



## Developing a female-only flock for artificial insemination purposes in ostriches: Progress and future directions



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### ABSTRACT

The development of a flock of females that can produce eggs and maintain egg production rate without the presence of males is a prerogative for a viable artificial insemination protocol in ostriches. Over six consecutive breeding seasons (May–December, 2009–2014), we recorded the egg production performance of 40 single-penned (ART) South African Black ostrich females (2–9 years of age), and compared these records with the egg production of 162 pair-mated females of comparable age from the breeding flock (BP). ART females laid significantly fewer eggs than BP females (mean  $\pm$  SEM:  $3.49 \pm 0.13$  eggs per month vs.  $4.64 \pm 0.09$  eggs per month respectively;  $P < 0.001$ ). Both groups showed a similar pattern of laying, with a peak production in July to September. The mean egg weight of ART females was significantly lower than those of BP females ( $1367 \pm 2.25$  g vs.  $1423 \pm 1.1$  g, respectively;  $P < 0.001$ ). Furthermore, female age significantly affected egg production and egg weight whereby BP females reached a peak egg production at 3 years of age, while in ART females, egg production was the highest at 5 years of age. Interestingly, the number of eggs produced, clutches and eggs per clutch of ART females were independent of visual stimulation from the males. These results indicate that male presence is not needed to ensure egg production. Continuous recruitment of young females based on human-friendly behaviour to breeding by artificial insemination from high egg production performance parents could improve egg production of the ART flock. Studies are also needed to gain a better understanding of underlying physiological mechanisms promoting spontaneous ovulation in this species.

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### 1. Introduction

The technology of artificial insemination (AI) has developed over the last 50 years to become very sophisticated,

particularly in the turkey and dairy industries (Bakst and Dymond, 2013). However, crucial prerequisites (i.e. routine collection of good quality semen, semen preservation for short and long term storage and effective insemination) for the implementation of this technology appear to have slowed down its expansion to many other livestock species. Studies aimed at developing an AI program in farmed ostriches have yielded some promising results

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highlighting the potential of this technology to assist with the further development of this fragile industry (Malecki et al., 2008; Cloete and Malecki, 2011). Animal-friendly methods for both semen collection of males (Rybnik et al., 2007) and the insemination of females (Malecki et al., 2008) have been developed. An ostrich-specific semen diluent was established more recently, allowing the preservation of semen for both short and long-term storage (Smith, 2016). It has been suggested in the past that ostriches are induced breeders, based on the propensity of pair-mated females to cease egg production when their male partners are removed from the mating paddocks (Cloete et al., 1998). Therefore, another important aspect for this technology to be implemented is to have female ostriches producing quality eggs and maintaining egg production rate in the absence of males (Malecki et al., 2008).

In the farming environment, the breeding season for ostriches depends on the photoperiod and climate and lasts about 6–8 months and sometimes even longer (Degen et al., 1994; Soley and Groenewald, 1999). They are predominantly seasonal breeders, following a distinctive pattern over the reproduction season, tending to peak in late winter-early spring, followed by a rest period of about a month, before showing a second although lower peak in early-summer (Degen et al., 1994; Fair et al., 2011). In the southern hemisphere it traditionally runs from winter (May/June) to summer (January/February) (Jarvis et al., 1985; Cloete et al., 1998). Ostriches are either mated using a free-range system with a male:female ratio of 6:10, or kept in pairs or trios (Lambrechts, 2004). Sexual maturity is reached in females between 18 and 24 months of age (Soley and Groenewald, 1999; Bunter, 2002). According to Cloete and Brand (2014) the average (SD) age of ostrich females at the production of the first egg was 733 (73) days. Ostrich females may potentially produce an egg every 2 days, but egg production is extremely variable between females (Cloete et al., 1998, 2004, 2008; Bunter, 2002). For instance, over an 8-month-period, a range of 0–121 eggs/hen/season was recorded within a pair-mated flock breeding system (Van Schalkwyk et al., 1996). The mean weight of ostrich eggs varies between 1300 and 1600 g, and has been demonstrated to be variable between females (Cloete et al., 2004; Fair et al., 2011), and to not only affect chick weight at hatching but also chick survival during the first month after hatching, a crucial factor in ostrich farming (Cloete et al., 2001; Bonato et al., 2009; Fair et al., 2011). Additionally, ostriches do lay in clutches, with variable intervals between successive ovipositions, potentially lasting days and sometimes weeks (Bunter, 2002; Malecki et al., 2008). Thus, selection for increased clutch length and a reduced interval between clutches could result in increased egg production.

As suggested above, the separation of the sexes in ostriches has quickly reduced the rate of lay of ostrich females, which could complicate the use of AI as a breeding strategy if females fail to reproduce without the presence of males (Cloete et al., 1998; Malecki et al., 2004). In many avian species, male presence is required to stimulate ovarian growth and egg laying and early work has illustrated the importance of auditory and visual stimuli of male courtship behaviour on ovarian development

(Matthew, 1938; Lehramn and Friedman, 1969). However, it was observed that domestic fowl and turkey hens would readily produce eggs in the complete absence of males, although egg production was higher when turkey hens were penned in the presence of toms than when they were completely isolated from males (Bhagwat and Craig, 1979; Jones and Leighton, 1987). In the case of the emu (a close relative to the ostrich) this problem could be solved if the male remains in sight of the female (Malecki and Martin, 2002). Interestingly, a reduction in laying after the separation of sexes in emus and ostriches is not observed in all females and is thought to be influenced by rearing conditions, whereby females developed sexual preferences for humans through the phenomenon of imprinting (Malecki et al., 1997; Bubier et al., 1998; Malecki et al., 2008). If this phenomenon is associated with laying, selective breeding could take advantage of the opportunity by specifically selecting females that can maintain egg production without males. This would be an essential step for breeding programs incorporating AI technology, as this will lead to more efficient use of sperm, while maximising the number of eggs per AI dose but also reducing the cost associated with keeping extra males while promoting rapid genetic improvement stemming from an increased selection differential on the male side.

As the reproductive performance of the female ostrich may exert a considerable influence on the outcome of an AI program, our aim was to study egg production, egg weight, clutch size and clutch number for human friendly single-penned females relative to females paired with males over 6 consecutive breeding seasons.

## 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), during the 2009–2014 breeding seasons (May to December). Monthly egg production performance of 40 South African Black ostrich females (aged 2–9 years of age) single-penned and displaying desirable behaviour towards humans (i.e. lack of fear, clucking, voluntarily crouch: ART; Bonato et al., 2013), and of 162 paired females from the breeding flock (BP) of comparable breed and age were recorded. The average age was of  $4.1 \pm 0.17$  years (mean  $\pm$  SEM; range 3.6–4.5 among years) for the ART females and of  $3.6 \pm 0.13$  years (range 3.2–4.0 among years) for the BP females over the six breeding seasons. The population is a well-known resource flock providing material for many previous studies on genetic and environmental factors affecting reproduction in pair-mated ostrich females (Cloete et al., 1998, 2004, 2008). During the first three breeding seasons (2009–2011), ART females were maintained in pens isolated from males physically and visually (but not audibly), while for the last three breeding seasons (2012–2014), females were able to see and hear males but were still physically separated from them. Eggs were collected on a daily basis in both the ART and BP groups, identified according to their camp, and weighed using an

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