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Research Article

Effect of semen collection method on fertility and hatchability rates and sperm penetration holes of ostrich (*Struthio camelus*)

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Abstract

The ostrich industry appears to be in a good position for the development of artificial insemination technology. This study was conducted to investigate the effect of semen collection methods on fertility and hatchability and sperm penetration holes of ostrich. A total of 9 males and 27 females, 3-7 years old, were randomly assigned to three treatment groups with 3 males and 9 females for each group. Three semen collecting methods including manual massage method, dummy method and teaser female method were compared for three consecutive months. Results revealed that the use of teaser female or dummy methods for collecting the semen from male ostrich resulted in significant increase ($P \leq 0.05$) infertility (%), hatchability from fertilize eggs (%), hatchability from set eggs (%), and sperm penetration holes and significant decrease ($P \leq 0.05$) in embryonic mortality (%) compared with manual massage method during the whole period of experiment. Development of the animal friendly methods (teaser female and dummy methods) for collecting semen from ostriches has advanced considerably in recent years. Semen collected by the teaser or dummy methods gave better results for fertility and hatchability rates and sperm penetration holes.

Keywords: Ostrich; Collection method; fertility and hatchability rates

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Introduction

At present, commercial ostrich (*Struthio camelus*) farming is based on a natural mating system. Ostriches are usually kept in pairs, trios or colonies with a low male to female ratio (Malecki et al., 2008). This practice is economically inefficient because it is difficult to artificially select for commercially important traits, as superior males can only mate with a few females in one season, and because of the severely inflated feed cost owing to all the surplus males that need to be maintained. Furthermore, inadequate egg production, great embryo mortality, poor chick survival, suboptimal and variable growth rates and poor responses to selective breeding are serious problems faced by ostrich farmers (Cloete et al., 1998). All traits of economic importance exhibit sufficient genetic variation for substantial progress to purposeful selection (Cloete et al., 2008 a&b). Yet this potential is not realized on an industry basis, owing to a lack of performance records linked to pedigree information. The ostrich production is also constrained by a very low male to female ratio of 1: 1.7 (Malecki et al., 2008), resulting in a severely inflated feed cost owing to all the surplus males that need to be fed. The development of an artificial insemination (AI) protocol in the industry could potentially overcome these limitations. With a reliable semen collection method, fertility of males

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could be determined and when combined with the artificial insemination of females, selection for specific traits could be facilitated and genetic improvement accelerated. Although a viable protocol for the semen collection has been established (Rybnik et al., 2007; Malecki et al., 2008), vital elements are still missing from the present knowledge including: the identification of elite males producing high quality ejaculates; a thorough knowledge of semen properties; the identification of an optimal semen diluent for the extension of ostrich semen; and the insemination dose and frequency.

In practice, semen is collected for evaluation of male fertility, diagnosis of fertility problems, semen preservation and artificial insemination. For such purpose, semen needs to be physiologically normal and attempts to develop a stress free method that would evoke normal ejaculation were subsequently made (Rozenboim et al., 1996, 1999, 2003; Ya-jie et al., 2001). In those attempts docile ostrich pairs that cooperated with the handlers were used and ejaculates were collected by interrupting mating with the artificial cloaca (AC) (Malecki et al., 1997a&b), although Rozenboim et al. (2003) found that the AC was stressful for their pairs and a vacuum system used for collecting semen from turkeys gave better results. Despite those attempts, the training method and the collection techniques have not been clearly established. Moreover, these methods require co-operation of a pair of birds that may not be reliable and therefore do not often guarantee ejaculates on demand. The method not involving a female, the nonteaser approach, was first developed in ratites for the emu (Malecki et al., 1997a). The method is based on development of desirable human-male bird interactions. In the ostrich, this concept was proven by Malecki and Martin (2005) who showed that those male ostriches that respond to humans by squatting and performing a ground display (kantling) are good candidates to non-teaser training and can be trained to mount the dummy (Malecki and Martin, 2005).

The success of ostrich farming depends largely on the production of fertile eggs, but scientific reports of fertility and hatchability of artificially incubated ostrich eggs in different countries show that hatchability results are highly variable (Deeming and Ar, 1999). Although hatchability of artificially incubated ostrich eggs can be as high as 80%, it is typically between 30% to approximately 60% (Deeming and Ar, 1999; Van Schalkwyk et al., 2000). Deeming and Ar (1999) have reported hatchability figures of fertile eggs as low as 11% in extreme situations. The ostrich egg is unusual for its large size, ranging between 1 - 2 kg, averaging at approximately 1.5 kg (Deeming et al., 1993). Although an ostrich egg is the largest of the living birds, it is also the smallest (1.5%) in proportion to the adult body weight (Badley, 1997). Several measures of productivity for farmed ostriches have been proposed and include the number of eggs laid per hen per year, the percentage of incubated eggs that are fertile (fertility), the percentage of fertile eggs that hatch (hatchability) and the percentage of hatched chicks that survive to a specified age (survival percentage) (More, 1996). Fertility and hatchability rates have been published and range from 10-60 and 27-67%, respectively in countries likes Australia, South Africa, Namibia and Zimbabwe (Cloete et al., 1998; Van Schalkwyk et al., 2000; Mushi et al., 2008).

This study will attempt to quantify the impact of semen collection methods using a manual massage method, a teaser female and a dummy on fertility and hatchability rates and sperm penetration holes of ostrich.

Materials and Methods

This study was conducted at a natural reserve for breeding ostriches in Babylon governorate from May to September 2014 (time of the ostrich breeding season). Male and female ostriches were selected for training 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. The ration was formulated on the farm according to a commercial specification and consisted of a whole grain mix, vitamin-mineral supplement and fresh alfalfa. The concentrate was given in the morning and the alfalfa in the afternoon while water was provided *ad libitum*.

The males used in training for manual 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 or just be held in the corner of the yard. 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 (Irons et al., 1996; Hemberger et al., 2001) of the semen into the glass beaker.

The males used in training with teaser females (n=3) were maintained in the breeding enclosures 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, and a horse rubber liner. A collecting vial was attached to a tapered rubber liner mounted on the end of the artificial vagina. 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. Semen was collected through artificial vagina using female teaser as described by Al-Daraji and Al-Shemmary (2015).

The males used in training with a dummy female (n=3) were maintained individually in enclosures. The artificial vagina was inserted into the dummy (Malecki and Martin, 2005; Rybnik et al., 2007). Semen was collected as described by Daraji and Al-Shemmary (2015).

Collected semen was evaluated semen quality parameters including semen volume, semen concentration, motility and dead sperm percentage (Daraji and S.A. Al-Shemmary (2015). The AI pool was made using equal numbers of fresh spermatozoa from each male. The concentration of spermatozoa in a pool was subsequently confirmed with a spectro-photometer. A total of 27 adult females were randomly assigned for three semen collection method. Insemination was performed with either AI gun used for cows or specially modified plastic syringe of sufficient length to reach the female vagina (Fig. 1&2). Two methods were followed to inseminate the females. In the first methods, in the standing position, the vagina was opened with lateral pressure and semen was deposited into the cloaca with the help of syringe or catheter (Fig. 3 & 4). In the second method, gentle stimulation was provided on the back of the female until fully crouched (Fig. 5). The speculum fitted with a lighting source was then inserted into the cloacae to see the vaginal opening easily. The insemination straw, mounted on a tuberculin syringe or catheter, was then introduced into the cloaca and inserted into the vagina until resistance was felt (approximately 1-2 cm in depth) (Fig. 6). Deeper insemination was not performed to avoid irritation and possible cessation of laying. A dose of sperm was then introduced into the vagina and then inseminating straw was gradually withdrawn.

The eggs (n=182) produced from all females involved in this study were collected from the second day after insemination of the hens. Eggs designated for hatching had no eggshell defects and their weight ranged from1150 to 1550 g. Eggs were kept in the incubator under standard conditions. Eggs were weighed before placing in the incubator. Until day 38, eggs were kept in the setter in which the temperature and relative humidity were kept at 36.6ºC and 20% respectively and rotated four times daily by an angle of 90° (Fig. 7). On day 39, the eggs were weighed before their transfer to the hatching compartment in which the temperature was 36°C and relative humidity 50%. After hatching, the chicks were assessed and unhatched eggs were opened in order to determine the number of unfertile eggs as well as eggs with dead embryos or unhatched chicks. Fertility (%), hatchability from fertilized and set eggs (%) and embryonic mortality

Fig. 1: The catheter used in artificial insemination of ostrich females

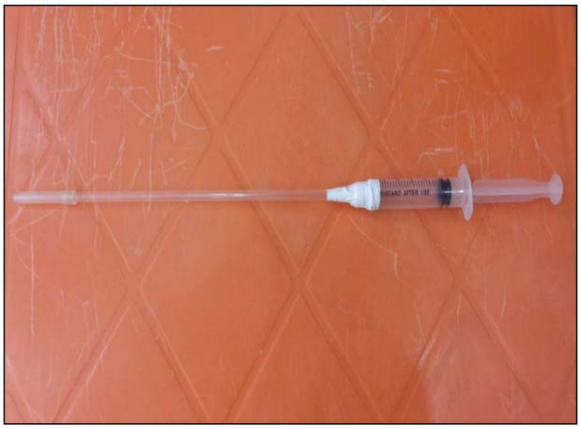

Fig. 2: The plastic syringe used for artificial insemination of ostrich females

rates were determined for three consecutive months on the basis of one hatch for each month.

The perivitelline-hole assay was used as an indicator of the effects of semen collection method on the toms' fertilizing ability (Bramwell et al., 1995; Donoghue, 1996). Eggs collected for 1 wk during each month of experiment were used for the perivitelline-hole assay. The chalazae were cut away using small dissecting scissors. The ovum was submerged in 1% (w/v) NaCl for 25s. A section of the perivitelline layer (PL), ~ 15 X 25 mm, was then cut to include the portion directly over the germinal disc (GD). The PL section was held with fine forceps and gently washed with phosphate-buffered saline (PBS), removing the remnants of the yolk. The PL section was then mounted on a glass microscope slide, fixed with four drops of 3% (w/v) paraformaldehyde (PFA), and allowed to stand for 15s until the slide was tipped to drain the PFA. One small drop of undiluted Schiffs' Reagent was allowed to stay on the slide until purple coloration appeared (several seconds). The slide was then tipped to drain excess stain. Under a bright field microscope

Fig. 3: Insemination of ostrich females in standing condition by using a catheter

Fig. 4: Insemination of ostrich females during standing by using a plastic syringe

using a 10X objective, fitted with a 10X eyepiece, the circular unstained holes caused by sperm digestion were easily detected. One field of view of holes created in the PL due to sperm penetration (SP) was counted (Donoghue et al., 1998).

The data were analyzed using an analysis of variance (ANOVA) with the general linear model procedure of the SAS program (SAS Institute, 2004). The means of variables were compared using Duncan's multiple- range test (Duncan, 1955).

Results

Data represented in Table I revealed that the percentage of fertility and hatchability from fertilized and set eggs tended to be lower (P≤0.05) for the manual massage method than dummy method or the teaser during all months of experiment. However, teaser method recorded the highest values (P≤0.05) regarding these three traits in comparison with other two methods (dummy or massage methods) during all months of experiments. Results also indicated that collecting semen from ostrich males during mating (teaser or dummy methods) appeared to yield less (P≤ 0.05) rate of embryonic mortality than when the manual massage is used during all months of experiment and concerning the total mean of this trait (Table 1). Moreover, the teaser method recorded the lowest (P≤0.05) mean and total embryonic mortality as compared with other two methods (dummy or massage methods) during all months of experiments.

Data obtained revealed that the method of semen collection from ostrich males affected the sperm penetration holes (P<0.05). Sperm penetration holes were higher in both teaser and dummy methods (Fig. 8) (P<0.05) in comparison with manual massage method during all months of experiments and regarding the total mean of this trait. Furthermore, teaser method recorded the highest value (P<0.05) of sperm penetration holes as compared with the other two methods (dummy or manual massage methods) during all months of experiment (Fig. 8).

Discussion

The best results achieved by teaser and dummy methods in comparison with manual massage method regarding fertility and hatchability rates and sperm penetration holes may be explained by the fact that teaser and dummy methods in term of semen quality are superior to manual massage method (Bonato et al., 2010; Rybnik et al., 2007; Malecki et al., 2007). Rybnik

Fig. 5: Female ostrich begins to crouch voluntarily after being followed. Crouching female emu stimulated by rubbing her sides and back

Fig. 6: Insemination of ostrich females during crouching by using a plastic siringe

et al. (2007) concluded that both methods (Teaser and dummy methods) are reliable, yield ejaculates of high quality and give reproducible results. Al-Daraji (1998) and Al-Daraji and Al-Hayani (2013) found significant positive correlation between qualitative and quantitative traits of semen and fertility and hatchability traits. Fertility of an egg is affected by factors directly related to the laying hen such her ability to mate successfully, stored sperm, ovulate and finally a suitable environment for the formation and development of embryo (Brillard, 2003). Fertility also depends on the ability of cock to mate successfully, quantity and quality of semen deposited (Brillard, 2003; Bobbo et al., 2013). McDaniel et al. (1995) and Bramwell et al. (1996) reported that there was highly significant positive correlation between sperm egg penetration and fertility and hatchability rates and the sperm egg penetration. Al-Daraji (2001) found that there was a strong positive correlation between sperm penetration values and fertility in White Leghorn × White Leghorn, New Hampshire×White Leghorn, Iraqi Brown × White Leghorn and Iraqi Barred×White Leghorn. The correlation for all breeds combined was also significant. In addition, SP was also positively correlated with hatchability and negatively correlated with embryonic mortality. Eslick and McDaniel (1992) demonstrated that poor semen characteristics resulted in low fertility and hatchability and high embryonic mortality. Bramwell et al. (1996), however, indicated that the lower number of active spermatozoa at the site of fertilisation in the male line may eventually lead to a more serious fertility problem if the mean SP numbers continue to diminish in succeeding generations. Following several generations of selection for specific traits, fertility may not change dramatically but the line may eventually become fixed at consistently low sperm penetration values. With a decreased number of active spermatozoa at the site of fertilisation, there is a diminishing chance of a single sperm cell penetrating the PL at precisely the right location and at the correct time to fertilise the ovum. If sperm penetration was monitored consistently, the genetic trend towards a lower number of spermatozoa active at the germinal disc region could be reversed or maintained before it affected fertility negatively. McDaniel et al. (1995) and Al-Daraji (1998) have demonstrated that SP is highly correlated with fertility and is an excellent predictor of fertility. Also, Malecki et al. (2004)

Table 1: Effect of semen collection method on fertility and hatchability rates of ostrich (Mean ± SE) of ostrich

Traits	First month			Second month			Third months			Total means		
	Manual massage	Dummy	Teaser	Manual massage	Dummy	Teaser	Manual massage	Dummy	Teaser	Manual massage	Dummy	Teaser
Fertility (%)	68.11 ± 2.15^{c}	76.45 ± 3.39^{b}	83.90 ± 3.92^{a}	73.87 ± 4.08^{c}	81.02 ± 4.55^{b}	88.67 ± 3.58^{a}	76.95 ± 2.85^{c}	86.83 ± 3.91^{b}	93.02 ± 5.18^{a}	72.97 ± 3.73^{c}	81.43 ± 4.08^{b}	88.53 ± 3.79^{a}
Hatchability of total eggs (%)	70.08 ± 2.15^{c}	79.55 ± 1.95^{b}	86.11 ± 3.30^{a}	74.97 ± 2.76^{c}	82.19 ± 1.92^{b}	90.17 ± 4.55^{a}	79.99 ± 2.34^{c}	88.53 ± 3.27^{b}	94.91 ± 4.81^{a}	75.01 ± 1.97^{c}	83.42 ± 2.56^{b}	90.39 ± 4.77^{a}
Hatchability of fertile eggs (%)	78.91 ± 2.15^{c}	84.82 ± 2.29^{b}	89.55 ± 1.97^{a}	84.95 ± 3.78^{c}	89.57 ± 1.96^{b}	94.80 ± 2.55^{a}	88.99 ± 1.87^{c}	93.89 ± 4.07^{b}	97.18 ± 3.56^{a}	84.28 ± 3.26^{c}	89.42 ± 1.71^{b}	93.84 ± 2.87^{a}
Embryonic mortality (%)	21.09 ± 1.11^{a}	15.18 ± 1.02^{b}	10.45 ± 0.99^{c}	15.05 ± 0.78^{a}	10.43 ± 0.49^{b}	5.2 ± 0.47^{c}	11.01 ± 0.58^{a}	6.11 ± 0.64^{b}	2.82 ± 0.09^{c}	15.72 ± 0.98^{a}	10.58 ± 0.53^{b}	6.16 ± 0.49^{c}

^{a,b,c}Means within a row and month of experiment with different superscript are different (P≤0.05)

Fig. 7: The eggs of ostrich inside the egg incubator

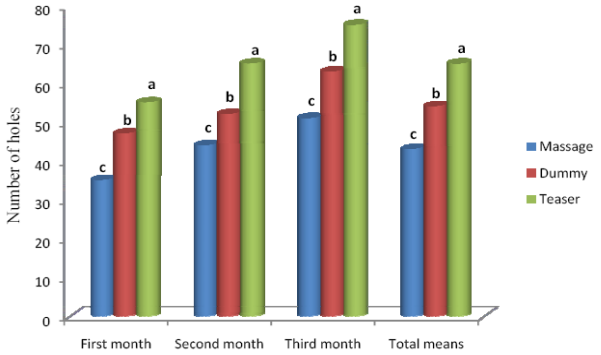

Fig. 8: Effect of semen collection method on number of sperm penetration holes in perivitelline layer of eggs collected from ostrich hens. ^{a,b,c} Means within each month of experiment with different superscript are significantly different (P≤0.05).

suggested that the long sperm storage duration in ostrich females, while being a useful breeding strategy in the wild, could also provide a basis for the establishment of a viable artificial insemination system for ostrich enterprises. Malecki and Martin (2003 a&b) have shown that the number of spermatozoa trapped on the perivitelline layer is positively correlated with fertility. It appears that a given number of spermatozoa must bind to and penetrate the perivitelline layer before fertilisation is successful. Therefore, as was found in the present study, any factor affecting SP should alter fertility and hatchability in oviposited eggs.

Conclusion

In conclusion, male ostriches develop desirable behaviour patterns that enable researchers to train them to collect semen. Teaser and dummy methods are more reliable and give better results regarding fertility and hatchability rates and sperm penetration holes in comparison with manual massage method.

References

Al-Daraji HJ (2001) Sperm–egg penetration in laying breeder flocks: a technique for the prediction of fertility. Br Poultry Sci 42: 266-270.

Al-Daraji HJ (1998) Effect of ascorbic acid supplementation on physiological and productive traits of Fawbro broiler breeders' flocks reared under hot climate. Ph. D. dissertation, College of Agriculture, University of Baghdad.

Al-Daraji HJ, Al-Hayani WK (2013) Effect of adding different levels of L-carnitine to diet on fertility and hatchability traits of guinea fowls. Tikrit J Agric Sci 13: 29-34.

Al-Daraji HJ, Al-Shemmary SA (2015) Effect of collection method on semen quality of ostrich

(*Struthio camelus*). Res Opin Anim Vet Sci 5: 108-115.

Badley AR (1997) Fertility, hatchability and incubation of ostrich (*Struthio camelus*) eggs. Poult Avian Biol Rev 8: 53-76.

Bobbo AG, Yahaya MS, Baba SS (2013) Comparative assessment of fertility and hatchability traits of three phenotypes of local chickens in Adamawa state. IOSR J Agric Veter Sci 4: 22-28.

Bonato M, Rybnik PK, Malecki IA, Cornwallis CK, Cloete SWP (2010) Between male variation in semen characteristics and preliminary results on the dilution of semen in the ostrich. South African J Anim Sci 40 (Issue 5, Supplement 1): 438-441.

Bramwell RK, McDaniel CD, Wilson JL, Howarth B (1996) Age effect of male and female broiler breeders on sperm penetration of the perivitelline layer overlying the germinal disc. Poultry Sci 75: 755-760.

Bramwell RK, Marks HL, Howarth B (1995) Quantitative determination of spermatozoa penetration of the Perivitelline layer of the hen's ovum as assessed on oviposited eggs. Poultry Sci 74: 1875-1883.

Brillard JP (2003) Practical aspects of fertility in Poultry. Wld's Poult Sci J 59: 441-446.

Cloete SWP, Brand Z, Bunter KL, Malecki IA (2008a) Direct responses in breeding values to selection of ostriches for live weight and reproduction. Aust J Exp Agric 48: 1314-1319.

Cloete SWP, Engelbrecht A, Olivier JJ, Bunter KL (2008b) Deriving a preliminary breeding objective for commercial ostriches: an overview. Aust J Exp Agric 48: 1247–1256.

Cloete SWP, Van Schalkwyk SJ, Brand Z (1998) Ostrich breeding-progress towards scientifically based strategy. In: Huchzermeyer, FW (Ed.), Ratites in a Competitive World. Proceedings of the 2nd International Scientific Ratite Conference. Oudtshoorn, South Africa, 21–25 September, pp: 55-62.

Deeming DCA, Ar A (1999) Factors affecting the success of commercial incubation. In: The Ostrich: Biology, Production and Health. Ed. Deeming DC. CABI Publishing, CAB International, Wallingford. pp: 159-190.

Deeming DC, Ayres L, Ayres FJ (1993) Observations on the commercial production of ostrich (*Struthio camelus*) in the United Kingdom: Incubation. Vet Rec 132: 602-307.

Donoghue A M (1996) The effect of twenty-four hour in vitro storage on sperm hydrolysis through the Perivitelline layer of ovipositioned turkey eggs. Poult Sci 75: 1035-1038.

Donoghue AM, Holsberger DH, Evenson DP, Froman DP (1998) Semen donor selection by in vitro sperm mobility increases fertility and semen storage in the turkey hen. J Androl 19: 295-301.

Duncann DB (1955) Multiple range and multiple F tests. Biometrics 11: 1-42.

Eslick ML, McDaniel GR (1992) Interrelationships between fertility and hatchability of eggs from broiler breeder hens. J Appl Poult Res 1: 156-159.

Hemberger MY, Hospes R, Bostedt H (2001) Semen collection, examination and spermiogram in ostriches. Reprod Dom Anim 36: 241-243.

Irons PC, Bertshinger HJ, Soley JT, Burger WP (1996) Semen collection and evaluation in the ostrich, in: Deeming, D.C. (Ed.) Improving our Understanding of Ratites in a Farming Environment, pp. 157-159 (Oxfordshire, Ratite Conference).

Malecki IA, Rybnik PK, Martin GB (2008) Artificial insemination for ratites: a review. Aust J Exp Agric 48: 1284-1292.

Malecki IA, Martin GB (2005) Development of reproductive technology and fertility assessment for the emu and ostrich farming. Rural Industries Research and Development Corporation, Australia (RIRDC Publication No 05/200).

Malecki IA, Martin GB (2003a) Distribution of spermatozoa in the outer perivitelline layer from above the germinal disc of emu and ostrich eggs. Reprod Fert Dev 15: 263-268.

Malecki IA, Martin GB (2003b) Sperm supply and egg fertilization in the ostrich (*Struthio camelus*). Reprod Dom Anim 38: 429-435.

Malecki IA, Rybnik PK, Horbanczuk JO, Lukaszewicz E, Naranowicz H (2007) Semen collection in the ostrich. Proceeding of the XIV World Ostrich Congress, Riga, Latvia, 19th-20th October.

Malecki IA, Cloete SWP, Gertenbach WD, Martin GB (2004) Sperm storage and duration of fertility in female ostriches (*Struthio camelus*). South Afr J Anim Sci 34 (3): 158-165.

Malecki IA, Martin GB, Lindsay DR (1997a) Semen Production by the Male Emu (*Dromaius novaehollandiae*). 1. Methods for semen collection. Poult Sci 76: 615-621.

Malecki IA, Martin GB, Lindsay DR (1997b) Semen Production by the Male Emu (*Dromaius novaehollandi*ae). 2. Effect of collection frequency on the production of semen and spermatozoa. Poult Sci 76: 622-626.

McDaniel CD, Bramwell RK, Wilson JL, Howarth B (1995) Fertility of male and female broiler breeders following exposure to elevated ambient temperatures. Poult Sci 74: 1029–1038.

More SJ (1996) The performance of farmed ostrich eggs in eastern Australia. Preventative Vet Med 29: 91-106.

Mushi EZ, Binta MJ, Chabo RG, Galetshipe O (2008) Problems associated with artificial incubation and

hatching of ostrich (*Struthio camelus*) eggs in Botswana. Res J Poult Sci 2: 21-26.

Rozenboim I, Navot A, Gvaryahu G, Degen A (1996) Collecting semen ostrich – a new and friendly method. Poultry Science Association, 85th Annual Meeting, July 8-12, 1996, Louisville, Knetucky, Abs. 72.

Rozenboim I, Navot A, Snapir N, Gvaryahu G, Rosenshtrauch A, Degen A (1999) A method for obtaining semen from the ostrich (*Struthio camelus*) and its quantitative and qualitative characteristics. In: Proceedings of the International Conference on Bird Reproduction. 22-24 September 1999, Tours, France (Poster).

Rozenboim I, Navot A, Snapir N, Gvaryahu G, Rosenshtrauch A, El Halawani ME, Gvaryahu G, Degen A (2003) Methods for collecting semen from the ostrich (*Struthio camelus*) and some of its quantitative and qualitative characteristics. Br Poult Sci 44: 607-611.

Rybnik PK, Horbanczuk JO, Naranowicz H, Lukaszewicz E, Malecki IA (2007) Semen collection in the ostrich (*Struthio camelus*) using a dummy or a teaser female. Br Poult Sci 48: 635–643.

SAS Institute (2004) SAS/STAT User's Guide. Relase Version 7.00. SAS Institute. Cary. North Carolina.

Van Schalkwyk SJ, Cloete SWP, Brown CR, Brand Z (2000) Hatching success of ostrich eggs in relation to setting, turning and angle of rotation. Brit Poult Sci 41: 46-52.

Ya-jie JI, Yan-bo Y, Wu-zi D (2001) Studies on ostrich semen character and semen storage at low temperature. J Econ Anim 5: 49-54.