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Between male variation in semen characteristics and preliminary results on the dilution of semen in the ostrich

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Abstract

This study is part of an ongoing project on artificial insemination in ostriches. The physical output of neat semen from four ostrich males was investigated and the effect of reconstituting semen with: 1) seminal plasma of the same male (SPS); 2) seminal plasma of another male (SPD), and 3) Dulbecco's Modified Eagles Medium (DMEM). Semen was collected daily from one or two pairs of males using the dummy female method, each pair being replicated twice. Spermatozoa viability in neat semen, SPS, SPD and DMEM was assessed using nigrosin-eosin staining and the proportions of live normal, live abnormal and dead sperm were determined. Semen volume (mean ± SE) was 1.27 ± 0.13 mL, the concentration of spermatozoa 3.68 ± 0.17 x 10^9 /mL and the number of spermatozoa 4.92 ± 0.64 x 10^9 /ejaculate. Furthermore, the live normal, live abnormal and dead spermatozoa in the neat semen were 61.2 ± 4.5%, 21.2 ± 2.7% and 17.7 ± 4.3% respectively. The ejaculate volume and the number of dead spermatozoa were not affected by collection time. However, the number of live abnormal spermatozoa increased through the day causing a reduction in live normal spermatozoa. Furthermore, re-suspending spermatozoa in DMEM reduced the number of live normal (31.4 ± 4.6%) and live abnormal spermatozoa (11.0 ± 2.7%) and increased the number of dead spermatozoa (57.6 ± 4.4%). In contrast, numbers of live spermatozoa were higher when suspended in seminal plasma and similar in SPS (53.9 ± 4.6%) and SPD (50.7 ± 4.6%). These are the first crucial steps to determining the optimum semen collection time and to improving the viability of diluted spermatozoa.

Keywords: Artificial insemination; diluent; spermatozoa morphology and viability; *Struthio camelus*
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Introduction

To date, the ostrich industry is still inefficient and is essentially characterized by an inadequate egg production, high embryo mortality, poor chick survival and suboptimal growth rates while selective breeding is not commonly practiced (Cloete *et al.*, 1998). All traits of economic importance exhibit sufficient genetic variation for substantial progress to purposeful selection (Cloete *et al*., 2008). Yet this potential is not realised 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 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; as well as the insemination dose and frequency. In this context, the physical output of neat semen from four ostriches was

examined and investigated the effect of reconstituting centrifuged semen on the morphology and viability of spermatozoa.

Materials and Methods

The study was carried out on four male ostriches (2 - 4 years of age), maintained at the Oudtshoorn Research Farm, in November 2008. Semen was collected daily (09:00 to 19:00) from one or two pairs of males using the dummy female method (Rybnik *et al*., 2007), each pair being replicated twice. Following copulation with the female dummy, ejaculates were collected and measured using a pipette (200 µL), and the concentration of spermatozoa was determined by counting sperm using a haemocytometer from 20 µL semen diluted 1 : 400 (v/v) with a phosphate buffered saline solution containing 10% formalin. The number of spermatozoa was subsequently calculated by multiplying semen volume and concentration (Malecki *et al*., 1997).

Within 15 min of collection time, semen was centrifuged for 1 minute at 10,000 rpm. All the seminal plasma from above the spermatozoa pellet was removed and centrifugations were repeated until no pellet was visible (adapted from Blesbois & Hermier, 1990). Spermatozoa were then re-suspended in the seminal plasma to a concentration of 10 - 15 million spermatozoa per mL with: (1) seminal plasma of the same male (SPS); (2) seminal plasma of another male (SPD); (3) DMEM (Dulbecco's Modified Eagles Medium). Samples of neat semen, SPS, SPD and DMEM were incubated for 15 min at room temperature before being mounted onto a glass slide. The proportion of live normal, live abnormal and dead spermatozoa was estimated after counting 500 spermatozoa stained with nigrosin-eosin.

To investigate potential male differences in semen output, three general linear mixed models (GLMM) were constructed in which ejaculate volume, concentration of spermatozoa and number of spermatozoa per ejaculate were entered as the response variables. Male number was entered as fixed factor, and time of collection as a random factor. Similarly, male variation in semen properties was investigated by conducting a GLMM in which live normal, live abnormal and dead spermatozoa were, this time, entered as response variables. Statistical analyses were performed using SPSS 17 (SPSS Inc., Chicago, K. USA).

Results and Discussion

The volume (mean ± SE) of semen collected was 1.27 ± 0.13 mL, the concentration of spermatozoa 3.68 ± 0.17 x 10^9 /mL and the number of spermatozoa per ejaculate of 4.92 ± 0.64 x 10^9. Both ejaculate volume and the number of spermatozoa were variable between males ($F_{1,3}$ = 6.26, P = 0.001 and $F_{1,3}$ = 4.78, P = 0.003 respectively; Figure 1), while the concentration of spermatozoa did not differ between males ($F_{1,3}$ = 1.32, P = 0.280). Furthermore, the live normal, live abnormal and dead spermatozoa in the neat semen were 61.2 ± 4.5%, 21.2 ± 2.7% and 17.7 ± 4.3% respectively. The ejaculate volume and the number of dead spermatozoa were not affected by the collection time. However, the number of abnormal spermatozoa appeared to increase through the day, at the expense of the live normal spermatozoa (Figure 2). Furthermore, no differences in the proportion of live normal, live abnormal and dead spermatozoa were detected between the four males (P >0.05). The number of live normal spermatozoa also did not differ between spermatozoa re-suspended in SPS (53.9 ± 4.6%) or in SPD (50.7 ± 4.6%). Re-suspending spermatozoa in DMEM had a negative effect on the viability of spermatozoa. A lower number of live normal spermatozoa (31.4% ± 4.6%), live abnormal spermatozoa (11.0 ± 2.7%) and a higher number of dead spermatozoa (57.6 ± 4.4%) than in seminal plasma were observed (Figure 3).

Conclusions

This study indicates that ejaculate volume and number of spermatozoa per ejaculate are variable between individuals, while concentration of spermatozoa is similar. The results suggest that collecting semen early in the day yields better quality ejaculates. This finding could be related to higher ambient temperatures experienced in the afternoon, and needs to be investigated further. The lack of a difference in the percentages of dead spermatozoa between semen reconstituted with the seminal plasma of the same male and of another male indicates that seminal plasma properties in ostriches may be similar across individuals. The DMEM had a detrimental effect on the viability of spermatozoa, which could have been caused by inadequate composition of that medium or removal of seminal plasma components that are essential to sperm function.

Analysing the chemical differences between seminal plasma and DMEM may therefore provide valuable insight into the factors regulating sperm viability. Further studies are thus warranted to determine the optimum semen collection time and post collection handling protocol of semen in order to improve the viability of stored ostrich spermatozoa.

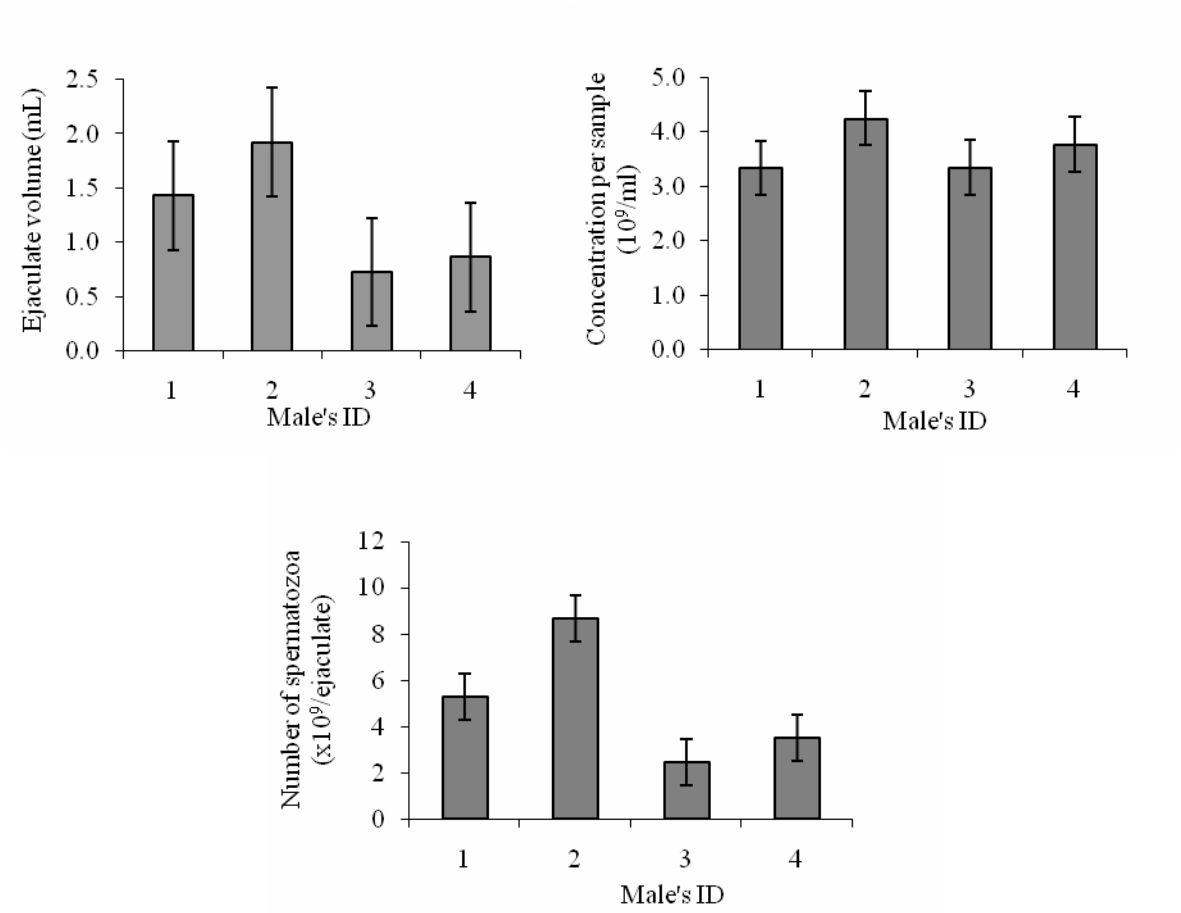

Figure 1 Ejaculate volume, concentration per sample and number of spermatozoa per ejaculate (mean ± SE) of four ostrich males.

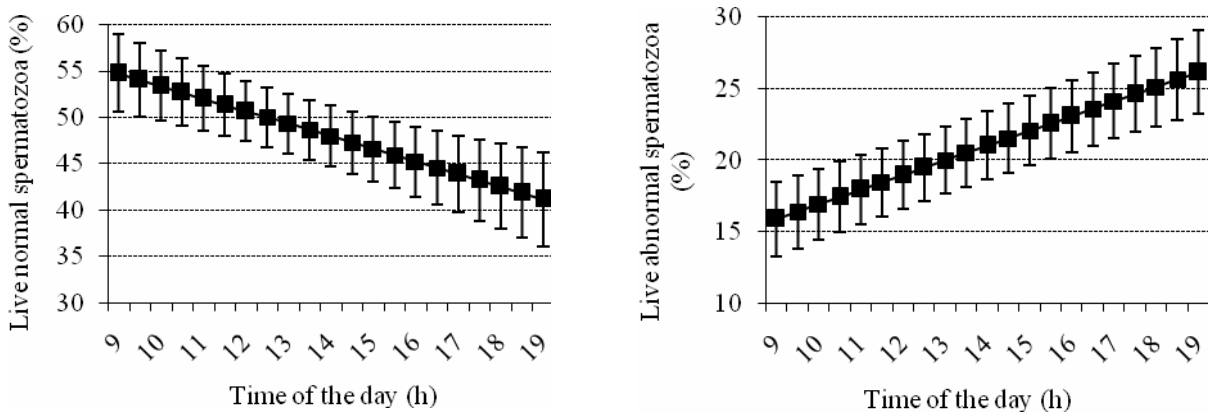

Figure 2 Percentages of live normal and live abnormal spermatozoa (mean ± SE) in relation to the time of collection.

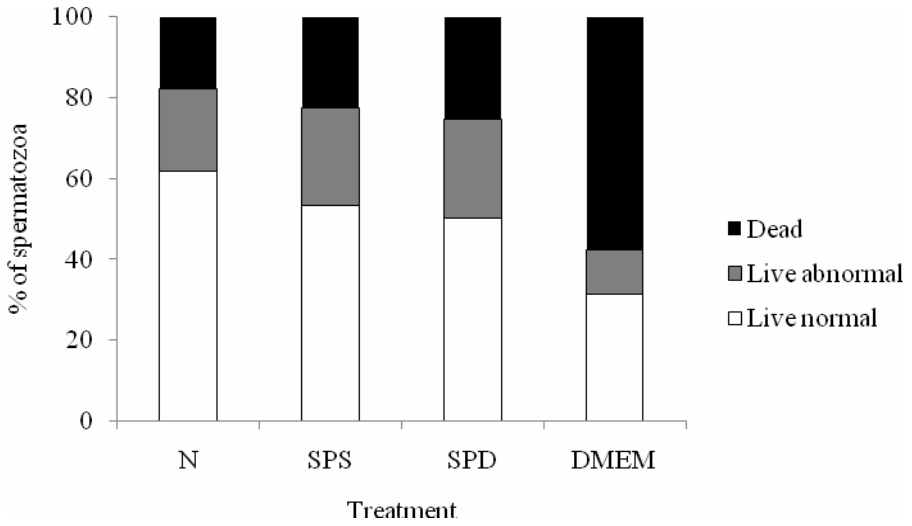

Figure 3 Percentages of live normal, live abnormal and dead spermatozoa in either neat semen (N), seminal plasma from the same male (SPS), seminal plasma from another male (SPD) or DMEM (Dulbecco's Modified Eagles Medium).

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