Animal Reproduction Science 151 (2014) 49–55

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Predicting ejaculate quality and libido in male ostriches: Effect of season and age

Maud Bonato[a,*], Irek A. Malecki[a,b], Paulina K. Rybnik-Trzaskowska[c], Charlie K. Cornwallis[d], Schalk W.P. Cloete[a,e]

[a] Department of Animal Sciences, University of Stellenbosch, Matieland 7602, South Africa
[b] School of Animal Biology M085, Faculty of Natural and Agricultural Sciences, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
[c] Department of Genetics and Animal Breeding, Warsaw University of Life Sciences, Ciszewskiego 8, 02-786 Warsaw, Poland
[d] Department of Biology, Lund University, SE-223 62, Lund, Sweden
[e] Directorate Animal Sciences: Elsenburg, Private Bag X1, Elsenburg 7607, South Africa

ARTICLE INFO

Article history:
Received 16 May 2014
Received in revised form 27 August 2014
Accepted 12 September 2014
Available online 28 September 2014

Keywords:
Semen quality
Artificial insemination
Struthio camelus
Poultry

ABSTRACT

The success of artificial breeding program depends largely on the reproductive performance of males. Male performance can vary with season and age impacting on quality and quantity of semen collected for artificial insemination purposes and therefore fertility of inseminated females. We examined variation in semen output and male libido of seven male ostriches (aged 2–5 years) over a period of 24 months. We collected ejaculates using a dummy female and measured semen characteristics (ejaculate volume, sperm concentration, number of spermatozoa per ejaculate, sperm motility and morphology) and male libido (willingness to mount the dummy). A total of 1006 ejaculates were collected. Across months, the volume of semen (mean ± SEM) ranged from 1.03 ± 0.12 mL to 1.85 ± 0.07 mL, the sperm concentration from 3.21 ± 0.12 × 10^9/mL to 4.16 ± 0.74 × 10^9/mL, and the number of spermatozoa from 3.42 ± 0.28 × 10^9 to 7.66 ± 0.47 × 10^9. The largest volume of ejaculates and the highest number of sperm were collected in spring. Ejaculates with higher number of normal sperm were also collected in spring–early summer, whereas ejaculates with higher numbers of live abnormal and dead sperm were collected in winter. Sperm motility was relatively constant over months, despite a reduction in summer (January–February), while male libido peaked in winter (June–July) and spring (October–November). Furthermore, we observed high individual variation between males for all variables tested, except for motility. These results indicate that collections conducted in spring yield higher number of spermatozoa, when the libido of males is also at a maximum. Therefore in this species seasonal variation in semen quality should be considered in breeding programmes by artificial insemination to maximise fertility.

1. Introduction

Commercial ostrich farming originally started in the 1800s in South Africa and has since undergone major development worldwide (Smit, 1964; Deeming and Bubier, 1999). However, poor productivity and low genetic improvement as a result of low fertility achieved by natural mating is still the main economic barrier highlighting the need to develop assisted reproductive techniques and selective breeding programs (Cloete et al., 1998; Malecki et al., 2008; Cloete and Malecki, 2011). The development of novel techniques to collect semen and inseminate females

* Corresponding author. Tel.: +27 44 272 6077; fax: +27 44 279 1910.
E-mail address: mbonato@sun.ac.za (M. Bonato).

http://dx.doi.org/10.1016/j.anireprosci.2014.09.015
0378-4320/

in a stress free manner has generated a new interest in implementing assisted reproduction technology in this species (Rybnik et al., 2007; Malecki et al., 2008; Malecki and Rybnik-Trzaskowska, 2011). To date, a few studies on ostrich semen have been conducted on different methods and frequency of semen collection (Hemberger et al., 2001; Rybnik et al., 2007; Bonato et al., 2011; Rybnik et al., 2012), as well as on sperm motility and survival after short-term storage (Malecki et al., 2008; Ciereszko et al., 2010; Bonato et al., 2010, 2012). However, little is known about the influence of season and male age on production of semen. Rybnik et al. (2012) reported that the production of ostrich semen in Poland varied with the year, age and month of the breeding season, although the breeding season was limited to four months. Additionally, all recent studies undertaken on ostrich semen have demonstrated high variation between individual males in semen yield and characteristics, as well as male sexual behaviour (Bonato et al., 2010, 2012; Rybnik et al., 2012), but the basis for this variation is still unclear.

Seasonality may potentially be an important factor underlying variation in semen production in ostriches. Ostriches are considered spring–summer breeders because their highest breeding activity coincides with an increase in daylight length (Degen et al., 1994; Soley and Groenewald, 1999). The timing and duration of the breeding season, however, was observed to vary in both the wild (Magige et al., 2009) and for farmed ostriches (Soley and Groenewald, 1999). For instance in the southern hemisphere, wild ostriches in Zimbabwe were observed to lay eggs from July to December (winter to beginning of summer) irrespective of rainfall, which appear to support the role of day length in regulating their breeding season (Jarvis et al., 1985). In South African commercial farms, the breeding season of ostriches usually runs from June (winter) to February (summer) (Jarvis et al., 1985; Cloete et al., 1998). In the northern hemisphere, the ostrich egg-laying season in Poland was strictly in spring and summer months (Horbanczuk and Sales, 1999), while in Israel egg laying was reported in every month of the year (Degen et al., 1994). Again it supports the role of day length but suggesting the ostrich might also be an opportunistic breeder (Deeming and Bubier, 1999). While most ostrich breeding activity is in the spring–summer months the impact of seasonality on the reproductive performance of ostriches is currently unclear.

In seasonally breeding species, male reproductive efficiency is restricted to a defined time of the year where sexual activity and semen output are maximised while being reduced or absent in other months. In birds, sexual behaviour and semen quality are parameters limiting male reproductive efficiency, as they can have a profound effect on fertility (Sexton, 1983; Saeid and Al-Soudi, 1975; Cheng et al., 2002; Nwakalor et al., 1988). Seasonal changes in sperm numbers, motility, viability and morphology have been reported in several farmed bird species (Shikabrown chicken: Obidi et al., 2008; duck: Penfold et al., 2000; emu: Malecki et al., 2000). Moreover, the production of semen and sperm in birds also depends on age. In several studies, younger males were found to produce significantly lower semen volumes than older males (white leghorn: Elagib et al., 2012; emu: Malecki et al., 1997), and there is limited data suggesting this also is the case in ostriches (Rybnik et al., 2012). All these aspects clearly highlight the need to understand the effect of season and age on the production of semen and sperm, and male libido in ostriches, in order to successfully carry out semen preservation and artificial insemination protocols.

Table 1
Age of male ostriches and their numbers over the 2 years of study.

Male age	2009	2010
(Years)		
2	3	0
3	3	3
4	1	3
5	0	1

As the reproductive performance of the male ostrich can exert a considerable influence on the outcome of an artificial breeding programme, the aim of the study was to assess sperm quality with regard to sperm number, motility, morphology, viability as well as male libido over a 24-month period, in order to establish peak semen production and quality periods.

2. Material and methods

2.1. Study population

The study was conducted at the Oudtshoorn Research Farm of the Western Cape Department of Agriculture, situated in the Klein Karoo, South Africa (33°63′ S,22°25′ E), from June 2009 to May 2011 on seven South African black ostrich males (2–5 years of age; Table 1). Prior to the experiment, males were trained to mount a dummy female and ejaculate into an artificial cloaca (Rybnik et al., 2007). Males were primarily selected based on their reaction to humans and their capacity to cooperate during the collection of semen. Ejaculates were collected twice a week over the entire 24-month period.

2.2. Semen measurements

Ejaculate 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 buffered saline solution containing 10% formalin. The number of sperm was then calculated by multiplying semen volume and sperm concentration. Sperm motility, subjectively assessed as collective motility was scored a 5-point scale (1: below 20% of motile sperm; 2: 20–40%; 3: 40–60%; 4: 60–80%; 5: 80–100%) by observing mass movement of 10 μL neat semen under a 20× objective (Allen and Champion, 1955). Finally, samples of neat semen were mounted onto a glass slide and the percentage of live normal (membrane intact with normal morphology, eosin impermeable), live abnormal (membrane intact, eosin impermeable but with alteration of normal morphology) and dead sperm (membrane damaged and eosin permeable) was estimated after counting 500 sperm stained with nigrosin-eosin (Lake and Stewart, 1978; Łukaszewicz et al., 2008) on duplicate slides. Sperm

forming a complete unit of a slightly curved head, mid-piece and tail were considered as live normal (as described by Soley and Roberts, 1994), while any sperm showing a deviance from this structure was considered as live abnormal (i.e. giant sperm, double-headed sperm, bent head, swollen head, broken head or tail, straight sperm, bent at mid-piece or tail; Du Plessis et al., in press).

2.3. Measurement of male libido

Male libido was evaluated as the willingness to mount the dummy female described previously by Bonato et al. (2011). Briefly, male libido was scored on a scale from 0 to 3 (0: no reaction; 1: approach with interest but no willingness to mount; 2: no courtship but willingness to mount the dummy; 3: courtship and willingness to mount the dummy). The number of collection attempts to a successful semen collection was also recorded.

2.4. Statistical analysis

To investigate the effects of month, year and male age on ejaculate volume, we conducted a general linear mixed model (GLMM) with ejaculate volume as the dependent variable, month, year (and their interactions) and male age were entered as fixed factors, while the male's breeding value for chick production was entered as a covariate, to control for potential genetic predisposition of certain males to produce higher quality ejaculates. Male identity, the interaction between male identity and male age, as well as the interaction between male identity and month were entered as random factors to control for repeated measures of individuals. Similar GLMM analyses were conducted with sperm concentration, total number of sperm, percentage of live normal sperm, live abnormal sperm, and dead sperm as dependent variables. Percentage of live normal, live abnormal and dead sperm were first arcsin transformed. The significance of fixed effects (factors and covariates) in the GLMM's were examined using Wald Type adjusted F statistics and the effect with the highest P value was sequentially dropped until only significant terms ($P < 0.05$) remained in the model (Jones and Taylor, 1999).

To assess the effect of month, year and male age on ostrich sperm motility, an ordered logit model was performed, given that motility was an ordered variable (values from 0 to 5). Motility was entered as the dependent variable, month, year (and their interactions) and male age were entered as fixed factors, while male's breeding value for chick production was entered as a covariate. Male identity was entered as a random factor to control for repeated measures of individuals. A similar analysis was conducted for male libido (values from 0 to 3).

All statistical analyses were performed using Genstat version 13 (VSN International Ltd., UK).

3. Results

3.1. Descriptive statistics of ejaculate variables

A total of 1006 ejaculates were collected over the 24-month period. The mean ejaculate volume (mean ± SEM) was 1.54 ± 0.03 mL, with a concentration of $3.33 \pm 0.04 \times 10^9$/mL, and a mean output of $5.36 \pm 0.13 \times 10^9$ sperm per ejaculation. The mean proportions of live normal, live abnormal and dead sperm were 75.32 ± 0.44%, 14.48 ± 0.34% and 10.20 ± 0.35%, respectively. The mean motility score was 4.37 ± 0.04 and the libido score was 2.84 ± 0.01. All dependent variables were affected by male identity ($P < 0.001$) and male age ($P < 0.05$) but not by their interaction ($P > 0.05$).

Table 2
The mean ± SEM of monthly ejaculate volume, sperm concentration and number of sperm in relation to month of collection in seven male ostriches over a 24-months period.

Month	Volume (mL)	Concentration ($\times 10^9$/mL)	Number of sperm ($\times 10^9$)
June	1.64 ± 0.31^{b}	2.86 ± 0.11^{a}	5.18 ± 0.47^{c}
July	1.35 ± 0.21^{a}	4.34 ± 0.16^{e}	5.61 ± 0.86^{c}
August	1.52 ± 0.26^{a}	3.23 ± 0.09^{c}	5.22 ± 0.68^{c}
September	1.39 ± 0.31^{a}	3.39 ± 0.11^{c}	5.06 ± 0.59^{b}
October	$1.81 \pm 0.17^{c,d}$	3.97 ± 0.12^{d}	7.27 ± 0.52^{h}
November	1.88 ± 0.16^{d}	$3.67 \pm 0.14^{c,d}$	7.18 ± 0.58^{h}
December	1.50 ± 0.27^{a}	3.06 ± 0.17^{b}	4.87 ± 0.64^{a}
January	1.64 ± 0.16^{b}	$3.12 \pm 0.15^{b,c}$	5.47 ± 0.64^{d}
February	$1.76 \pm 0.24^{b,c}$	3.43 ± 0.14^{c}	6.11 ± 0.57^{f}
March	1.79 ± 0.21^{c}	3.18 ± 0.11^{c}	6.04 ± 0.53^{e}
April	1.61 ± 0.31^{b}	3.32 ± 0.13^{c}	6.39 ± 0.56^{g}
May	1.66 ± 0.31^{b}	3.24 ± 0.12^{c}	5.17 ± 0.51^{c}

[a,b]Means with different superscripts within the column and factor differ at $P < 0.05$.

3.2. Quantitative analysis: effects of month of collection, male age and year on ejaculate volume, concentration and sperm numbers

The greatest ejaculate volume was collected in October–November (spring months) and April (autumn; $F_{11,\ 994} = 3.42$, $P < 0.001$; Table 2), while the concentration of sperm was higher in September–October (spring months; $F_{11,\ 994} = 8.51$, $P < 0.001$; Table 2) and higher numbers of sperm were also collected in September–October ($F_{11,\ 994} = 3.34$, $P = 0.001$; Table 2). Although a similar pattern was observed across months, a difference in ejaculate volume and concentration were noted between years, whereby larger ejaculates and higher concentrations of sperm were collected in year 2 (June 2010–May 2011), as compared to year 1 (June 2009–May 2010) (ejaculate volume: year 1: 1.37 ± 0.04; year 2: 1.80 ± 0.04; $F_{1,\ 1005} = 15.04$, $P < 0.001$; concentration: year 1: $3.07 \pm 0.06 \times 10^9$/mL; year 2: $3.58 \pm 0.05 \times 10^9$/mL, $F_{1,\ 1005} = 26.47$, $P < 0.001$). A difference in the total number of sperms per ejaculate was also noted between years ($P < 0.05$). Semen volume and numbers of sperm per ejaculate varied significantly with male age ($F_{3,\ 1002} = 3.23$, $P < 0.001$; $F_{3,\ 1002} = 2.99$, $P = 0.010$, respectively; Table 3) as 3–5-year-old males produced larger ejaculates with more sperm, and across months ($F_{3,\ 994} = 3.23$, $P < 0.001$ and $F_{3,\ 994} = 7.48$, $P < 0.001$, respectively) than 2-year-old male.

Table 3
The mean ± SEM of ejaculate volume, sperm concentration and number of sperm in relation to age in seven male ostriches over a 24-months period.

Male age (Years)	Volume (mL)	Concentration ($\times 10^9$/mL)	Number of sperm ($\times 10^9$)
2	1.02 ± 0.18^a	3.70 ± 0.11^d	4.33 ± 0.35^a
3	1.61 ± 0.05^b	3.48 ± 0.04^c	5.87 ± 0.15^d
4	1.82 ± 0.05^c	2.97 ± 0.07^b	5.62 ± 0.23^b
5	2.07 ± 0.13^d	2.58 ± 0.19^a	5.85 ± 0.59^c

[a,b]Means with different superscripts within the column and factor differ at $P < 0.05$.

3.3. Qualitative analysis: effect of month of collection, male age and year on the motility and viability of sperm

Motility of sperm was affected by month of collection ($F_{11,\ 994} = 5.14$, $P < 0.001$; Table 4), with higher motility scores found in September–December (spring–early summer months), but no difference was observed between years ($P > 0.05$). Motility scores were also found to increase with the age of the male ($F_{3,1002} = 3.72$, $P = 0.002$; Table 4).

Similarly, both viability and morphology of sperm were affected by month of collection (% of live normal sperm: $F_{11,\ 994} = 6.03$, $P < 0.001$; % of live abnormal sperm: $F_{11,\ 994} = 6.40$, $P < 0.001$ and % of dead sperm: $F_{11,\ 994} = 4.31$, $P < 0.001$; Table 4). Ejaculates with higher number of normal sperms were collected in October–December (spring-early summer months), whereas ejaculates with higher number live abnormal and dead sperms were collected in June–August (winter months). In addition, a difference between the two years was observed for all three variables (% of live normal sperm: $F_{1,1005} = 9.89$, $P = 0.002$; % live abnormal sperm: $F_{1,1005} = 68.85$, $P < 0.001$; % dead sperm: $F_{1,1005} = 2.99$, $P = 0.04$). Sperm morphology and viability were also affected by male age (% live normal sperm: $F_{3,1002} = 6.01$, $P < 0.001$; % live abnormal sperm: $F_{3,1002} = 3.01$, $P < 0.001$; % dead sperm: $F_{3,1002} = 2.27$, $P < 0.001$; Table 5), with the percentage of live sperm increasing with age, while both the percentage of live abnormal and dead sperm decreasing with age.

3.4. Effect of month of collection, male age and year on number of successful collections and male libido

Out of 1344 potential semen collections over the 24-months period, only 1173 attempts were performed (due to wet/cold or hot weather conditions impacting upon the semen collections), of which a total of 1006 ejaculates were collected. The monthly rate of success varied across months ($chi^2 = 37.42$, $df = 11$, $P < 0.001$), ranging from 72% (July) to 94% (November). No difference was observed between years ($P > 0.05$). Male libido was significantly affected by month of collection ($F_{11,\ 994} = 2.26$, $P < 0.001$; Table 6) whereby male libido was higher from May to July (late autumn–winter) and September–November (spring). No difference was observed between the two years ($P > 0.05$). Finally, libido score increased with the age of males ($F_{3,\ 1002} = 5.91$, $P < 0.001$).

4. Discussion

The findings in this study demonstrate that season and age have a significant influence on male libido and semen production of male ostriches. Although it is

Table 4
The mean ± SEM of monthly sperm motility, percentage of live normal sperm, percentage of live abnormal sperm and percentage of dead sperm in relation to month of collection in seven male ostriches over a 24-months period. Sperm motility was estimated as collective motility by observing a mass movement and scored on a point scale from 1 to 5 (1: below 20% of motile sperm; 2: 20–40%; 3: 40–60%; 4: 60–80%; 5: 80–100%).

Month	Motility	% live normal	% live abnormal	% dead
June	4.23 ± 0.13^b	69.75 ± 1.27^a	22.70 ± 0.87^c	15.59 ± 1.06^b
July	4.56 ± 0.21^b	72.58 ± 1.78^a	13.27 ± 1.23^b	14.14 ± 1.48^b
August	4.43 ± 0.09^b	71.26 ± 1.01^a	15.78 ± 0.69^b	13.01 ± 0.84^b
September	4.24 ± 0.11^b	76.68 ± 1.29^a	13.95 ± 0.89^b	9.27 ± 1.08^a
October	4.72 ± 0.11^b	81.00 ± 1.31^b	10.71 ± 0.91^a	8.36 ± 1.09^a
November	4.85 ± 0.21^b	81.02 ± 1.57^b	10.00 ± 1.08^a	8.90 ± 1.31^a
December	4.81 ± 0.28^b	$80.74 \pm 1.92^{a,b}$	11.20 ± 1.32^a	8.08 ± 1.60^a
January	3.87 ± 0.15^a	72.43 ± 1.80^a	14.36 ± 1.24^b	13.19 ± 1.50^b
February	4.13 ± 0.14^a	75.91 ± 1.58^a	16.16 ± 1.09^b	7.94 ± 1.32^a
March	4.82 ± 0.11^b	78.45 ± 1.28^a	14.41 ± 0.89^b	7.18 ± 1.07^a
April	4.52 ± 0.12^b	73.92 ± 1.42^a	14.20 ± 0.98^b	$12.08 \pm 1.19^{a,b}$
May	$4.18 \pm 0.11^{a,b}$	75.23 ± 1.31^a	12.25 ± 0.91^b	14.76 ± 1.09^b

[a,b]Means with different superscripts within the column and factor differ at $P < 0.05$.

Table 5
The mean ± SEM of sperm motility, percentage of live normal sperm, percentage of live abnormal sperm and percentage of dead sperm in relation to age in seven male ostriches over a 24-months period. Sperm motility was estimated as collective motility by observing a mass movement and scored on a point scale from 1 to 5 (1: below 20% of motile sperm; 2: 20–40%; 3: 40–60%; 4: 60–80%; 5: 80–100%).

Male age (Years)	Motility	% live normal	% live abnormal	% dead
2	4.23 ± 0.13^a	74.33 ± 1.29^a	12.60 ± 0.91^c	13.07 ± 1.07^c
3	4.51 ± 0.05^c	76.53 ± 0.54^b	12.02 ± 0.38^c	11.45 ± 0.45^b
4	4.36 ± 0.08^b	77.31 ± 0.85^c	11.22 ± 0.59^b	11.47 ± 0.59^b
5	4.57 ± 0.19^d	79.40 ± 2.17^d	10.00 ± 1.52^a	10.60 ± 1.79^a

[a,b]Means with different superscripts within the column and factor differ at $P < 0.05$.

Table 6
The mean ± SEM of monthly libido scores of seven male ostriches over a 24-months period. Male libido was evaluated as the willingness to mount the dummy female and scored on a scale from 0 to 3 (0: no reaction; 1: approach with interest but no willingness to mount; 2: no courtship but willingness to mount the dummy; 3: courtship and willingness to mount the dummy).

Month	Libido score
June	2.94 ± 0.05^{b}
July	2.99 ± 0.08^{b}
August	2.68 ± 0.03^{a}
September	2.84 ± 0.04^{a}
October	2.93 ± 0.04^{b}
November	2.93 ± 0.05^{b}
December	2.84 ± 0.09^{a}
January	2.79 ± 0.06^{a}
February	2.86 ± 0.05^{a}
March	2.88 ± 0.04^{a}
April	2.70 ± 0.05^{a}
May	2.93 ± 0.04^{b}

[a,b]Means with different superscripts within the column and factor differ at $P < 0.05$.

possible to collect semen from ostriches all year around, collections in spring (September–October) yielded more semen than in winter. Similarly, the viability of sperm was higher in spring, while sperm motility was relatively constant over months, despite a reduction in summer (January–February). Male libido peaked in winter (June–July) and spring (October–November). Furthermore, the quantity and quality of sperm collected (in terms of number of sperm, morphology, viability and motility), as well as the male libido were found to increase with age.

In birds, patterns of photoperiod, rainfall and temperature are considered to be environmental cues that trigger physiological changes characteristic of the breeding season (Burger, 1949; Farner and Follett, 1966; Ruiz de Elvira et al., 1982). For instance, in Guinea fowl (*Numida meleagris meleagris*), semen quantity and quality increases with the approach of the breeding season (rainy season), but decreases with the onset of the dry season eventually leading to no ejaculate collection during the dry season (Nwakalor et al., 1998). Similarly in the emu, a close relative of the ostrich, the breeding season of males is restricted to autumn and winter months, while females begin laying in autumn, reaching a peak egg production in winter (June–July) and terminate in spring (Malecki et al., 1998). Hence, reproduction in the emu shows a strong seasonality, whereby the reproductive system seems to be 'switched' on after the summer solstice, and 'switched' off with the onset of the dry season. Interestingly, we did not detect such a clear seasonal pattern in our study and, although the quantity and quality of semen varied across months, viable semen could be collected all year around.

Several studies have also suggested that the regulation of the male ostrich sexual function may depend on day length and temperature changes, as testicular activity may be enhanced at a specific period of the year when day length is long (Degen et al., 1994; Soley and Groenewald, 1999). For instance, Degen et al. (1994) showed that testosterone levels (which stimulates the production and maturation of sperm in males) increase 1 month after the onset of the breeding season, and remain elevated for approximately 4 months, before showing lowest levels at the end of the breeding season. Our observations of a higher ejaculate volume, sperm concentration and sperm number in spring when day length increases and the lowest values observed in winter when day length is shortest suggest a pattern of elevated androgen levels to occur in spring. Our results are also consistent with previous studies on ostriches, which used different methods of semen collection and been carried out at a different latitude (Hemberger et al., 2001; Rybnik et al., 2012), whereby peak sperm production were found at the beginning of spring, while the lowest values were found towards the end of the breeding season in autumn.

There was a clear seasonal pattern in semen quality with live normal sperm reaching a peak (81%) in the middle of spring, while the lowest values (varying between 68% and 72% of live normal sperm) were observed in winter. Seasonal variation in semen quality has been documented in several birds. For instance, in the chicken (Cecil and Bakst, 1986) and the northern pintail (Penfold et al., 2000), the number of morphologically normal sperm increased as the season progressed, while higher number of abnormal sperms were mostly observed at the beginning of the breeding season. The rapid production of morphologically normal sperm is likely to be important for reproductive success as a high number of morphologically abnormal sperms correlate well with infertility (Kamar and Baldredin, 1959; Wishart and Palmer, 1986; Soley et al., 1991). In that sense, we observed higher number of abnormal and dead sperms at the beginning of the breeding season (which also correspond to winter months), while lower numbers were observed later on.

Environmental stress such as extreme climatic conditions (particularly high temperatures) has been shown to adversely affect fertility (Jensen et al., 1992). For instance, McDaniel et al. (1996) reported that an ambient temperature exceeding 31 °C depressed sperm motility, viability and fertilization potential in broilers. In this study, sperm motility score was lower in summer (January–February), as compared to other times of the year. Summer months in the Klein Karoo are characterized by hot and dry weather conditions with daytime temperatures frequently reaching 40 °C. Similar results were found in white leghorn chickens, whereby sperm motility was lower during the dry and hot season and higher during the rainy and cold season (Kamar and Badreldin, 1989; Elagib et al., 2012). Interestingly, an increase of dead sperm was observed during winter months, which could potentially result from lower androgen levels associated with a decrease of photoperiod (Degen et al., 1994; Madekurozwa et al., 2002), or an inappropriate processing of sperm during semen collection at low ambient temperature, and needs to be investigated further.

A large amount of variation was observed between males in all semen variables analysed as well as on male libido, which is in accordance with previous studies (Bonato et al., 2010, 2011; Rybnik et al., 2012). The results indicate that it should be feasible to select males for their potential to produce and supply sperm for more than one female within the context of an artificial insemination programme. Additionally, age was also found to affect

all these variables whereby older males (3–5 years old) produced larger ejaculate volumes with more sperm than younger males (2 years old). Similar results were found in the emu, whereby 2-year-old emus produced less semen than 3–5 years old emus (Malecki et al., 1997). Interestingly, the motility and the percentage of live normal sperm was also higher in older males, suggesting that the all-round quality of semen may improve with age. In all poultry species, quality parameters change with the age of males, with the semen of older birds having significantly lower motility, viability and mass movement than younger birds, ultimately leading to a progressive decline in fertility (Bakst and Cecil, 1992). In the ostrich, males may take 3–4 years to mature (Jensen et al., 1992; Soley and Groenewald, 1999), and 9-year-old males still show good fertilization success in natural mating conditions (Lambrechts, 2004; Bunter and Graser, 2000). Hence, semen parameters presented in this study are mainly characteristics of young males, and more data from older males is needed to establish when sperm senescence occurs in this species.

The existence of seasonal, age and male effects might have profound implications on cryopreservation protocols, a crucial aspect of assisted reproductive technology in this species. Season may affect sperm freezability via changes in the enzymatic or biochemical composition of semen (Datta et al., 1980). This could directly affect the permeability and toxicity of cryoprotectants and increase the susceptibility of sperm to freezing damage (Blesbois et al., 1992; Blesbois and Brillard, 2007). For instance, in free-range roosters, the season of collection influenced almost all frozen-thawed sperm motility values: the percentage of immotile frozen-thawed sperm was lower in spring-collected samples compared to those collected during the autumn and winter (Santiago-Moreno et al., 2012). In addition, the proportion of various lipid components in sperm has been shown to be age-related and to influence freezability, and ultimately the cryopreservation process (Cerolini et al., 1997; Long et al., 2010). Interestingly, Long et al. (2010) also found evidence that sperm from elite rooster lines differ in their ability to undergo cryopreservation while retaining sperm function. All these elements stress the impact of seasonal variation in ostrich sperm quantity and quality, as well as the male and male age effects onto the development of a viable protocol for assisted reproduction technology in this species.

5. Conclusions

Although it is possible to collect ejaculates from ostriches all year around, there is seasonal variation in semen quantity and quality with the highest semen quality during the spring months (October–November), when male sexual behaviour was also high. Such seasonal variation must be considered during assisted reproductive technology, when collecting ejaculates for the purpose of semen preservation or artificial insemination, so as to optimise male use and maximise fertility. Individual variation between males in semen characteristics also make it necessary to perform a semen evaluation to distinguish between males differing in their semen output and quality. Ultimately, further studies are needed to establish specific lines of sires with high semen output combined with docile behaviour. Finally, additional knowledge is needed to evaluate the effect of seasonality on sperm freezability in this species to maximize the percentage of motile and viable sperm after the freeze-thawing process.

Acknowledgments

We express our sincere gratitude to the Western Cape Department of Agriculture and the Oudtshoorn Research Farm for the usage of the resource flock and facility. We are also grateful to Stefan Engelbrecht and Basie Pfister for assistance in taking care of the birds, as well as Cecelia Mutlow at the Klein Karoo Laboratory for assistance with laboratory equipment. Funding was provided by the Western Cape Agricultural Research Trust, the South African Ostrich Business Chamber and the National Research Foundation (NRF) (TP201107263032) of South Africa through their THRIP program. M. Bonato was supported by a Claude Leon Postdoctoral Fellowship.

References

Allen, C.J., Champion, L.R., 1955. Competitive fertilization in the fowl. Poult. Sci. 34, 1332–1342.
Bakst, M., Cecil, H., 1992. Effects of modification of semen diluent with cell culture semen replacements on fresh and stored turkey semen quality and hen fertility. Poult. Sci. 71, 754–764.
Blesbois, E., Brillard, J.P., 2007. Specific features of in vivo and in vitro sperm storage in birds. Animal 1, 1472–1481.
Blesbois, E., de Reviers, M., 1992. Effect of different fractions of seminal plasma on the fertilising ability of fowl spermatozoa stored in vitro. J. Reprod. Fertil. 95, 263–268.
Bonato, M., Rybnik, P.K., Malecki, I.A., Cornwallis, C.K., Cloete, S.W.P., 2010. Between male variation in semen characteristics and preliminary results on the dilution of semen in the ostrich. S. Afr. J. Anim. Sci. 40, 438–441.
Bonato, M., Rybnik-Trzaskowska, P.K., Malecki, I.A., Cornwallis, C.K., Cloete, S.W.P., 2011. Twice daily collection yields greater semen output and does not affect male libido in the ostrich. Anim. Reprod. Sci. 123, 258–264.
Bonato, M., Cornwallis, C.K., Malecki, I.A., Rybnik-Trzaskowska, P.K., Cloete, S.W.P., 2012. The effect of temperature and pH on the motility and viability of ostrich sperm. Anim. Reprod. Sci. 133, 123–128.
Bunter, K.L., Graser, H.U., 2000. Genetic Evaluation for Australian Ostriches—A Report for the Rural Industries Research and Development Corporation. Rural Industries Research and Development Corporation, Australia.
Burger, J.W., 1949. A review of experimental investigations on seasonal reproduction in birds. Wilson Bull. 61, 211–230.
Cecil, H.C., Bakst, M.R., 1986. Serum testosterone concentration during two breeding cycles of turkeys with low and high ejaculate volumes. Dom. Anim. Endocrinol. 3, 27–32.
Cerolini, S., Kelso, K.A., Noble, R.C., Speake, B.K., Pizzi, F., Cavalchini, L.G., 1997. Relationship between spermatozoan lipid composition and fertility during aging of chickens. Biol. Reprod. 57, 976–980.
Cheng, F.P., Guo, T.J., Wu, J.T., Ursem, P.J.F., Colenbrander, B., Fung, H.P., 2002. Annual variation in semen characteristics of pigeons (*Columba livia*). Poult. Sci. 81, 1050–1056.
Ciereszko, A., Rybnik, P.K., Horbanczuk, J.O., Dietrich, G.J., Deas, A., Slowinska, M., Liszewska, E., Malecki, I.A., 2010. Biochemical characterization and sperm motility parameters of ostrich (*Struthio camelus*) semen. Anim. Reprod. Sci. 122, 222–228.
Cloete, S.W.P., Van Schalkwyk, S.J., Brand, Z., 1998. Ostrich breeding—progress towards scientifically based strategy. In: Huchzermeyer, F.W. (Ed.), Ratites in a Competitive World. Proceedings of the 2nd International Scientific Ratite Conference, 21–25 September. Oudtshoorn, South Africa, pp. 55–62.
Cloete, S.W.P., Malecki, I.A., 2011. Breeder welfare: the past, present and future. In: Glatz, P., Lunam, C., Malecki, I. (Eds.), The Welfare of Farmed Ratites. Springer-Verlag, Berlin, Heidelberg, Germany, pp. 13–44.

Datta, I.C., Prabhu, G.A., Khan, A.G., 1980. Influence of genotype and season upon phosphomonoesterase and transaminase activity in semen plasma of the fowl *Gallus domesticus*. Indian J. Exp. Biol. 18, 1195–1198.
Deeming, D.C., Bubier, N.R., 1999. Behaviour in natural and captive environments. In: Deeming, D.C. (Ed.), The Ostrich: Biology, Health and Production. CAB. Cambridge, International University Press, pp. 83–104.
Degen, A.A., Weil, S., Rosenstrauch, A., Kam, M., Dawson, A., 1994. Seasonal plasma levels of luteinizing and steroid hormones in male and female domestic ostriches (*Struthio camelus*). Gen. Comp. Endocr. 93, 21–27.
Du Plessis, L., Bonato, M., Smith, M., Malecki, I.A., Cloete, S.W.P., Soley, J., 2014. A morphological classification of sperm defects in the ostrich (Struthio camelus). Anim. Reprod. Sci., http://dx.doi.org/10.1016/j.anireprosci.2014.09.003.
Elagib, H.A.A., Musharaf, N.A., Makawi, S.A., Mohamed, H.E., 2012. The effects of age and season on semen characteristics of white leghorn cocks under Sudan conditions. Int. J. Poult. Sci. 11, 47–49.
Farner, D.S., Follett, B.K., 1966. Light and other environmental factors affecting avian reproduction. J. Anim. Sci. 25, 90–118.
Hemberger, M.Y., Hospes, R., Bostedt, H., 2001. Semen collection, examination and spermiogram in ostriches. Reprod. Dom. Anim. 36, 241–243.
Horbanczuk, J.O., Sales, J., 1999. Reproduction as a crucial problem in ostrich breeding. World Poult. 15, 28–30.
Jarvis, M., Jarvis, C., Keffen, R., 1985. Breeding seasons and laying patterns of the southern African *ostrich Struthio camelus*. Ibis 127, 442–449.
Jensen, J.M., Johnson, J.H., Weiner, S.T., 1992. Husbandry and Medical Management of Ostriches, Emus and Rheas. Wildlife and Exotic Animal TeleConsultants, College Station, TX.
Jones, M.C., Taylor, P.C., 1999. Statistical Modelling Using GENSTAT. Arnold, London.
Kamar, G.A.R., Baldredin, A.L., 1959. Sperm morphology and viability. Acta Anat. 39, 81–83.
Lake, P.E., Stewart, J.M., 1978. Artificial Insemination in Poultry. Ministry of Agriculture Fisheries and Food, Bulletin 213. Her Majesty's Stationery Office, London.
Lambrechts, H., (PhD thesis) 2004. Reproductive Efficiency of Ostriches (*Struthio camelus*). University of Bloemfontein.
Long, J.A., Bongalhardo, D.C., Pelaez, J., Saxena, S., Settar, P., O'Sullivan, N.P., Fulton, J.E., 2010. Rooster semen cryopreservation: effect of pedigree line and male age on post-thaw sperm function. Poult. Sci. 89, 966–973.
Łukaszewicz, E., Jerysz, A., Partyka, A., Siudzińska, A., 2008. Efficacy of evaluation of rooster sperm morphology using different staining methods. Res. Vet. Sci. 85, 583–588.
Madekurozwa, M-C., Chabvepi, T.S., Matema, S., Teerds, J., 2002. Relationship between seasonal changes in spermatogenesis in the juvenile ostrich (*Struthio camleus*) and the presence of the LH receptor and 3β-hydroxysteroid dehydrogenase. Reproduction 123, 735–742.
Magige, F.J., Stokke, B.G., Sortland, R., Roskaft, E., 2009. Breeding biology of ostriches (*Struthio camelus*) in the Serengeti ecosystem, Tanzania. Afr. J. Ecol. 47, 400–408.
Malecki, I.A., Martin, G.B., Lindsay, D.R., 1997. Semen production by the emu (*Dromaius novaehollandiae*). 2. Effect of collection frequency on the production of semen and spermatozoa. Poult. Sci. 76, 622–626.
Malecki, I.A., Martin, G.B., O'Malley, P.J., Meyer, G.T., Talbot, R.T., Sharp, P.J., 1998. Endocrine and testicular changes in a short-day seasonally breeding bird, the emu (*Dromaius novaehollandia*) in Southern Australia. Anim. Reprod. Sci. 53, 143–155.
Malecki, I.A., Beesley, J.R., Martin, G.B., 2000. Changes in the characteristics of emu semen with season. Br. Poult. Sci. Suppl. 41, S18.
Malecki, I.A., Rybnik, P.K., Martin, G.B., 2008. Artificial insemination technology for ratites: a review. Aust. J. Exp. Agric. 48, 1284–1292.
Malecki, I.A., Rybnik-Trzaskowska, P.K., 2011. Natural mating and artificial insemination. In: Glatz, P., Lunam, C., Malecki, I. (Eds.), The Welfare of Farmed Ratites. Springer-Verlag, Berlin, Heidelberg, Germany, pp. 45–64.
McDaniel, C.D., Bramwell, R.K., Wilson, J.C., Howarth, B., 1996. Fertility of male and female broiler breeders following exposure to elevated ambient temperature. Poult. Sci. 74, 1029–1033.
Nwakalor, L.N., Okeke, G.C., Njoku, D.C., 1988. Semen characteristics of the guinea fowl *Numida meleagris meleagris*. Theriogenology 29, 545–554.
Obidi, J.A., Onyeanusi, B.I., Rekwot, P.I., Ayo, J.O., Dzenda, T., 2008. Seasonal variations in seminal characteristics of Shikabrown breeder cocks. Int. J. Poult. Sci. 7, 1219–1223.
Penfold, L.M., Wildt, D.E., Herzog, T.L., Lynch, W., Ware, L., Derrickson, S.E., Monfort, S.L., 2000. Reprod. Fertil. Dev. 12, 229–235.
Ruiz de Elvira, M.C., Herndon, J.G., Wilson, M.E., 1982. Influence of estrogen-treated females on sexual behaviour and testosterone levels of a social group of Rhesus monkeys during the non-breeding season. Biol. Reprod. 26, 825–834.
Rybnik, P.K., Horbanczuk, J.O., Naranowicz, H., Lukaszewicz, E., Malecki, I.A., 2007. Semen collection in the ostrich (*Struthio camelus*) using a dummy or a teaser female. Br. Poult. Sci. 48, 635–643.
Rybnik, P.K., Horbanczuk, J.O., Naranowicz, H., Lukaszewicz, E., Malecki, I.A., 2012. The ostrich (*Struthio camelus*) ejaculate—effects of the method collection, male age, month of the season, and daily frequency. Br. Poult. Sci. 53, 134–140.
Saeid, J.M., Al-Soudi, K.A., 1975. Seasonal variation in semen characteristics of white leghorn, New Hampshire and indigenous chicken in Iraq. Br. Poult. Sci. 16, 97–102.
Santiago-Moreno, J., Castano, C., Toledamo-Diaz, A., Coloma, M.A., Lopez-Sebastian, A., Prieto, M.T., Camp, J.L., 2012. Influence of season on the freezability of free-range poultry semen. Reprod. Dom. Anim. 47, 578–583.
Sexton, T.J., 1983. Maximizing the utilization of the male breeder: a review. Poult. Sci. 62, 1700–1710.
Smit, D.J.v.Z., 1964. Ostrich Farming in the Little Karoo. Pamphlet No. 358. Department of Agricultural Technique Services, Pretoria, Republic of South Africa.
Soley, J.T., Bertschinger, H.J., Roberts, D.C., de Lange, D.H., Burger, W.P., 1991. Correlation between semen quality and egg fertility in ostriches. In: Seminar on Ostrich Research, Oudtshoorn, South Africa, July, pp. 45–47.
Soley, J.T., Roberts, D.C., 1994. Ultrastructure of ostrich (*Struthio camelus*) spermatozoa II. Scanning electron microscopy. Ondersterpoort J. Vet. Res. 61, 239–246.
Soley, J.T., Groenewald, H.B., 1999. Reproduction. In: Deeming, D.C. (Ed.), The Ostrich: Biology, Health and Production. CAB. International University Press, Cambridge, pp. 129–158.
Wishart, G.J., Palmer, F.H., 1986. Correlation of the fertilizing ability of semen from individual male fowls with sperm motility and ATP content. Br. Poult. Sci. 27, 97–102.