

## Factors related to shell deaths during artificial incubation of ostrich eggs

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### ABSTRACT

The ostrich industry experiences a high rate of embryonic mortalities during artificial incubation of eggs. Embryonic deaths were studied from data recorded on 37 740 fertile eggs incubated artificially during the 1998–2005 breeding seasons. Roughly 10 000 eggs that sustained embryonic mortalities were classified according to the stage and nature of death, i.e. before 21 days of incubation, after 21 days of incubation, deaths after pipping and rotten eggs. Although infection may have played a role in ~1300 rotten eggs, no detailed knowledge of the pathogens involved was available. The remainder of deaths could not be related to pathogens and the deaths were thus generally referred to as non-infectious. The overall level of embryonic mortality in all the eggs studied was 28.5 %. Overall embryonic mortality was affected by incubator, with higher levels (57.0 %) found in eggs incubated in an African Incubator<sup>®</sup> and also in eggs that were transferred between incubators during incubation (38.1 %). Overall embryonic mortality also increased in eggs produced by older females. Eggs produced in the autumn had the highest level of embryonic mortality at 53.6 %, whereas eggs produced in the winter had a marginally higher level of embryonic mortalities of 29.2 % compared with eggs produced during summer (27.4 %). Eggs produced by South African (SA) Black males crossed to Zimbabwean Blue females had high levels of embryonic losses of 45.7 %. The embryonic mortality of eggs produced by SA Blacks or Zimbabwean Blue breeding birds subjected to pure breeding was similar at ~33–34 %, but embryonic mortality was improved in eggs produced by Zimbabwean Blue males crossed to SA Black females (27 %). Embryonic mortality was increased in eggs that were set directly (32.0 %) or subjected to longer than 6 days of storage (43.5 %). Embryonic mortality was affected by year. The results that were obtained will assist in determining non-infectious factors that have a negative effect on hatching success. Steps can thus be taken to eliminate such factors that may compromise hatching success.

**Key words:** female age, genotype, incubator, ostrich, season, storage time.

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### INTRODUCTION

Artificial incubation has become an essential part of any commercial poultry enterprise<sup>3</sup>. Ideally, every fertile egg should produce a healthy hatchling. In reality this situation is never achieved in a commercial hatchery. Despite substantial advances in incubator design and incubation techniques since the ostrich industry in South Africa began in the 1800s, problems with embryonic mortality during artificial incubation is still one of the main

constraints to the development of the ostrich industry world-wide<sup>6,13</sup>. Optimum incubator temperature used in automatic incubators is normally defined as that required to achieve maximum hatchability<sup>17</sup>. The physiological requirements of the developing ostrich embryo are met by the control of temperature<sup>35</sup>, humidity<sup>30</sup>, gaseous environment<sup>37</sup> and the turning of eggs<sup>36</sup>. Although hatchability of artificially incubated ostrich eggs can reach 80 %, it is typically between 30 % to approximately 60 %<sup>13,36</sup>. Deeming and Ar<sup>13</sup> have reported hatchability of fertile eggs as low as 11 % in extreme situations. Shell-deaths thus contribute to a large extent to the low hatching rates in the ostrich industry. Embryonic mortality in ostrich eggs usually occurs either early or late in the incubation period, with relative few deaths in mid-term<sup>4</sup>.

The age of the female appears to be one of many factors influencing the number of eggs produced as well as hatchability.

Females start egg production at an age of 2–2.5 years and peak egg and chick production are achieved at 8–9 years of age. This peak was followed with a general decline in reproductive performance at greater ages, which was more pronounced for chick production than for egg production<sup>11</sup>. Ostrich females have a longer economic life compared with the other poultry species<sup>22</sup>, therefore making it difficult to compare ostrich breeds with the small domestic poultry species traditionally used for egg production. In both broiler breeders and quail, young females have a higher proportion of early embryonic deaths than mature females<sup>20,27,38</sup>. For ostriches the opposite seems to be true in the sense that embryonic survival decreases over successive laying seasons<sup>3</sup>.

Ostriches are generally regarded as seasonal breeders, with the commencement of the breeding season coinciding with an increase in photoperiod<sup>22</sup>. According to Lambrechts<sup>23</sup>, peak production for ostriches in the southern hemisphere occurs between winter (July) and summer (January). Genetic make-up is one of the factors influencing the performance of individuals and by selecting for certain traits, genetic improvement may be achieved<sup>26</sup>. Egg quality is also reported to have significant genetic components<sup>29</sup>. Fertility in turkeys is influenced genetically, with strain and variety differences that are apparent<sup>3</sup>.

Pre-incubation storage leads to morphological changes in the blastoderm and to a lower growth rate of the embryo in small domestic poultry<sup>16,24</sup>. Albumen quality is compromised by prolonged storage time<sup>3</sup>. A proportionate increase in early embryonic mortality occurs with an increased storage time of duck and quail eggs<sup>25,38</sup>. This coincides with results of Deeming and Ar<sup>13</sup>, reporting a lower hatchability in ostrich eggs that could be attributed to an increase in early mortalities for eggs stored between 12 and 14 days. Ar and Gefen<sup>2</sup>, Badley<sup>3</sup>, Sahan *et al.*<sup>28</sup> and Hassan *et al.*<sup>19</sup> also reported an increase in early embryonic mortalities for eggs stored for extended periods, up to 10 days and longer.

The main objective of the present study was to investigate non-infectious factors that potentially influence embryonic

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mortality in ostrich eggs, specifically genotype, female age, year and season of production, storage time prior to setting eggs in the incubator and the incubator used.

## MATERIALS AND METHODS

### Animals

Eggs were derived from the commercial ostrich breeding flock maintained at the Oudtshoorn Experimental farm in the Klein Karoo region of South Africa. The origin of the ostrich flock and the general management procedures implemented have been described previously<sup>8,32</sup>. Data for this study were derived from the 1998–2006 breeding seasons. In total, 48 027 eggs were produced by the flock during this period. The data were edited to exclude infertile eggs ( $n = 10\,173$ ), eggs not set because of breakages and cracks in the shell ( $n = 1495$ ), eggs with defects, i.e. too small, soft or chalky shells, etc. ( $n = 805$ ) and eggs not set for various other reasons ( $n = 1178$ ). The latter category included eggs left as nest eggs, eggs left with breeding pairs that were used for chick rearing, as well as eggs used in other experiments. Details regarding the genotype, female age, date of lay, year and season of lay, storage time, and specific incubator used were known for individual eggs and were evaluated for the remaining 37 740 eggs in an attempt to derive robust trends involving the influence of various non-infectious factors on embryonic mortality.

Unless specified otherwise, each breeding bird received a ration of 2.5 kg dry matter per day throughout the breeding season, which lasted from the beginning of June until the end of January for most years. The exceptions to this were the 1999 breeding season (when the birds were also retained in the breeding paddocks for February) and 2002 (when some breeding pairs were left in the mating paddocks throughout the year, as part of a separate experiment described by Lambrechts<sup>25</sup>).

During 2003, Zimbabwean Blue breeders were introduced to the flock and mated in various combinations with South African Black males and South African Black females<sup>5</sup>. Data that were recorded for 2003–2006 thus involved various combinations of the 2 purebred bloodlines as well as the reciprocal crosses between them. Because Zimbabwean Blue birds were heavier than their South African Black contemporaries<sup>5</sup>, they were assumed to have a larger maintenance requirement and therefore received 2.8 kg feed/breeding bird per day.

### Egg storage and incubation

Methods for collection, sanitation and storage at the experimental site are well documented<sup>33,34</sup>. Briefly, eggs were collected daily, weighed and identified by date and paddock (female) of origin. The surface of each egg was sterilised by 20 min of ultraviolet exposure and labelled with a permanent marker. During the breeding season, eggs were stored for no more than 6 days at a temperature of 17 °C and relative humidity (RH) of 75 %. At the beginning and end of the season, however, there were insufficient eggs to occupy the available incubator space optimally. These eggs were consequently stored for periods not exceeding 20 days. Eggs were artificially incubated at 36 °C and 24 % RH in Buckeye<sup>®</sup>, Prohatch<sup>®</sup>, Natureform<sup>®</sup> or African International<sup>®</sup> incubators. A practice of moving eggs between incubators during the incubation process was necessary because of limited space within incubators and the large numbers of eggs being produced each week during peak production. Space in individual incubators dictated the placement of eggs. The Buckeye<sup>®</sup> and Prohatch<sup>®</sup> incubators were preferred as the primary incubators throughout the laying season. The other incubators were primarily used during peak-laying in winter and spring to accommodate the overflow. This resulted in the occasional use of a combination of incubators for a single setting of eggs. The capacity and operation of the incubators, with the exception of the African Incubator<sup>®</sup> (which was more recently acquired) have been described by Cloete *et al.*<sup>10</sup>. The African Incubator<sup>®</sup> has a capacity of 1000 eggs and was operated at 36 °C and a RH of 24 %. During incubation, the eggs were turned through an angle of 60° hourly and eggs were treated as described by Van Schalkwyk *et al.*<sup>36</sup>.

### Statistical methods

Overall embryonic deaths were classified as embryonic deaths from 0 to day 21 of incubation (1st half), after 21 days of incubation (2nd half), post-pipping and rotten eggs. Eggs not showing any macroscopic development (blastoderm size <3 mm) were regarded as infertile and those with embryonic development that had ceased (blastoderm size >3 mm) as 1st-half embryonic deaths<sup>36</sup>.

It is conceded that infection may have played a role in ~1300 rotten eggs. However, no detailed knowledge of the pathogens involved was available. The remainder of deaths could not be related to pathogens, and the deaths were thus referred to as non-infectious.

Chi square procedures were used to

assess the effects of genotype, female age, year and season of lay, storage time, as well as of the incubator used<sup>31</sup>. The Bonferoni correction was applied to all analyses, since all analyses involved multiple comparisons. Embryonic deaths during the 2nd half of the incubation period and deaths post-pipping were expressed relative to all fertile eggs, or relative to the eggs still being incubated after candling at 21 days (i.e. after infertile eggs and eggs with embryonic mortalities during the 1st half of the incubation period were removed). Both sets of figures are presented but it needs to be stressed that the conclusions derived from this study were largely independent of this classification.

## RESULTS

### Genotype

Since the Zimbabwean Blue birds were introduced to the experimental farm during 2003, only the production seasons of 2003–2006 were considered. Eggs produced by South African Black females mated to Zimbabwean Blue males had the lowest proportion of embryonic mortality (0.270; Table 1). The latter proportion is significantly lower than those derived for purebred South African Black eggs (0.338) and from an uncharacteristically high proportion of overall embryonic deaths in the South African Black male × Zimbabwean Blue female combination (0.457) ( $P < 0.05$ ). Embryonic deaths during the 1st half and 2nd half of incubation, as well as rotten eggs were the main contributors to the poor performance of eggs produced by the latter breed combination compared with those eggs produced by Zimbabwean Blue males mated to South African Black females in particular. Overall shell deaths were fairly consistent across seasons for South African Blacks (ranging from 0.331 in summer to 0.341 in the winter), and the 2 crossbred combinations (ranges of 0.267–0.277 in the Zimbabwean Blue × South African Black combination and 0.439–0.472 in the South African Black × Zimbabwean Blue combination). By contrast, embryonic mortalities of pure Zimbabwean Blue eggs were affected by season ( $\chi^2 = 6.431$ ; degrees of freedom = 3;  $P < 0.05$ ). Closer scrutiny of the data revealed that embryonic mortalities tended to be lower during summer (0.239) than in winter (0.365) and in spring (0.302). However, no significant differences between seasons were detected after the Bonferoni correction was applied to the data, owing to the low number of eggs produced in summer ( $n = 61$ ).

Table 1: Proportions of overall and classified embryonic mortality in relation to genotype for the 2003–2005 breeding seasons.

Genotype	Category							Overall
	Number of eggs	Embryonic deaths – 1st half	Embryonic deaths – 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	
Overall	19 925	0.073	0.207	0.223	0.012	0.016	0.042	0.333
Black male × Black female	15 102	0.072 <sup>a</sup>	0.211 <sup>b</sup>	0.227 <sup>b</sup>	0.011	0.015	0.044 <sup>b</sup>	0.338 <sup>b</sup>
Blue male × Blue female	894	0.097 <sup>b</sup>	0.178 <sup>a,b</sup>	0.197 <sup>a,b</sup>	0.020	0.028	0.034 <sup>a,b</sup>	0.329 <sup>b</sup>
Black male × Blue female	976	0.122 <sup>c</sup>	0.255 <sup>c</sup>	0.291 <sup>c</sup>	0.015	0.025	0.065 <sup>c</sup>	0.457 <sup>c</sup>
Blue male × Black female	2 953	0.059 <sup>b,c</sup>	0.177 <sup>a</sup>	0.188 <sup>a</sup>	0.012	0.015	0.022 <sup>a</sup>	0.270 <sup>a</sup>
$\chi^2$		51.735	35.446	46.134	7.458	9.035	44.854	122.028

<sup>a,b,c</sup>Denote significant ( $P < 0.05$ ) differences in columns.

Critical  $\chi^2$  ( $P = 0.05$ ) for 3 degrees of freedom = 7.815.

\*Expressed relative to eggs still being incubated after candling at 21 days.

### Female age

The proportion of overall embryonic mortality increased with female age ( $P < 0.05$ ; Table 2). Both embryonic deaths during the 1st and 2nd half of incubation were proportionally increased in older females, the effect being more pronounced for deaths during the 2nd half of incubation ( $P < 0.05$ ). Embryonic mortalities belonging to other classifications were less clearly related to female age, although a small number of significant differences were also found for those eggs for which a cause of mortality could not be ascribed (Table 2). Embryonic mortality post-pipping, and rotten eggs, were largely independent of female age ( $P > 0.05$ ). However, the eggs produced by the oldest female age category of >10 years appeared to sustain higher levels of spoilage than those produced by some younger age groups ( $P < 0.05$ ).

### Year

Significant variation was found for the overall embryonic survival of eggs produced in different years. Significant

( $P < 0.05$ ) variation between years was also found for the classified stages and causes of embryonic mortality. These results were not tabled, for reasons that are outlined in the Discussion.

### Season

Overall, embryonic mortality was particularly high in a small number of eggs produced during autumn ( $P < 0.05$ ; Table 3). The overall proportion of embryonic deaths was marginally lower ( $P < 0.05$ ) in eggs produced during summer (December–February) than in eggs produced during winter (June–August), at 0.274 and 0.292, respectively. Compared with the eggs produced out of season during autumn, however, the latter difference is of a small magnitude. Season had no effect on embryonic mortalities that occurred post-pipping (Table 3).

### Storage time

The overall proportions of embryonic mortality were higher ( $P < 0.05$ ) at 32.0 % in freshly laid eggs that were set directly without storage and in those eggs stored

for >6 days (43.5 %; Table 4). The deleterious effect of a short storage time was confined to the late embryonic mortalities. Embryonic survival in general (except for deaths post-pipping) was adversely affected by prolonged storage ( $P < 0.05$ ).

### Incubator

Information on the incubator used to incubate specific eggs was not recorded during the 1998 breeding season. The influence of the incubator on embryonic deaths could consequently only be considered for eggs produced subsequently (Table 5). Overall, embryonic mortalities were higher (57.0 %) for eggs incubated in the African Incubator<sup>®</sup> and in those incubated in combinations (38.1 %) ( $P < 0.05$ ; Table 5). The overall embryonic mortality of eggs incubated in the Prohatch<sup>®</sup> incubator were also somewhat higher than those incubated in the Buckeye<sup>®</sup> or Natureform<sup>®</sup> incubators. Shell deaths during the 1st half of incubation were particularly high in the African Incubator<sup>®</sup>, whereas shell deaths during the 2nd half of the incubation period were higher in

Table 2: Proportions of overall and classified embryonic mortality in relation to female age from 2 to >10 years.

Age	Category							Overall
	Number of eggs	Embryonic deaths – 1st half	Embryonic deaths – 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	
Overall	37 740	0.060	0.178	0.189	0.011	0.014	0.036	0.285
2 years	2 100	0.044 <sup>a</sup>	0.119 <sup>a</sup>	0.125 <sup>a</sup>	0.013	0.016	0.038 <sup>a,b</sup>	0.214 <sup>a</sup>
3 years	5 477	0.051 <sup>a</sup>	0.158 <sup>b</sup>	0.167 <sup>b</sup>	0.010	0.013	0.034 <sup>a</sup>	0.253 <sup>b</sup>
4 years	5 921	0.051 <sup>a</sup>	0.171 <sup>b,c</sup>	0.180 <sup>c,b</sup>	0.012	0.016	0.032 <sup>a</sup>	0.266 <sup>b,c</sup>
5 years	5 732	0.060 <sup>a,b</sup>	0.165 <sup>b</sup>	0.175 <sup>c,b</sup>	0.012	0.016	0.041 <sup>a,b</sup>	0.277 <sup>b,c</sup>
6 years	5 092	0.064 <sup>a</sup>	0.179 <sup>b,c</sup>	0.192 <sup>c</sup>	0.007	0.009	0.033 <sup>a</sup>	0.283 <sup>c</sup>
7 years	4 529	0.070 <sup>b</sup>	0.181 <sup>b,c</sup>	0.195 <sup>c</sup>	0.011	0.014	0.038 <sup>a</sup>	0.299 <sup>c,d</sup>
8 years	3 485	0.062 <sup>a</sup>	0.219 <sup>d</sup>	0.233 <sup>a,c</sup>	0.011	0.016	0.034 <sup>a</sup>	0.326 <sup>d,e</sup>
9 years	2 402	0.078 <sup>b</sup>	0.230 <sup>d</sup>	0.249 <sup>a,d</sup>	0.009	0.013	0.031 <sup>a</sup>	0.348 <sup>e</sup>
10 years	1 599	0.058 <sup>a</sup>	0.187 <sup>b,d</sup>	0.198 <sup>d,c</sup>	0.015	0.020	0.036 <sup>a,b</sup>	0.295 <sup>c,d</sup>
>10 years	1 403	0.076 <sup>b</sup>	0.205 <sup>c,d</sup>	0.222 <sup>d</sup>	0.014	0.020	0.059 <sup>b</sup>	0.355 <sup>d,e</sup>
$\chi^2$		56.047	166.296	186.891	15.101	14.162	33.154	205.679

<sup>a,b,c,d,e</sup>Denote significant ( $P < 0.05$ ) differences in columns.

Critical  $\chi^2$  ( $P = 0.05$ ) for 9 degrees of freedom = 16.919.

\*Expressed relative to eggs still being incubated after candling at 21 days.

Table 3: Proportions of overall and classified embryonic mortalities in relation to the season of production.

Season	Category							Overall
	Number of eggs	Embryonic deaths – 1st half	Embryonic deaths – 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	
Overall	37 740	0.060	0.178	0.189	0.011	0.014	0.036	0.285
Winter	13 766	0.062 <sup>b,c</sup>	0.171 <sup>a</sup>	0.183 <sup>a</sup>	0.012	0.015	0.047 <sup>b</sup>	0.292 <sup>b</sup>
Spring	16 425	0.059 <sup>b</sup>	0.183 <sup>a</sup>	0.194 <sup>a</sup>	0.011	0.015	0.026 <sup>a</sup>	0.279 <sup>a,b</sup>
Summer	7 260	0.053 <sup>a,b</sup>	0.174 <sup>a</sup>	0.184 <sup>a</sup>	0.010	0.013	0.037 <sup>b</sup>	0.274 <sup>a</sup>
Autumn	289	0.159 <sup>d</sup>	0.294 <sup>b</sup>	0.350 <sup>b</sup>	0.007	0.013	0.076 <sup>c</sup>	0.536 <sup>c</sup>
$\chi^2$		58.289	33.695	47.980	1.525	1.236	108.969	100.051

<sup>a,b,c,d</sup>Denote significant ( $P < 0.05$ ) differences in columns.

Critical  $\chi^2$  ( $P = 0.05$ ) for 3 degrees of freedom = 7.815.

\*Expressed relative to eggs still being incubated after candling at 21 days.

Table 4: Proportions of overall and classified embryonic mortalities in relation to storage time from collection to setting.

Storage time	Category							Overall
	Number of eggs	Embryonic deaths – 1st half	Embryonic deaths – 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	
Overall	34 289	0.060	0.177	0.189	0.010	0.013	0.036	0.283
Set directly	4 956	0.057 <sup>a,b</sup>	0.218 <sup>d</sup>	0.231 <sup>c</sup>	0.013	0.018 <sup>b</sup>	0.032 <sup>a</sup>	0.320 <sup>d</sup>
1 day	4 808	0.058 <sup>a,b</sup>	0.179 <sup>b,c</sup>	0.191 <sup>b</sup>	0.011	0.015 <sup>a,b</sup>	0.035 <sup>a</sup>	0.284 <sup>b,c</sup>
2 days	4 791	0.049 <sup>a</sup>	0.170 <sup>a,b,c</sup>	0.179 <sup>a,b</sup>	0.	0.013 <sup>a,b</sup>	0.033 <sup>a</sup>	0.263 <sup>a,b,c</sup>
3 days	4 512	0.058 <sup>a,b</sup>	0.152 <sup>a</sup>	0.169 <sup>a,b</sup>	0.012	0.015 <sup>a,b</sup>	0.039 <sup>a</sup>	0.261 <sup>a</sup>
4 days	4 950	0.058 <sup>a,b</sup>	0.150 <sup>a</sup>	0.160 <sup>a</sup>	0.007	0.009 <sup>a</sup>	0.037 <sup>a</sup>	0.253 <sup>a</sup>
5 days	4 575	0.065 <sup>b</sup>	0.181 <sup>b,c</sup>	0.193 <sup>b</sup>	0.007	0.009 <sup>a</sup>	0.038 <sup>a</sup>	0.291 <sup>c,d</sup>
6 days	4 808	0.066 <sup>b</sup>	0.174 <sup>a,b,c</sup>	0.186 <sup>b</sup>	0.010	0.013 <sup>a,b</sup>	0.033 <sup>a</sup>	0.282 <sup>c</sup>
>6 days	889	0.107 <sup>c</sup>	0.243 <sup>d</sup>	0.272 <sup>c</sup>	0.004	0.007 <sup>a,b</sup>	0.081 <sup>b</sup>	0.435 <sup>d</sup>
$\chi^2$		51.390	129.631	129.798	18.867	20.850	58.288	179.548

<sup>a,b,c,d</sup>Denote significant ( $P < 0.05$ ) differences in columns.

Critical  $\chi^2$  ( $P = 0.05$ ) for 7 degrees of freedom = 14.067.

\*Expressed relative to eggs still being incubated after candling at 21 days.

both the African Incubator<sup>®</sup> and in eggs set in combinations of incubators ( $P < 0.05$ ). Although other significant ( $P < 0.05$ ) differences between designations were also found for other categories of embryonic deaths, the magnitude of these differences were modest compared to those above. The only category that was not affected by incubator was those deaths that occurred post-pipping, after

the eggs had been transferred to the hatcher.

## DISCUSSION

### Genotype

In the breeding programme, Zimbabwean Blues have been introduced to produce offspring with an improved live weight<sup>14</sup> and an improved carcass

weight<sup>5</sup>. However, the effect of cross-breeding on egg production and fertility also needs to be considered. Embryonic mortality as a result of genetic problems can negatively influence hatchability, but such an effect has not yet been recorded in ostriches<sup>3</sup>. The unexpectedly high level of embryonic deaths in the SA Black × Zimbabwean Blue combination is a cause of concern, especially since the best

Table 5: Proportions of overall and classified embryonic mortalities in relation to the incubator used during the 1999–2005 breeding seasons

Incubator	Category							Overall
	Number of eggs	Embryonic deaths – 1st half	Embryonic deaths – 2nd half	Second half (live)*	Death post-pipping	Death post-pipping (live)*	Rotten	
Overall	34 221	0.060	0.177	0.188	0.001	0.013	0.036	0.283
Buckeye <sup>®</sup>	23 196	0.053 <sup>a</sup>	0.161 <sup>a</sup>	0.169 <sup>a</sup>	0.009	0.012	0.036 <sup>b</sup>	0.259 <sup>a</sup>
Prohatch <sup>®</sup>	7 368	0.061 <sup>b</sup>	0.200 <sup>b</sup>	0.213 <sup>b</sup>	0.011	0.015	0.033 <sup>b</sup>	0.305 <sup>b</sup>
African Incubator <sup>®</sup>	718	0.273 <sup>c</sup>	0.258 <sup>c</sup>	0.354 <sup>d</sup>	0.007	0.015	0.032 <sup>a,b</sup>	0.570 <sup>d</sup>
Natureform <sup>®</sup>	473	0.076 <sup>a,b</sup>	0.127 <sup>a</sup>	0.137 <sup>a</sup>	0.013	0.016	0.015 <sup>b</sup>	0.230 <sup>a</sup>
Combinations	2 466	0.062 <sup>a,b</sup>	0.251 <sup>c,d</sup>	0.267 <sup>c</sup>	0.011	0.015	0.058 <sup>a</sup>	0.381 <sup>c</sup>
$\chi^2$		599.733	200.959	273.667	2.949	4.624	41.312	497.835

<sup>a,b,c,d</sup>Denote significant ( $P < 0.05$ ) differences in columns.

Critical  $\chi^2$  ( $P = 0.05$ ) for 4 degrees of freedom = 9.488.

\*Expressed relative to eggs still being incubated after candling at 21 days.

hatchability results in absolute terms were achieved in the reciprocal cross. Further research is required to enable a better understanding of this phenomenon, since it cannot be readily explained at present. Differences between the pure-bred genotypes and the Zimbabwean Blue × SA Black cross were of a smaller magnitude, albeit also significant ( $P < 0.05$ ). In the comparison with the breeds and crosses, it should also be considered that SA Black females overall had a markedly higher overall egg and chick production than Zimbabwean Blue females, irrespective of the genotype of the sire<sup>5</sup>.

### Female age

In the resource population used, overall hatchability was lower in 2-year-old females, most likely due to lower levels of fertility<sup>11</sup>. Although the oldest age groups were still capable of good egg production, chick production declined owing to higher levels of embryonic mortality<sup>7,11</sup>. The latter trend coincides with our findings in that young females had the lowest percentage of embryonic deaths during the 1st half and 2nd half of incubation. There was also an increase in overall embryonic deaths. The data suggest that females older than 8–10 years should be culled in breeding operations. The higher shell deaths can be related to changes in egg weight and shell quality with hen age, which is presumed to ultimately influence the hatchability of eggs<sup>7,11</sup>.

### Year

Even though year affected the hatchability of ostrich eggs, year effects are generally transient and unpredictable. Year effects may depend on typical climatic conditions; variation in the chemical composition of the raw materials used to compose diets and managerial regimes occurring for that specific year. Although eggs being incubated are shielded from changes in atmospheric climate conditions by a climate controlled environment, it is conceivable that ambient climatic conditions may affect eggs prior to incubation. However, such effects are poorly understood at present and are still being investigated. It would suffice to state that year effects are not repeatable and average performance in a given year cannot be predicted with any reasonable accuracy. Such effects have, therefore, little practical application, except for the identification of possible long-term trends.

### Season

The highest overall shell deaths of 53.6 % in autumn are, in the context of ostrich production, probably irrelevant because autumn usually falls in the normal

rest period of the breeders. Furthermore, most of the relatively small number of eggs designated as autumn eggs was acquired only over a single season. Moreover, since only part of the flock was involved, eggs had to be stored for slightly longer periods than usual to utilise incubator space optimally. The other seasons were represented throughout the experimental period, and results are probably more representative. In the other seasons, shell deaths were highest during winter at 29.2 %. From this level, embryonic deaths declined by 1.8 % towards summer (Table 3). These results differ from those of Wilson *et al.*<sup>39</sup> that hatchability for the set number of eggs decreased linearly as the breeding season progressed. In this study the hatchability of fertile eggs increased as the breeding season progressed from winter to summer. Whereas the summer season resulted in the lowest proportion of embryonic deaths for ostrich eggs in the present study, the winter season resulted in the best hatching results for duck eggs<sup>9</sup>. The slight increase in embryonic mortality early in the breeding season should be balanced against the higher overall egg production in the winter and spring seasons compared with the summer. Egg output was found to taper off in the period leading to and after the summer solstice<sup>15,23</sup>. This effect is clearly discernible in the egg numbers provided in Table 3.

### Storage time

Embryonic mortalities in the 1st half of the incubation period increased nearly 2-fold in eggs stored for >7 days. Sahan *et al.*<sup>28</sup> correspondingly found that late embryonic deaths increased from 14.3 % in eggs stored for 1 day to 18 % in eggs stored for 10 days. In this study, embryonic mortalities in the 2nd part of incubation increased by 4.1 % after 6 days of storage, corresponding to the proportion of mortalities in eggs that were set directly. Ar *et al.*<sup>2</sup> also reported a significant increase in rotten ostrich eggs after 7 days of storage. In this study, rotten eggs increased by about 5 % for eggs stored for more than 6 days. The results suggest that fresh eggs have higher embryonic deaths in the 2nd half of the incubation period compared with eggs stored between 1 and 6 days. Understandably this was also reflected in higher overall embryonic mortalities in freshly-packed eggs. These results are in accordance with findings by Ar *et al.*<sup>2</sup>, Narahari *et al.*<sup>25</sup>, Fassenko *et al.*<sup>16</sup>, Deeming<sup>12</sup>, Wilson *et al.*<sup>39</sup> and Horbańczuk<sup>21</sup> who reported that hatchability decreased as the duration of pre-incubation storage time for ostrich eggs increased.

### Incubator

As with other species of birds, the physiological requirements of the developing ostrich embryo can be met during artificial incubation by provision of appropriate temperature<sup>34</sup>, humidity<sup>30</sup>, the correct gaseous environment<sup>37</sup> and the proper turning of eggs<sup>36</sup> in automatic incubators. There are currently a number of commercially available ostrich incubators on the market, ranging from wooden incubators which provide only temperature control and air circulation to those that allow electronic control of all variables. In our study, all the incubators were of the latter type. All the incubators were set to provide the same conditions with regard to temperature, 36 °C and RH, 24 %. Despite this, there was differential embryonic mortality. The African Incubator<sup>®</sup> had the highest proportion of shell deaths in the 1st half of the incubation period and also the highest overall embryonic deaths. Because this incubator was mainly used during winter and spring, when egg production outstripped the capacity of the other incubators, this trend could not be attributed to usage during seasons when high levels of embryonic mortalities were expected. Conversely, its undesirable performance could rather be attributed to temperature gradients caused by suboptimal incubator design, which was evident in the fluctuation in temperature readings taken in its compartments throughout the breeding season. Exposure to heat stress can have a significant influence on hatchability of eggs<sup>1</sup> and high temperatures can lead to an increase in early- and late-embryo mortalities<sup>3,17,18</sup>. The same detrimental effect on hatchability was also shown in ostrich eggs, where severe temperature gradients were shown to exist in forced-draught wooden incubators<sup>35</sup>. The use of a combination of incubators resulted in a significantly higher level of late embryonic mortalities (25.1 %). We did not find any corresponding literature in other avian species, but it seems reasonable to postulate that the increased handling of these eggs may have contributed to this result.

### CONCLUSIONS

This study did not attempt to clarify infectious causes of embryonic mortality of ostrich eggs, since the pathogens involved in a minority of rotten eggs were not identified. It has to be conceded that embryonic mortalities probably originate from a number of multi-factorial causes, which cannot be dealt with exhaustively in a single paper. However, a number of non infectious effects related to embryonic mortality of ostrich eggs could be identi-

fied. Some have direct and immediate application, such as the culling of females older than 8–10 years from the breeding flock, the storage of eggs for only 1–6 days where at all practicable, and to assure that all incubators are set optimally for maximised hatching success. It also seems to be a good management practice to minimise the transfer of eggs being incubated between incubators. Furthermore, an extended research effort is indicated as far as the observed bloodline effects are concerned. Such studies may play a major role in the better understanding and eventual resolution of the problem of production losses in commercially incubated ostrich eggs.

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