely (Irons et al., 1996; Hemberger et al., 2001). Rozenboim et al. (1999 & 2003), attempted semen collection with the help of an artificial vagina during mating in the presence of the handler. However, the artificial vagina was stressful to the birds while the vacuum system (a tygon tube connected to a small vacuum device) was the most reliable technique (Rozenboim et al., 2003). Ya-jie et al. (2001), on the other hand, reported using docile ostrich pairs that cooperated with the handlers and collected ejaculates by interrupting mating with an artificial vagina used for emus (Malecki et al., 1997a&b). Even though these attempts have demonstrated that a behavioural approach to collect semen is feasible, the training method and the collection technique have not been clearly established. Preliminary studies suggested that male ostriches that court humans mount the dummy (Malecki and Martin, 2005). Recently, we established new ostrich-friendly receptive female (the teaser method) and dummy female (the dummy method) methods (Al-Daraji and Al-Shemmary, 2015). Once trained, the males respond to the crouching female or "dummy" female stimulus, allowing the routine collection of ejaculates (Rybnik et al., 2007). In present study, we assessed semen collection using a massage method, a teaser female and a dummy.

Materials and Methods

This study was conducted at a natural reserve for breeding ostriches in Babylon governorate from May to September, 2014 (breeding season). Male and female ostriches selected for training were from a breeding flock consisting of 90 adult ostriches (3 to 7 years old). The birds were given an ostrich breeder ration containing 22.8% crude protein and 2719 Kcal ME/kg which was formulated and prepared on the farm according to a commercial recommendation and consisted of a whole grain mix, vitamin-mineral supplement and fresh alfalfa. The concentrate was offered in the morning and the alfalfa in the afternoon. Water was provided *ad libitum*.

The males used in training for massage method (n=3) were maintained separately in the breeding enclosures. The male ostrich were confined in a specially constructed crush in order to prevent injury to the bird and the handlers (Fig. 1) or without a crush (Fig. 2). Once the male was restrained, the phallus was extruded out of the cloaca and held down with one hand, using a cloth to allow a firmer grip. The fingers of the right hand were introduced into the phallic groove and passed deeply into the cloaca until the semen papillae were located. Gentle massage of the papillae led to ejaculation and semen ran down the

Fig. 1: Semen collection from male ostrich confined in a specially constructed crush by massage method

phallic groove (Irons et al., 1996; Hemberger et al., 2001) and then the flowing out semen was collected in glass beaker (Fig. 1 & 2).

The males used in training with teaser females (n=3) were maintained in the breeding enclosures together with their females as separate breeding units of one male and one female. The artificial vagina was locally manufactured and made of a PVC tube 11 cm in diameter and 41 cm long. A collecting vial was attached to a tapered rubber liner mounted at the end of the artificial vagina (Fig. 3).

On the outside there were four strips of firm dense foam glued to the surface lengthwise for tight fitting into the PVC tube of the dummy. The collector appeared near the holding yard and walks towards the teaser to induce crouching (Fig. 4). The male ostrich approaches the crouching female and attempts to mount her and the collector stood with artificial vagina in his hand near the female and approached the birds from behind when they started mating (Fig. 5). While the male was attempting to penetrate the female's cloaca with his partially extruded phallus, the artificial vagina

Fig. 2: Semen collection from male ostrich held in the corner of the yard by massage method

Fig. 3: The artificial vagina for collecting semen from the male ostrich

was placed next to the female's vent and the phallus was directed into the artificial vagina (Fig. 6). Once intromission was achieved in the artificial vagina the phallus became erected and the male ejaculated (Fig. 7) while arching his neck and swinging his head sideways and grunting (Malecki et al., 1997; Rybnik et al., 2007).

The males used in training with a dummy female (n=3) were maintained individually in enclosures. The enclosure was adjacent to a covered area between the two houses with a decked floor area. The covered area was used for training and was accessible via a swinging

Fig. 4: The collector appears near the holding yard and walks towards the teaser to induce crouching

gate. Two enclosures shared one undercover area. The artificial vagina was inserted into the dummy (Fig. 8) (Malecki and Martin, 2005; Rybnik et al., 2007) and the collector approached the male enclosure. Once the collector opened the gate and put a dummy on the floor the male came up with his wings lifted and attempted to mount the dummy (Fig. 9). The collector lowered himself while the male mounted putting the right leg on the dummy, spreading and waving the wings and swinging the head from side to side. When the male achieved intromission in the artificial vagina, he ejaculated while arching his neck forward and swinging his head sideways and grunting, a sequence of behaviour performed during normal copulation or a teaser method collection (Fig. 10 & 11). Once ejaculation was completed the male dismounted and left the floor.

Semen characteristics were evaluated fortnightly for three consecutive months. Ejaculated volume was measured with an automatic pipette, and sperm concentration was determined with a haemocytometer in 20 µl semen diluted 1:400 (v/v) with a phosphate

Fig. 5: The male ostrich approaches the crouching female and attempts to mount her and the collector stands with artificial vagina in his hand near the female and approaches the birds from behind when they start mating process

Fig. 6: While the male is attempting to penetrate the female's cloaca with his partially extruded phallus, the artificial vagina is placed next to the female's vent and the phallus is directed into the artificial vagina

buffered saline solution containing 10% formalin. The number of sperm was subsequently calculated by multiplying semen volume and sperm concentration (Malecki et al., 1997). Sperm motility was estimated as collective and individual motility (Al-Daraji, 2007 a & b). This was subjectively assessed percentage of motile sperm at the time of collection is commonly used as a measure of ejaculate quality. Finally, samples of neat semen were mounted onto a glass slide and the proportion of live normal, live abnormal and dead sperm (Fig. 12 & 13) was estimated after counting 300 sperm stained with nigrosin-eosin (Al-Daraji, 2007 a&b).

Statistical comparisons were based on 18 semen samples (each sample derived from an individual bird), for each experimental period which represented one month. The data was assessed by analysis of variance using the General Linear Model method (SAS, 2000). Test of significance for the difference between means was done by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Results revealed that using teaser female method for collection of semen from male ostriches recorded the highest ($P \leq 0.05$) ejaculated volume, spermatozoa concentration, total number of spermatozoa, mass motility and individual motility during the whole period of experiment followed by the dummy and massage method respectively (Table 1). Results also indicated that massage method recorded the highest values ($P \leq 0.05$) of dead sperms, abnormal sperms and abnormal acrosome during the whole period of experiment and concerning the total mean of these traits followed by dummy method whereas teaser female method recorded the lowest values for these traits (Table 2).

Male ostriches can be trained to ejaculate into the artificial vagina using a teaser or a dummy female. Following training, semen can be collected routinely

Fig. 7: Semen of male ostrich that collected by artificial vagina

Fig. 8: Dummy with the artificial vagina used to train male ostriches

and the quality and quantity of semen collected with the artificial vagina appears normal. The methods provide a good alternative to the manual massage method as the birds can express their sexual behaviour. Despite the fact that ostriches are large birds and difficult to handle,

Fig. 9: Male comes up with his wings lifted and attempts to mount the dummy

some adult ostriches demonstrated that they are suitable for training by showing behaviour such as curiosity and a close distance with humans. Little fear or aggression and more expression of sexual behaviour were noticed. Habituating them to semen collection procedures and presence of human can lead to development of behavioural patterns that facilitate training (Rybnik et al., 2007). The crouching stimulus is essential in training the teaser method because the males need to be induced to mount the female. In addition this 'stimulus-response' relationship allows the time of mating to be controlled (Malecki et al., 1997) which is essential for effective semen collection.

Quality of semen obtained with the teaser or a dummy female appears similar to that reported by Bertshinger et al. (1992), Ya-jie et al. (2001), Rozenboim et al. (2003) or Rybnik et al. (2007) and greater than when the manual massage was used (Hemberger et al., 2001). The teaser female method seems to have yielded greater ejaculated volume than the dummy female; the latter method is not less successful. Development of the animal friendly method for collecting semen from ostriches has advanced considerably in recent years.

Table 1: Effect of collection method on semen characteristics (Mean ± SE) of ostrich males

Semen traits	First month			Second month			Third months			Total means		
	Massage	Dummy	Teaser	Massage	Dummy	Teaser	Massage	Dummy	Teaser	Massage	Dummy	Teaser
Ejaculate volume (ml)	0.85 ± 0.02 [c]	1.07 ± 0.11 [b]	1.31 ± 0.23 [a]	0.89 ± 0.05 [c]	1.11 ± 0.27 [b]	1.49 ± 0.26 [a]	0.91 ± 0.07 [c]	1.16 ± 0.29 [b]	1.52 ± 0.31 [a]	0.88 ± 0.06 [c]	1.11 ± 0.25 [b]	1.44 ± 0.42 [a]
Sperm Concentration (10^9/ml)	3.01 ± 0.33 [c]	3.57 ± 0.19 [b]	3.99 ± 0.56 [a]	3.15 ± 0.42 [c]	3.62 ± 0.39 [b]	4.02 ± 0.29 [a]	3.20 ± 0.50 [c]	3.88 ± 0.38 [b]	4.25 ± 0.35 [a]	3.12 ± 0.17 [c]	3.69 ± 0.14 [b]	4.08 ± 0.40 [a]
Total number of spermatozoa (10^9)	2.55 ± 0.60 [c]	3.82 ± 0.48 [b]	5.22 ± 0.63 [a]	2.80 ± 0.39 [c]	4.01 ± 0.52 [b]	5.98 ± 0.34 [a]	2.91 ± 0.28 [c]	4.50 ± 0.47 [b]	6.46 ± 0.44 [a]	2.75 ± 0.52 [c]	4.11 ± 0.32 [b]	5.88 ± 0.36 [a]
Mass motility (%)	70. 31 ± 3.11 [c]	82.22 ± 5.22 [b]	86.51 ±3.99 [a]	72.46 ± 4.28 [c]	86.60 ± 6.39 [b]	90.15 ± 5.88 [a]	78.49 ± 4.48 [c]	89.63 ± 3.37 [b]	93.78 ± 4.99 [a]	73.75 ± 3.91 [c]	86.15 ± 4.61 [b]	90.14 ± 6.02 [a]

[a,b,c]Means within a row and month of experiment with different superscript are different (P≤ 0.05).

Table 2: Effect of collection method on semen characteristics (Mean ± SE) of ostrich males

Semen traits	First month			Second month			Third months			Total means		
	Massage	Dummy	Teaser	Massage	Dummy	Teaser	Massage	Dummy	Teaser	Massage	Dummy	Teaser
Individual motility (%)	74. 25 ± 1.22 [c]	85.41 ± 3.45 [b]	89.63 ± 2.81 [a]	76.16 ± 3.77 [c]	87.59 ± 5.25 [b]	91.98 ± 4.11 [a]	79.96 ± 2.90 [c]	90.81 ± 6.02 [b]	94.08 ± 4.87 [a]	76.79 ± 3.92 [c]	87.93 ± 5.15 [b]	91.89 ± 4.42 [a]
Dead spermatozoa (%)	21.01 ± 1.05 [a]	16.21 ± 0.89 [b]	9.53 ± 1.12 [c]	23.95 ± 1.33 [a]	17.81 ± 0.86 [b]	10.75 ± 0.79 [c]	25.08 ± 1.60 [a]	18.45 ± 1.07 [b]	12.15 ± 0.92 [c]	23.34 ± 1.90 [a]	17.49 ± 1.18 [b]	10.81 ± 0.92 [c]
Abnormal spermatozoa (%)	26.47 ± 1.39 [a]	19.35 ± 0.85 [b]	10.29 ± 0.77 [c]	28.31 ± 1.07 [a]	20.40 ± 1.16 [b]	11.09 ± 0.79 [c]	31.04 ± 1.95 [a]	21.99 ± 1.66 [b]	14.02 ± 1.04 [c]	28.60 ± 2.31 [a]	20.58 ± 1.91 [b]	11.80 ± 0.68 [c]
Abnormal acrosomes (%)	12.53 ± 0.51 [a]	7.48 ± 0.66 [b]	3.29 ± 0.28 [c]	14.08 ± 0.98 [a]	8.99 ± 0.91 [b]	4.87 ± 0.87 [c]	16.22 ± 1.08 [a]	11.97 ± 0.93 [b]	6.95 ± 0.88 [c]	14.27 ± 1.19 [a]	9.48 ± 1.26 [b]	5.03 ± 0.73 [c]

[a,b,c]Means within a row and month of experiment with different superscript are different (P≤ 0.05).

Fig. 10: Male start courting with a dummy

Fig. 11: When the male achieves intromission in the artificial vagina he ejaculates while arching his neck forward and swinging his head sideways

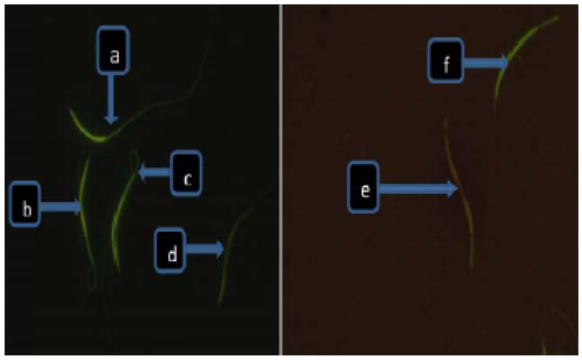

Fig. 12: (a, d and f) live normal sperms (b and c) live abnormal sperms and (e) dead sperm (100 ×)

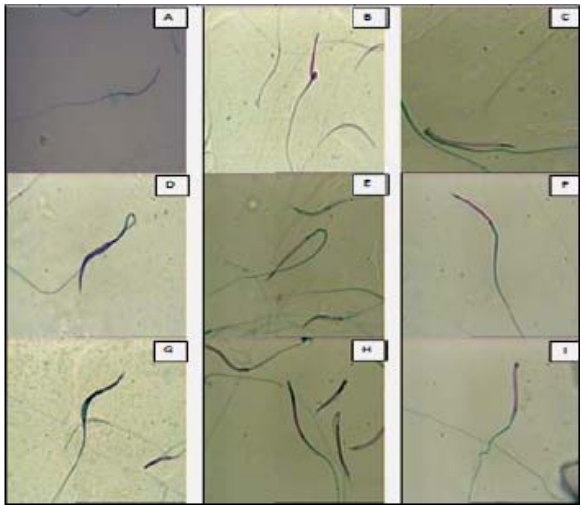

Fig. 13: (A) normal sperm, (B and E) curled midpiece, (C) broken midpiece (D) bent head and tail, (F) bent midpiece, (G) giant sperm head, (H) elongated head, (I) Sperm acrosome and tail abnormality (100 ×)

Normal ejaculates can be collected regularly but the males need to be trained in a human friendly method (Rybnik et al., 2007). Semen collected by the teaser or dummy method is of good quality and quantity and is suitable for storage. While our results and those of others indicate normal values for ostrich semen, the daily output of semen and spermatozoa are yet to be determined. The ostrich industry appears to be in a good position for development of the artificial insemination (AI) technology although adoption of the AI technology in ostrich breeding will mean substantial changes to the current industry structure (Bonoto et al., 2010).

It was concluded from this study that teaser or dummy methods appeared to yield better quality of semen than manual massage.

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