IMPROVEMENT OF SEMEN QUANTITY AND QUALITY IN OSTRICHES BY OXYTOCIN INJECTION BEFORE SEMEN COLLECTION

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Abstract

The effect of intravenous oxytocin injection before semen collection on semen quality was investigated in ostriches. Six clinically healthy ostriches, aged 3-5 years, previously trained for semen collection by manual stimulation were included in the experiment. These ostriches were routinely used for semen collection. Prior to oxytocin injection, they were subjected to 2 successive semen collections, 4 days apart. Then, they rested for another 4 days after the second semen collection. On the day of the third semen collection, the birds were intravenously injected with 5 IU of oxytocin via phallic vein. Semen collection was attempted 5 min after the injection. Four days after the third semen collection, ejaculates were again obtained from the birds. Seminal characteristics included in the evaluation were semen volume, percentage of motile sperm, sperm concentration, percentages of live, abnormal, and hyo-osmotic swollen spermatozoa.

Semen volume was increased from 0.27 ± 0.08 and 0.23 ± 0.06 ml (8 and 4 day before injection, respectively) to 0.95 ± 0.17 ml ($p < 0.05$) on the day of oxytocin injection and returned to 0.26 ± 0.08 ml 4 days later. Sperm motility was also increased from 18.13 ± 9.26 and 23.75 ± 9.76% (8 and 4 day before injection, respectively) to 57.5 ± 12.54 ($p < 0.05$) on the day of injection and returned to 23.75 ± 10.68% 4 days after injection. Sperm concentration expressed similar results, and higher concentration was obtained on the day of injection (753.13 ± 354.94 × 10^6 cells/ml) compared to 8 and 4 days before and 4 days after injection (62.25 ± 31.68, 143.06 ± 102.91 and 70.78 ± 32.33 × 10^6 cells/ml, respectively, $p < 0.05$). Oxytocin enhanced the percentages of live and membrane integrity in the same manner. The percentage of live spermatozoa was higher with oxytocin injection (73.50 ± 12.55%) than the others (ranged 21.63-44.25%, $p < 0.05$). The percentage of swollen sperm (hypo-osmotic swelling test) in the day of oxytocin injection (65.25 ± 13.10%) was higher than the others (ranged 15.13-31.31%, $p < 0.05$). No significant effect was found on percentage of abnormal sperm. We concluded that oxytocin injection before semen collection improved most seminal characteristics in ostriches. Whether or not the injection depleted sperm reserve may need further study.

Keywords: Oxytocin, semen quality, motility, semen collection, ostrich

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Introduction

Although reproductive system has been intensively investigated in domestic poultry, very few reproductive technologies are applicable for ostriches *(Struthio camelus)*. Mating system needs at least 1 male per 2-3 females, the high ratio that does not allow efficient management and reproductive systems to develop for farming. Artificial insemination is one of the major approaches to improve production and enhance reproductive efficiency (Malecki *et al*., 2008). Semen collection in ostriches has been described and well documented (Soley and Groenewald, 1999; Hemberger, *et al*., 2001). This allows assisted reproductive technology to be studied and developed both for semen preservation and artificial insemination. Regardless of collection methods, semen quality and quantity are the main purposes to be achieved.

Spermatogenesis in ostriches is more or less similar to that of chicken. There are 2 types of spermatognia, type A and type B, in seminiferous epithelium of ostriches (Soley and Groenewald, 1999). They have pale nuclei and line on basement membrane. Spermatogonia divide and give rise to primary spermatocytes which enter first meiotic division. They appear in various stages of first meiotic division and are prominent cells in germinal epithelium of seminiferous tubules. Primary spermatocytes divide and give rise to secondary spermatocytes which have relatively large nucleus about 6 μm in diameter. Secondary spermatocytes divide and spermatids are the result of completion of meiotic division. There are 8 stages of spermiogenesis described in ostriches (Soley and Groenewald, 1999). Spermatids change in morphology during these 8 stages and spermatozoa are the final products of the process. The developing germ cells during all stages of spermatogenesis are joined together by intercellular bridges similar to those of birds and mammals.

To maximize spermatozoa harvesting, high frequency of collection may be applied as far as no injury occurs to male ostriches and no dramatic effect occurs to semen quality. Alternatively, oxytocin has been used to facilitate semen collection, at least in bulls (Palmer *et al*., 2004), rams (Bozkurt *et al*., 2007) and men (Rezk *et al*., 2004). Oxytocin causes contraction of smooth muscle in female although its function in males is unclear. It has important roles, in female mammals, at least in lactation and parturition. In males, it is believed that oxytocin is involved in sexual behavior such as penile erection and copulatory behavior (Carter, 1992; Argiolas and Melis, 2004). Oxytocin induces penile erection via sensitive brain area at paraventricular nucleus of the hypothalamus. In ostriches, anterior pituitary is not directly connected to the hypothalamus as in mammals. Thus, releasing hormones secreted from the base of the hypothalamus is conveyed via blood stream to anterior pituitary gland. The posterior pituitary gland of ostrich, however, is connected to the hypothalamus as in mammals (Soley and Groenewald, 1999). It secretes neurohormones including oxytocin. To the best of our knowledge, very little information concerning use and application of oxytocin for semen collection in ostriches is available. The aim of this study was thus to investigate whether oxytocin injection before semen collection could increase semen quantity and quality.

Materials and Methods

Animals

Six months before experiment, ostriches *(Struthio camelus)*, aged 3-5 years, have been trained for semen collection by manual stimulation (Figure 1). Six birds were selected from healthy, trained ostriches for this experiment. Those male ostriches were raised under common management system and separated from female ostriches.

Ostriches were routinely used for semen collection. The birds were subjected to 2 successive semen collections, 4 and 8 days prior to oxytocin injection. Ostriches rested for another 4 days after the second semen collection. On the day of the third semen collection, the birds were intravenously injected with 5 IU of oxytocin via phallic vein. To

allow full effect of oxytocin, ejaculates were collected from the birds 5 min after hormone injection. Four days after the third semen collection, ejaculates were again obtained from the birds. Seminal characteristics included in the evaluation were semen volume, percentage of motile sperm, sperm concentration, percentages of live, and abnormal and hyo-osmotic swollen spermatozoa.

Semen Collection and Evaluation

Semen collection following Soley and Groenewald, 1999; Hemberger, *et al.*, 2001) was conducted in the morning in a quiet environment. Each of the ostriches was held in a plucking crush similar to breeding box for artificial insemination in cattle. Soft bag was applied to head of ostriches to calm down. Once the bird was in the device, the collector accessed to the back end. The phallus was retracted, rinsed with warm water and held in position by an assistant. Soft and clean cloth was used for a firmer grip of the phallus. A collector stimulated seminal papillae with fingers to empty semen from ductus deferens. Ejaculation occurred after a few minutes of stimulation and semen ran down the phallic groove where it was collected into a clean glass container.

Figure 1. Semen collection by manual stimulation in ostriches. The phallus was held in position away from posterior end and a track of white colored semen (arrow) was collected into the beaker

Immediately after collection, semen was held at room temperature (25°C) before evaluation. Semen sample was subjectively evaluated for sperm progressive motility under a light microscope at magnification of 400× (Carl Zeiss Axiostar Plus microscope). To reduce variation, motility evaluation was conducted by the same person in all semen samples and expressed as the percentage of progressively motile sperm cells. The percentages of sperm viability were evaluated after staining with a vital dye, 1.67% eosin Y in 10% nigrosin solution (Hancock, 1951; Chalah and Brillard, 1998). An equal volume of semen sample was mixed with staining solution at 25ºC and smear was made after 30 sec of staining. The smear was air dried before evaluation by counting at least 200 cells under a light microscope in the same day of preparation.

Morphology was assessed by staining semen sample with William's stain. Semen sample was smeared on a clean glass slide, air dried and fixed in 95% absolute alcohol for 5 min. The slide was dipped in chloramine T for 1 min and then dipped a few times in 95% absolute alcohol and in carbolfuchsin stain for 8 min before being washed in running water. The slide was air dried and evaluated for sperm size, shape and characteristics of head and tail under the light microscope at 400×. At least 200 spermatozoa were counted and percentage of abnormal spermatozoa calculated.

Semen concentration was determined by diluting a semen sample with citrate chloramine T solution at the ratio of 1:100. The diluted semen was loaded in counting chambers of a haemocytometer. The sperm cells were count and concentration calculated. For hypo-osmotic swelling test, 2 µl of semen was mixed with 20 µl of a 100 mOsm solution (containing 0.49 g of sodium citrate and 0.9 g of glucose in 100 ml of distilled water). The solution was incubated at 25ºC for 1 h before a drop of mixture was placed on a slide, covered with a cover glass and membrane structures of spermatozoa were evaluated under a microscope at 400×.

Statistical Analysis

Data were tested for normal distribution with Kolmogorov Smirnov one sample test before being analysed. Wilcoxon test was used as non-parametric statistics of data analysis. Differences were considered significant at $P < 0.05$. All statistics was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, U.S.A.).

Results and Discussion

Oxytocin injection improved most seminal characteristics of ostriches and the values returned to base line at day 4 after injection. Semen volume collected by manual stimulation 8 and 4 days (day -8 and -4) before oxytocin injection were 0.27 ± 0.08 and 0.23 ± 0.06 ml respectively ($p > 0.05$; Table 1). The volume increased to 0.95 ± 0.17 ml ($p < 0.05$) on the day of oxytocin injection and returned to 0.26 ± 0.08 ml 4 days after injection. Sperm motility was increased from 18.13 ± 9.26 and 23.75 ± 9.76% (day -8 and -4, respectively) to 57.5 ± 12.54 ($p < 0.05$) on the day of injection and returned to 23.75 ± 10.68% 4 days after injection.

Sperm concentration on the day of injection (753.13 ± 354.94 × 10^6 cells/ml) was higher than those of 8 and 4 days before and 4 days after injection (62.25 ± 31.68, 143.06 ± 102.91 and 70.78 ± 32.33 × 10^6 cells/ml, respectively, $p < 0.05$). The percentage of live spermatozoa was higher with oxytocin injection (73.50 ± 12.55%) than the others (ranged 21.63-44.25%, $p < 0.05$). The percentage of swollen sperm (hypo-osmotic swelling test) in the day of oxytocin injection (65.25 ± 13.10%) was higher than the others (ranged 15.13-31.31%, $p < 0.05$). No significant effect was found on percentage of abnormal sperm.

Techniques for semen collection in emu and ostriches have been developed, such as artificial cloaca with the dummy or the teaser (Malecki, *et al*., 1997a; Rybnik *et al*., 2007), and manual stimulation technique (Soley and Groenewald, 1999; Hemberger, *et al*., 2001). The artificial cloaca technique requires dummy or the teaser and training of birds starting at young ages. We used manual stimulation technique because the available ostriches were mature and familiar with human and handling.

There are some approaches to increase sperm production and sperm output in emu and ostriches. Frequency of semen collection has been proved to increase sperm output. In emu, collection at twice daily frequency resulted in higher sperm output than that of once daily (Malecki, *et al*., 1997b). In ostriches, collection at 6 h interval yielded more sperm output than

Table 1. Semen volume, % motility, semen concentration and total sperm compared before and after oxytocin administration*. Data was shown as mean ± SE

	Days before/after oxytocin injection			
	-8	**-4**	**0**	**4**
Volume (ml)	0.27 ± 0.08[a]	0.23 ± 0.06[a]	0.95 ± 0.17[b]	0.26 ± 0.08[a]
Motility (%)	18.13 ± 9.26[a]	23.75 ± 9.76[a]	57.50 ± 12.54[b]	23.75 ± 10.68[a]
Concentration (×10^6/ml)	62.25 ± 31.68[a]	143.06 ± 102.91[a]	753.13 ± 354.94[b]	70.78 ± 32.33[a]
Total sperm (×10^9 cells)	0.01 ± 0.08[a]	0.04 ± 0.03[a]	0.69 ± 0.33[b]	0.02 ± 0.01[a]
Live sperm (%)	21.63 ± 11.07[a]	31.44 ± 11.33[a]	73.50 ± 12.55[b]	44.25 ± 13.14[a]
Hypo-osmotic swelling test (%)	15.13 ± 8.43[a]	25.75 ± 9.83[a]	65.25 ± 13.10[b]	31.13 ± 10.41[a]
Abnormal head (%)	21.65 ± 10.95	9.69 ± 2.31	17.31 ± 6.64	15.75 ± 6.39
Abnormal tail (%)	20.31 ± 10.16	11.44 ± 3.30	13.38 ± 3.44	12.44 ± 1.24

* *Different superscripts within the same row demonstrate significant differences ($p < 0.05$)*

those of 24 and 48 h intervals, while sperm motility and viability were not affected (Bonato *et al*., 2011). This approach is safe, useful and applicable when time and facilities are available. Semen quality in our study was similar to other reports. Semen volume obtained from untreated ostriches (3-5 years) in this study (0.23 to 0.27 ml) was comparable to 4-6 years-old ostriches (0.1 to 1.5 ml) reported by Hemberger *et al*. (2001) and adult ostriches (0.8 to 1.8 ml; Khumpim *et al*., 2010). The sperm motility in our study (18.13 to 23.75 in untreated birds) was also comparable to semen of 3-5 years-old ostriches diluted with BPSE (30.42; Rattanawaree *et al*., 2010). Sperm concentration of untreated ostriches in our study (62.25 to 143.06 × 10^6 sperm/ml) was, however, higher than another report (3.9 to 36.0 × 10^6 sperm/ml; Hemberger *et al*., 2001). Semen quality of untreated ostriches is thus comparable to those reported by other investigators.

Oxytocin is believed to have both endocrine and paracrine roles in male reproduction. Its roles have been investigated in many species including catfish (Viveiros *et al*., 2003), bulls (Palmer *et al*., 2004), rams (Bozkurt *et al*., 2007) and men (Rezk *et al*., 2004). We found that oxytocin increased most seminal characteristic in ostriches as well. This is supported by the studies in rabbits (Fjellstrom *et al*., 1968) and rams (Bozkurt *et al*., 2007) while there was no such effect in dogs (Traas and Kustritz, 2004). Our results indicate that oxytocin enhances semen quality and quantity in ostriches. It has proved that semen collection in ostrich yields better semen quality and quantity with oxytocin injection prior to collection. Further study may be needed to elucidate mechanism of oxytocin on semen collection in various species.

Conclusions

It may be concluded oxytocin injection before semen collection in ostriches improves most seminal characteristics in ostriches. This provides an alternative method for efficient harvesting of spermatozoa. Whether or not the injection depleted sperm reserve may need further study.

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